D 2013

INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR
UNIVERSIDADE DO PORTO

SALMONELLA IN SWINE IN PORTUGAL

CHARACTERIZATION OF RISK FACTORS AND MODELLING INFECTION

CARLA SOFIA CORREIA GOMES
TESE DE DOUTORAMENTO APRESENTADA AO
INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR DA UNIVERSIDADE DO PORTO
CIÊNCIAS VETERINÁRIAS

CARLA SOFIA CORREIA GOMES

Salmonella in Swine in Portugal:
Characterization of Risk Factors and Modelling Infection

Doctoral Degree in Veterinary Sciences
Thesis submitted to the
Instituto de Ciências Biomédicas Abel Salazar
University of Porto

Tutor: Doutor João Niza Ribeiro
Category: Invited Assistant Professor
Afiliation: Instituto de Ciências
Biomédicas Abel Salazar
University of Porto.

Co-tutor: Doutora Madalena Vieira-Pinto
Category: Assistant Professor
Afiliation: Departamento de Ciências Veterinárias,
Universidade de Trás-os-Montes e Alto Douro.

Co-tutor: Doutor Pavel Brazdil
Category: Full Professor
Afiliation: Faculdade de Economia
University of Porto.

Preface

The aims of this PhD project were to identify and quantify the risk factors associated with *Salmonella* spp. in the Portuguese swine sector; and to develop a simulation model of the disease in order to evaluate the cost-benefit of control measures for *Salmonella* spp. at farm level. Note that in Portugal, no surveillance programme for *Salmonella* in pigs is currently in place.

This PhD project was funded by the Institute for Science and Technology (Fundação para a Ciência e a Tecnologia – FCT), Portugal. The data for the risk factors analysis was provided by the Portuguese Veterinary Authorities (Direcção Geral de Alimentação e Veterinária). In addition, the data for the transmission parameters estimation manuscript was provided by Danish Agriculture & Food Council, in Denmark. Other organizations have also contributed by allowing us to use their data and these are listed in the Materials and Methods section.

As a PhD student I was registered and worked at the Department of Population Studies within the Institute of Biomedical Sciences Abel Salazar (ICBAS – University of Porto) in Portugal. From October to December of 2011 (three months) I visited the College of Engineering, Mathematics and Physical Sciences, of the University of Exeter, under the supervision of Prof. Trevor Bailey.

The thesis is divided in four chapters: Chapter 1 – Introduction; Chapter 2 – Material and Methods used in the various studies; Chapter 3 – Results, where the different manuscripts (published/submitted/drafted) are presented; and Chapter 4 – Discussion and Conclusion, which links the different studies and provides a final conclusion.

Manuscript 4 is currently under review by a scientific journal.

Manuscript 5 has not been yet submitted to any scientific journal due its dependency on the results from Manuscript 4.

I hope you enjoy reading the thesis!

Acknowledgements

This PhD has been a long and not always straightforward project. There were ups and downs, a lot of sacrifice to develop my statistical skills, filled with exciting new people, new places and a new job at the end!

When I felt “lost” I had the luck of being surrounded by amazing people that tried to guide me! The lack of data and delays in getting the data almost drove me crazy but a PhD is not for solving world’s mysteries (I bear in mind that)! A PhD is a learning process and at the end I really can say that I have developed my knowledge and skills compared to the beginning of this journey!

Where to start when they are so many people to thank! Probably with my formal and also informal supervisors: Prof. Niza, Prof. Madalena, Prof. Pavel, Prof. Lis and Prof. Trevor – thanks for all the help, guidance and for being present to support and develop my work.

My ex-colleagues from ICBAS: Niza, Denisa, Pedro, Corália, Alda, Rui and the entire group – this group has made me grown, they have bet on me and always allowed me to increase my knowledge. Without all of you I dare to say that I would not have any success in this journey. Other friends on ICBAS or linked to ICBAS also gave me encouragement: Ricardo, Marta, Madalena, Estela, Joana among others. Thanks for all the support.

A big thank to Theo! Theo has a big heart and allowed me to take advantage of his skills and shared with me his knowledge! It was fun to develop the model with him especially in the parts where he allowed me to “kill” the pigs! I think it was a great experience for both of us!

A thank to Carol, Harriet, Selene and Theo for proof-reading the thesis.

I thank to all my friends that kept my life on track and were always there to support me: Tânia, Mariana, Sofia, Margarida, Fábio, Sónia, Marta, Renata, Paula, Cristina, Pennie, Carol among others (this page is too short to list all of you) – life is a journey and I really enjoy having the pleasure of doing the journey with all of you!

My family, my parents, my siblings, my aunt… I have an amazing family, always present, always making me stronger! No words can describe how much honored I feel for belonging to this family. A big thank from me!

Last but not the least, Paulo you have been my support all these years! Keep being it! I’m a lucky girl!

SUMMARY

Salmonellosis is one of the major causes of food-borne disease in the world. The EU Regulation (EU Regulation No 2160/2003) requires its Member States (MS) to implement control measures in order to reduce the prevalence in food production species, including pigs. To set the reduction target each MS carried out baseline surveys to estimate the *Salmonella* spp. prevalence in some food production animals. In pigs, a baseline study at abattoir level (collection of lymph nodes of pigs slaughtered) and another at herd level (collection of pen faecal samples of breeding pigs) were performed. During these cross-sectional studies, information regarding herd management practices and potential risk factors linked to *Salmonella* was collected. Data from these baseline studies in Portugal was used in this thesis for a risk factor analysis where some risk factors were highlighted to be linked with increased risk at abattoir or at farm level. The study at abattoir level identified the following risk factors: abattoir region and sample collection time. Region of the herd, size of the herd (in terms of sows), management of breeding boars, source of semen, rodents control, number of animals per pen, breeding sector room, and source of feed were identified as influential risk factors in the herd level study.

Salmonella serotypes were divided in two groups: serotype Typhimurium and *S.* Typhimurium-like strains with the antigenic formula: 1,4,[5],12:i:-., and other serotypes. A categorical risk factor model was implemented to assess whether the risk factors were the same between the groups of serotypes. For the breeding pigs dataset, the group "Typhimurium" was associated with the stock density (number of breeding pigs and number of pigs per pen), the characteristics of the pig (age of breeding sows), and the source of semen. On the other hand, the group "other serotypes" was associated with region of the herd, source of semen, control of rodents, breeding sector room and source of feed. The risk factors for group Typhimurium suggest a contagious pattern and the risk factors for other serotypes appear to be related to environmental factors.

Each European MS should ideally implement control programmes to reduce the prevalence of *Salmonella* spp.. However in practice, the control of this agent in the swine sector has proved to be difficult and expensive at farm level, so the evaluation of the efficiency of control strategies for this agent has become an important and stringent issue. With this aim in mind we developed a stochastic model which simulates the agent spread inside a farrow-to-finish herd which can be used to test control measures in terms of cost-benefit. Some preparatory work was performed to estimate the transmission parameters to be used in the simulation model using data from a published longitudinal study which followed *S.* Typhimurium infected cohorts. Our model allowed for sensitivity and specificity of the tests used in the longitudinal study to be included, as well as for unobserved cohort

effects and time-dependent effects. The simulation model tried to mimic what is happening in a herd, in terms of management practice and was linked with an infection model which simulates the infections states for each sow/pig. The parameters which most influenced the infection state of sows at farrowing/suckling were: the transmission rate from susceptible (S) to infectious (I), from I to carrier (R), and from R to S; when applied to the sow-compartment. On the other hand, the parameters which most influenced the infection state of pigs for slaughter (fattening pigs) were: the transmission rate from S to I and the transmission rate from I to R, when applied to the pig-compartment; the transmission rate from R to S applied to sows at gestation and the piglets' protective factor. Several control measures can be recommended to influence these parameters in an attempt to control the proportion of infectious animals. The simulation model potentially allows quantification of cost-benefit control measures if linked to an economic model. The simulation model is flexible enough to introduce changes in the parameter distributions or values if future research and legislation so require. At the same time the model can be adapted to different types of production (e.g. breeding units, finisher units) as it was built in a compartmental way.

SUMÁRIO

A salmonelose é uma das doenças de origem alimentar mais frequente no mundo. A legislação europeia (Regulamento EU No 2160/2003) impõe aos Estados Membros (EM) a implementação de medidas de controlo com o objectivo de reduzir a prevalência de *Salmonella* spp. em espécies animais produtoras de alimentos para consumo humano, incluindo os suínos. De modo a definir uma meta de redução para este agente, cada EM levou a cabo estudos de base para estimar a prevalência da *Salmonella* spp. em animais de produção. Nos suínos os estudos base de prevalência foram efectuados nos matadouros (recolha de linfonódulos de porcos abatidos) e a nível das explorações com animais de reprodução (recolha de amostras compostas de fezes de animais reprodutores). Estes estudos transversais também recolheram informação relacionada com o maneio na exploração e com potenciais factores de risco para este agente que foram utlizados neste trabalho. Os factores de risco encontrados no estudo dos matadouros foram a região do matadouro e a hora de recolha das amostras. No estudo efectuado a nível das explorações com animais de reprodução, os factores de risco encontrados foram os seguintes: região da exploração, tamanho da exploração (número de porcas reprodutoras), maneio dos varrascos, origem do sémen, controlo de roedores, número de animais por parque, fase da produção de animais reprodutores, e a origem do alimento. Posteriormente os serotipos foram divididos em dois grupos: serotipo Typhimurium e seu semelhante com a fórmula antigénica: 1,4,[5],12:i:-., e outros serotipos. Para estimar se os factores de risco eram iguais entre estes dois grupos, foi efectuada uma análise logística categórica. Nesta análise, para os porcos reprodutores, o grupo "Tyhimurium" foi associado com o aumento da densidade de animais (número de animais reprodutores e número de animais por parque), a idade das porcas reprodutoras e a origem do sémen. O grupo "outros serotipos" foi associado com a região da exploração, a origem do sémen, o controlo de roedores, a fase da produção de animais reprodutores, e a origem do alimento. Estes resultados indicam que o grupo "Typhimurium" está associado a um padrão contagioso, e o grupo "outros serotipos" está associado a factores ambientais.

Cada EM deve implementar o seu programa de controlo para redução da prevalência deste agente. Contudo, na prática, o controlo de *Salmonella* em suínos tem sido de implementação difícil e com custos excessivos a nível do sector primário. Sendo assim, é importante avaliar a eficácia e adequabilidade das medidas de controlo existentes. Com este objectivo foi desenvolvido um modelo estocástico que simula a dinâmica da infecção por *S.* Typhimurium numa exploração em ciclo fechado e que pode ser utlizado para testar o custo-benefício das medidas de controlo. Para tal foi preciso estimar parâmetros

de transmissão da infecção. Com esse objectivo foram utilizados os dados de um estudo que seguiu grupos de animais infectados com *S.* Typhimurium ao longo do tempo. Os resultados foram ajustados para a sensibilidade e especificidade dos testes de diagnóstico utlizados, para o efeito de grupo e para o efeito de dependência de alguns parâmetros com o tempo. O modelo de simulação desenvolvido tem em consideração o maneio dos animais e ao mesmo tempo a dinâmica de infecção para cada animal. Os parâmetros que influenciaram mais o estado de infecção das porcas reprodutoras na maternidade foram os parâmetros de transmissão de susceptíveis para infecciosos, de infecciosos para portadores, e de portadores para susceptíveis, caso sejam alterados em todas as fases reprodutivas (cobrição, gestação e maternidade). Por outro lado os parâmetros que influenciaram mais o estado de infecção nos porcos de engorda foram os parâmetros de transmissão de susceptíveis para infecciosos e de infecciosos para portadores, caso sejam alterados em todas as fases de produção (maternidade, recria e engorda); o parâmetro de transmissão de portadores para susceptíveis caso seja alterado a nível da gestação; e o factor de protecção imunitário dos leitões na maternidade. Várias medidas de controlo, que influenciam estes parâmetros, podem ser aplicada para diminuir a proporção de animais infectados. O modelo de simulação pode ser utilizado para estimar os custos-benefícios de medidas de controlo se acoplado a um modelo económico. Este modelo de simulação é flexível o suficiente para introduzir mudanças nos parâmetros e suas distribuições se assim for necessário. Também pode ser adaptado a diferentes tipos de explorações (ex. unidades de engorda, unidades de reprodução) uma vez que foi construído em compartimentos.

Contents

PREFACE .... **III**

ACKNOWLEDGEMENTS .... **V**

SUMMARY .... **VII**

SUMÁRIO .... **IX**

CONTENTS .... **XI**

LIST OF FIGURES AND TABLES .... **XIII**

ACROYNMS .... **XV**

CHAPTER 1 - INTRODUCTION **1**

1.1 RELEVANCE OF THE STUDY 3

1.2. SALMONELLA – THE AGENT: LITERATURE REVIEW 4

1.2.1 Characterization 4

1.2.2 Pathogenesis and virulence 5

1.2.3 Infection by Salmonella spp. in humans 5

1.2.4 Salmonella spp infections in animals 9

1.2.5 Diagnostic methods 12

1.2.5.1 Detection of the immune response 12

1.2.5.2 Detection of the agent 14

1.3 - RISK FACTORS FOR SALMONELLA SPP. PRE-HARVEST AND HARVEST LEVEL 16

1.3.1 Farm risk factors 16

1.3.2 Risk factors on transport, lairage and slaughter associated with meat contamination 19

1.4 – SURVEILLANCE SYSTEMS AND CONTROL MEASURES FOR SALMONELLA SPP. IN SWINE 20

1.4.1 Control/Prevention 20

1.4.1.1 Pre-harvest control 21

1.4.1.2 Control at harvest level 26

1.4.2 Surveillance and monitoring systems 30

1.4.2.1 Countries with low prevalence (e.g. Sweden and Norway) 30

1.4.2.2 Countries with medium or high prevalence (Denmark, United Kingdom) 32

1.5. ECONOMIC ANALYSIS OF SALMONELLOSIS COSTS 33

1.5.1 Cost analysis of human cases to society 33

1.5.2 Cost-benefit analysis of controlling Salmonella in swine production 34

1.6. INFECTION MODELS 36

1.7. EUROPEAN UNION POLICY FOR SALMONELLA SPP. MANAGEMENT 39

1.8. OVERVIEW OF THE PIG SECTOR IN PORTUGAL 39

1.8.1. The livestock and pig sector *39*
1.8.1.1 World and in Portugal 40
1.8.1.2 Type of production 41
1.8.2 Characterisation of the pork food chain in Portugal *42*
1.9. Objectives of the PhD research 44
CHAPTER 2 – MATERIALS AND METHODS 45
2.1 Risk factors analyses for the farm and abattoir studies (Objective 1) 47
2.1.1 Slaughter Pigs risk factors study *47*
2.1.2 Breeding Pigs risk factors study *48*
2.2 Production and infection model (Objective 2) 51
2.2.1 Transmission parameters estimation *51*
2.2.2 Production and infection model *56*
CHAPTER 3 – RESULTS 69
Manuscript 1 71
Manuscript 2 81
Manuscript 3 91
Manuscript 4 107
Manuscript 5 141
CHAPTER 4 – DISCUSSION AND CONCLUSIONS 177
Conclusions 184
REFERENCES 187

List of Figures and Tables

Table 1: Reported cases of non-typhoid salmonellosis in humans (report type, number of cases, confirmed cases in the EU) 6
Table 2: Reported cases of non-typhoid salmonellosis in humans by serovar (10 most common) 7
Table 3: Baseline study (EU Regulation 2160/2003) in lymph nodes of pigs slaughtered in EU and Norway between 2006-2007 showing the prevalence of different serotypes 11
Table 4: Baseline study (EU Regulation 2160/2003) in breeding pigs in EU and Norway showing the prevalence of positive breeding holdings..........11
Table 5: Baseline study (EU Regulation 2160/2003) in breeding pigs in EU and Norway showing the prevalence of positive production holdings..........12
Table 6: Risk/Protection factors for Salmonella spp. infection, adapted from 17
Table 7: Transport, lairage and slaughter process risk factors 20
Table 8: Results of a cost–benefit analysis of four different national strategies against Salmonella in Danish pork 35
Table 9: Summary of cost-benefit analysis of the four scenarios..........36
Table 10: Infection models for Salmonella spp. infection in pigs found in the literature 38
Table 11: Pork meat market in Portugal (units in 1000tons)..........40
Table 12: Number of animals in Portugal (unit 1000heads)..........41
Table 13: Livestock production in Portugal (unit tons)..........41
Table 14: Type of swine herds in 2005 (FPAS, unpublished)..........42
Table 15: Distribution of the number of pigs per region in 2010 (1,000 animals) 42
Table 16: Distribution of the number of industrial herds (raising pigs for commercial purpose) by region in Portugal in 2009 (DGAV, unpublished) 42
Table 17: Number of abattoirs and volume of slaughter per region (between October 2010 and August 2011) 43
Table 18: Distribution of abattoirs that slaughter piglets per region (between October 2010 and August 2011) 43
Table 19: Distribution of cutting plants, minced meat and meat preparations plants, and butchers per region in 2009 (DGAV unpublished). 44
Figure 1: Scheme of the production model..........58
Figure 2: SIR model and its transition between states..........62

Acroynms

BCR	Benefit-cost ratio
BUGS	Bayesian inference Using Gibbs Sampling project
CRAN	Comprehensive R Archive Network
DALY	Disability Adjusted Life Year
DG SANCO	Directorate-General for Health and Consumers
DME	Danish mixed ELISA
DNA	Deoxyribonucleic acid
CI	Confidence Interval
EFTA	European Free Trade Association
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
GLMM	Generalized linear mixed model
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis of Cirtical Control Points
ISO	International Organisation for Standardization
kGy	Kilogray
LPS	Lipopolysaccharides
MCMC	Monte Carlo Markov Chain
MJE	Meat-juice ELISA
MLST	Multilocus sequence typing
MS	Member States
NSSCP	Norwegian Salmonella surveillance and control programme
OD	Optical density
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
PRRS	Porcine Reproductive and Respiratory Syndrome
SIR	Susceptible – Infectious – Resistant
SSCP	Single-strand conformation polymorphism
UK	United Kindgom
USA	United States of America
VNTR	Variable number tandem repeat
WHO	World Health Organization
ZAP	British Zoonosis Action Plan Salmonella Programme
ZNCP	Zoonosis National Control Plan

Chapter 1 - Introduction

1.1 Relevance of the study

Salmonellosis is one of the major causes of food-borne disease in the world. In 2010 99,020 human cases of salmonellosis were reported in the European Union (EU) [1]. In other regions this number might be similar but probably underestimated, as many cases are not reported. Beyond the human implication of this disease, *Salmonella* spp. is an important pathogen for animal production worldwide, although for the pig sector it is mainly a cause of subclinical disease. Additionally, the emergence of strains resistant to antibiotics is a problem to animal and human health. The contribution of pork products to the total burden of human salmonellosis cases varies between countries but it is estimated to be around 10% [2]. An EU Regulation (EU Regulation No 2160/2003) imposes on the Member States (MS) the implementation of control measures to reduce the prevalence in food production species including pigs. To set the reduction target each MS carried out baseline surveys to estimate the *Salmonella* spp. prevalence in some food production animals. The objective of the surveys was to obtain comparable data for all MS through harmonized sampling and testing schemes. In pigs a baseline study was done at abattoir level (collection of lymph nodes from slaughtered pigs) and another at herd level (collection of pen faecal samples from breeding pigs). These cross-sectional studies also collected information regarding herd management practices and potential risk factors linked to this agent. After setting the reduction target each MS will be responsible for establishing an effective national control programme adjusted for the country-specific characteristics, such as the risk factors, the disease prevalence and the financial implications for stakeholders.

The data generated by the baseline surveys was expected to enable the identification and quantification of potential risk factors. These factors could then be used in the development of programmes and procedures that reduce *Salmonella* spp. shedding in pig herds economically and effectively. All this information should be available before *Salmonella* reduction programmes are implemented at herd level, to enable farmers to make informed choices, enhance public health and avoid unnecessary costs [3].

The control of *Salmonella* in primary production has been enforced by food safety systems as the major source of human cases is food-borne. The need for global cooperation in the control of *Salmonella* was underlined by World Health Organization (WHO), as *Salmonella* infection threatens the live animal, feed and food trade. Therefore, *Salmonella* control is a challenge for the veterinary services, for producers, and the food industry as they aim to produce safe food. The whole food chain (from farm to fork) should implement control measures against this food-borne pathogen [4]. In practice, the control of this agent in the swine sector has proved to be difficult and expensive at farm level [5].

Consequently the evaluation of the efficiency of control strategies for this agent has become an important and stringent issue, as stated in recent reports [6].

1.2. Salmonella – the agent: literature review

1.2.1 Characterization

The genus of *Salmonella* was first identified in 1885 by Theobald Smith and Daniel Salmon. They isolated *Salmonella* Choleraesuis from the pig bowel and identified the agent responsible for enterocolitis in pigs [7]. The bacteria *Salmonella* belongs to the family *Enterobacteriaceae*. It is a genus with more than 2579 serovars that is highly adapted to animal and human hosts. It is a motile Gram-negative bacteria, facultative anaerobic, non-spore forming, rod-shaped (2-4 x 0.5 μm), and non-capsulated with fimbriae and flagela. The genus *Salmonella* consists of two species, *Salmonella enterica* and *Salmonella bongori*. The *Salmonella enterica* is divided in six subspecies: *S. enterica* subspp. *enterica*, *S. enterica* subspp. *salamae*, *S. enterica* subspp. *arizonae*, *S. enterica* subspp. *diarizonae*, *S. enterica* subspp. *houtenae*, and *S. enterica* subspp. *indica*. These species and subspecies can be distinguished on the basis of differential characteristics using biochemical tests and lisogenization, which have been supported by DNA-DNA hybridization studies and serological tests [8-10]. Each subspecies is divided into serotypes/serovars, which are determined according to their antigenic structure, which is composed of three principal antigens [8-10]: somatic antigens, flagellate antigens, and capsular antigens. The agglutination characteristics of antigens are used to differentiate more than 2579 different serotypes of *Salmonella*, according to Kauffmann-White scheme [10].

Salmonella enterica includes more than 99% of the identified serotypes of which 59% belongs to subspecies *enterica*. This subspecies includes the main clinical relevant serotypes [10].

The way serovars are classified has evolved with time. In practice, for *S. enterica* subspp. *enterica*, the subspecies name (subspp. *enterica*) does not need to be indicated as only serovars of this subspecies bear a name. Serovars of other subspecies of *S. enterica* and those of *S. bongori* are designated only by their antigenic formula [10]. Serovars that are frequently isolated in human or veterinary medicine have historically been given names denoting the syndrome (e.g. *S.* Typhi), host-specificity (e.g., *S.* Choleraesuis) or the geographical origin of the first isolation of the new serovar (e.g., *S.* Dublin) [4].

Salmonellae cause disease in both humans and animals. The serovar *S.* Typhi and most *S.* Paratyphi strains, which cause serious systemic infections in humans, are specific human pathogens. These pathogens have no animal reservoir [4].

1.2.2 Pathogenesis and virulence

Salmonellae are typically acquired through consumption of contaminated food or water. After passage through the stomach, the bacteria colonize the intestine, interacting with and translocating across the intestinal epithelium via three routes: (i) active invasion of enterocytes; (ii) invasion into specialized epithelial cells called M cells; and (iii) through dendritic cells that intercalate epithelial cells by extending protrusions into the gut lumen. Interaction of *Salmonellae* with the epithelium and the underlying resident immune cells, leads to the production of proinflammatory cytokines and chemokine, which subsequently recruit and activate other immune cells such as neutrophils, macrophages, dendritic cells, and T/B cells [11].
After consumption the bacteria is frequently exposed to low pH in the stomach, bile antimicrobial effect, decreasing oxygen supply, normal gut flora and metabolites, intestinal peristalsis, and cationic antimicrobial peptides present on the surface of epithelial cells. These encounters with stressful environments induce the expression of a number of genes whose products are essential for *Salmonella* to invade the intestinal epithelium and infect the host [4].

1.2.3 Infection by *Salmonella* spp. in humans

All Salmonella serovars are considered potentially pathogenic to humans, some more virulent than others. Human *Salmonella* infection can lead to two clinical conditions: enteric fever (typhoid and paratyphoid) and enterocolitis by non-typhoid bacteria [12]. Non-typhoid salmonellosis is considered a zoonosis. Human non-typhoid salmonellosis is characterized by a local enterocolitis. The incubation period varies between 5 hours and 7 days, and the clinical symptoms begin 12 to 36 hours after the infection. A shorter incubation period is associated with a major infectious dose or an increased susceptibility to the pathogen. The clinical symptoms include diarrhoea, nausea, abdominal pain, moderate fever and chills. The diarrhoea ranges from mild to severe with severe dehydration. Vomiting, prostration, anorexia and headaches can also occur. The symptoms last for 2 to 7 days. Sometimes systemic infections occur in the young, the elderly and the immunocompromised. Death is rare. Some people became carriers and some still shed *Salmonella* spp. after 3 months. Non-typhoid salmonellosis can cause

chronic diseases, such as reactive arthritis, and neurologic and neuromuscular diseases [8, 12]. Around 7% to 66% of humans are subclinical carriers [4].

Epidemiology of human cases

In 2010 around 99,020 confirmed cases of human salmonellosis in the EU were reported. The EU incidence was around 21.5 cases for 100,000 people, a decrease compared to the previous year (Table 1). The serovars more frequently isolated were *Salmonella* Enteritidis and *Salmonella* Typhimurium in 2009-2010 (Table 2) (EFSA 2012).

Table 1: Reported cases of non-typhoid salmonellosis in humans (report type, number of cases, confirmed cases in the EU)[1]

Country	2010				2009	2008	2007	2006
	Report type	Cases	Confirmed cases	Confirmed cases/ 100.000	Confirmed cases			
Austria	C	2179	2179	26.0	2775	2310	3375	4787
Belgium	C	3169	3169	29.2	3113	3831	3973	3693
Bulgaria	A	1217	1153	15.2	1247	1516	1136	-
Cyprus	C	137	136	16.9	134	169	158	99
Czech Republic	C	8456	8209	78.1	10480	10707	17655	24186
Denmark	C	1608	1608	29.1	2130	3669	1662	1662
Estonia	C	414	381	28.4	261	647	430	453
Finland	C	2422	2422	45.3	2329	3126	2737	2574
France	C	7184	7184	11.1	7153	7186	5510	6008
Germany	C	25306	24833	30.4	31395	42909	55400	52575
Greece	C	300	299	2.6	403	1039	706	825
Hungary	C	6246	5953	59.4	5873	6637	6578	9389
Ireland	C	356	349	7.8	335	447	440	420
Italy	C	2730	2730	4.5	4156	3232	4499	5164
Latvia	C	951	881	39.2	795	1229	619	781
Lithuania	C	1962	1962	58.9	2063	3308	2270	3479
Luxemburg	C	211	211	42.0	162	202	163	308
Malta	C	160	160	38.7	124	161	85	63
Netherlands	C	1447	1447	13.6	1205	1627	1245	1667
Poland	A	9732	9257	24.3	8521	9149	11155	12502
Portugal	C	207	205	1.9	220	332	482	387
Romania	C	1291	1285	6.0	1105	624	620	-
Slovakia	C	5171	4942	91.1	4182	6849	8367	8242
Slovenia	C	363	363	17.7	616	1033	1346	1519
Spain	C	4420	4420	38.4	4304	3833	3658	5117
Sweden	C	3612	3612	38.7	3054	4185	3930	4056
United Kingdom	C	9670	9670	15.6	10479	11511	13802	14055
Total EU		**100921**	**99020**	**21.5**	**108614**	**131468**	**152001**	**164011**
Iceland	C	34	34	11.0	35	134	93	116
Liechtenstein	C	-	-	-	-	0	1	14
Norway	C	1370	1370	25.7	1235	1941	1649	1813
Switzerland	C	1179	1179	15.1	1323	2051	1802	1798

A: aggregated data report; C: case-based report

Table 2: Reported cases of non-typhoid salmonellosis in humans by serovar (10 most common)[1]

10 serovars most commonly isolated					
2010			**2009**		
Serovar	**N**	**%**	**Serovar**	**N**	**%**
Enteritidis	43,563	45.0	Enteritidis	53,382	52.3
Typhimurium	21,671	22.4	Typhimurium	23,759	23.3
Infantis	1,776	1.8	Infantis	1,616	1.6
Typhimurium, monophasic 1,4,[5],12:i:-	1,407	1.5	Newport	760	0.7
Newport	831	0.9	Virchow	736	0.7
Kentucky	780	0.8	Derby	671	0.7
Virchow	685	0.7	Hadar	507	0.5
Derby	665	0.7	Kentucky	460	0.5
Mbandaka	470	0.5	Saintpaul	452	0.4
Agona	444	0.5	Bovismorficans	433	0.4
Outros	24,453	25.3	Outros	19,225	18.8
Total	**96,745**	**100**	**Total**	**102,001**	**100**

This incidence data (Table 1 and Table 2) can lead us to consider what foods are associated with the transmission of the infection. Furthermore what is the attributed risk for pork meat and pork products?

Fosse and colleagues [13] study has quantified the consumer risk concerning different pathogenic agents in Europe (data from the old 15 MS). In this study the human incidence for *Salmonella enterica* was quantified to be 51,537 cases per 100,000 habitants/year, with an attributed risk of 6.6% for pork meat (which results in an incidence rate of 3,374 cases of salmonellosis per 100,000 habitants/year due to pork meat). Beyond that, *Salmonella* was the agent which demonstrated higher risk values than other pathogens (like *Yersinia enterocolitica*, *Listeria monocytogenes* and *Camplylobacter* spp.).

Danish studies showed that the majority of the human cases are due to eggs (47.1% of cases, 95% confidence interval (CI): 43.3-50.8) and pork products (9% of cases, 95% CI: 7.8-10.4) [14].

In Netherlands, in 2006, the outbreaks of human salmonellosis cases were attributed to broiler meat (12%), eggs (33%), pork meat (18%, 477 cases) and beef meat (12%). The remaining outbreaks (25%) were of unknown origin [15].

Salmonella is additionally spread between countries by humans as a result of food-borne infections acquired abroad. The overall importance of this route of transmission may reflect the prevalence of *Salmonella* contamination on food (including food of animal origin) in a particular country. In low-prevalence countries, such as Finland, Norway and Sweden, more than 80% of human cases of salmonellosis are considered to be acquired abroad [4].

Antimicrobial resistance

Antimicrobial resistance is a daunting public health threat impacting both on human and animal health and it is a cause for concern wherever antimicrobial agents are in use (in hospitals, in the community, on farms, etc.). The use of antimicrobial agents in food animals results in antimicrobial resistance among pathogenic and commensal bacteria in these animals, and the resistant bacteria (or the resistant genetic determinants) may then be transmitted to humans through the food supply or by direct contact with animals. Antimicrobial resistance is emerging and spreading among some food-borne bacteria. *Campylobacter* and *Salmonella* are two examples of food-borne pathogens in which increasing resistance, particularly to fluoroquinolones and third generation cephalosporins, is a concern. Multidrug resistance is also a worrying possibility, particularly among *Salmonella*. Multidrug-resistant *S.* Typhimurium type 104 (DT104) and multidrug-resistant *S.* Newport have both caused recent food-borne outbreaks [16-18]. Pathogenic bacteria are not the only concern when considering antimicrobial resistance in bacteria with food animal reservoirs. Commensal bacteria are a less obvious threat, but can also be transferred from animals to humans through the food supply or through direct contact. These bacteria may carry transferable genetic determinants of resistance and serve as a reservoir of resistance genes for pathogenic bacteria.

In Europe, a study done between 2000 and 2004, in 134,310 non-typhoidal *Salmonella* human isolates, showed an increase in the overall resistance from 57% to 66%. In contrast there was a decline (18% to 15%) in the proportion of isolates showing multidrug-resistance (resistance to four or more antimicrobials). *Salmonella* Enteritidis resistance to nalidixic acid increased from 10% to 26% (probably related to the consumption of contaminated eggs). For *S.* Typhimurium, although the overall occurrence of resistance has been relatively unchanged over the 5-year period, there has been an overall decline in the occurrence of resistance to chloramphenicol and tetracyclines mainly by the overall reduction in the occurrence of the multi-resistant phage type (DT) 104 [19].

Several studies done in *Salmonella* isolates from animal sources showed a global increase in resistance [20-22]. In the USA the resistance was higher for sulphametazole (53%) and tetracyclines (60%) in *Salmonella* Agona pig isolates [20]. In Germany, between 2000 and 2002, 11,911 strains of *Salmonella* in animals, feed, food and environmental samples were isolated and typed. All were tested for their resistance to 17 antimicrobial drugs. Around 63% of the isolates showed some resistance and 40% were multi-resistant (resistant to more than one antimicrobial). The isolates of *Salmonella* Typhimurium DT104 in pigs and cattle and their resulting food products, were multi-resistant in 98% to 94% of the times respectively [23]. In Spain, in 290 *Salmonella* isolates from faeces of apparently healthy finishing pigs and 192 *Salmonella* isolates from faeces

of finishing pigs with diarrhoea, 90.3% resistance was detected in healthy animals and 95.3% in ill animals. Resistance was common in isolates of serogoup B of serovar Typhimurium and its monophasic variant 5,12:i:-. In 50% of the isolates multi-resistance was detected (defined in this study as resistance to more than 4 antimicrobials) [24]. When antimicrobial resistance data from herds that usually use antibiotics is compared, to data from herds that do not usually use them, it is common to see an increase of antimicrobial resistance linked to the antibiotics consumption [25, 26].

1.2.4 *Salmonella* spp infections in animals

As in humans, animals infected by *Salmonella* may or may not develop disease. Serovars that cause disease in a specific animal species are: *Salmonella* Abortus ovis (sheep), *Salmonella* Choleraesuis (pigs), *Salmonella* Gallinarum (poultry), *Salmonella* Abortus equi (horses), and *Salmonella* Dublin (cattle) [4]. These serovars cause disease in the species to which they are adapted and are considered less pathogenic to people. However, when humans become infected with the abovementioned serovars, they might cause severe septicaemia. These host adapted serovars primarily cause abortions or severe gastroenteritis in their animal host. [4].
A group of more frequently isolated serovars, such as *S.* Typhimurium, *S.* Enteritidis, *S.* Hadar and *S.* Infantis (among others), affect both humans and animals. In food production animals, these serovars may cause clinical disease (septicaemia, acute enteritis or chronic enteritis) or subclinical disease. In the subclinical form of the disease, the animal may either have a latent infection or become a temporary or persistent carrier [4]. For most of the food production animal species, *Salmonellae* usually establish a clinically inapparent infection of variable duration, with consequences in terms of public health. However, under various stress conditions, serovars that are usually non-pathogenic may also cause disease in food animal species [4].
The farm prevalence, depending on animal species and region, may vary from 0% to 90% [4].

Salmonella infection in pigs
Transmission of *Salmonella* between pigs is thought to occur mainly via the faecal–oral route. Depending on the inoculation dose, infection of pigs with *Salmonella* Typhimurium may result in clinical signs and faecal excretion of high numbers of bacteria [27]. Some studies show that the upper respiratory tract and lungs may be a portal of entry as well, and in recent reports, the airborne transmission of *Salmonella* Typhimurium in weaned pigs over short distances was found, but may be serotype dependent [28, 29]. The

palatine tonsils are often heavily infected in pigs and should be considered as a potential source of *Salmonella* contamination during slaughter. Following ingestion, *Salmonella* must survive the low pH of the stomach. When the pigs are fed a coarsely ground meal, this will result in slow emptying of the stomach and consequently a longer time in the acidic environment, reducing the number of surviving bacteria [30]. Bacteria that survive passage through the stomach travel to the small intestine, where they encounter other antibacterial factors including bile salts, lysozyme and defensins. Even though *Salmonella* Typhimurium can be highly resistant against the direct antibacterial effects of bile salts, these salts repress the invasion of *Salmonella* in epithelial cells [31]. Following adhesion, *Salmonella* invades the intestinal epithelium. Infection of pigs with *Salmonella* Typhimurium may result in long-term asymptomatic carriage of these bacteria. Since this carrier state in pigs is difficult to detect in live animals, either by bacteriological or serological methods [32], these pigs can bias monitoring programmes. Stress-induced excretion of *Salmonella* Typhimurium by carrier pigs transported to the slaughterhouse may cause contamination of transport and holding pens, resulting in pre-slaughter transmission of *Salmonella* to non-infected pigs [33, 34]. Various bacterial (*Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*) and viral infections (porcine reproductive and respiratory syndrome virus (PRRSV), Aujeszky's disease virus) can induce immunodeficiency in pigs. These infections may lead to an easier colonization by *Salmonella*, increased shedding or even higher mortality rates in pigs [35].

Prevalence of Salmonella spp. in pigs

The prevalence of this agent varies between countries due to the diagnostic tests and sampling strategies used. After EU Regulation 2160/2003, the EU decided to carry out baseline surveys to estimate the *Salmonella* spp. prevalence in some food production animals. The objective of the surveys was to obtain comparable data for all MS through harmonized sampling and testing schemes. In pigs the baseline studies were done at abattoir level (collection of lymph nodes from slaughtered pigs) and at herd level (collection of pen faecal samples from breeding pigs). The results of these studies showed that the prevalence and the serotype profile varies between countries (Tables 3, 4, and 5). The serovars most isolated in slaughtered pigs in Europe and in Portugal are *S.* Typhimurium, *S.* Derby and *S.* Rissen [36]. In breeding and production holdings *S.* Typhimurium and *S.* Derby continue to be the most isolated serovars in EU. However in Portugal, *S.* Rissen and *S.* London are more predominant than *S.* Derby [37].

Table 3: Baseline study (EU Regulation 2160/2003) in lymph nodes of pigs slaughtered in EU and Norway between 2006-2007 showing the prevalence of different serotypes [36]

Country	N	*Salmonella* spp.	S. Typhimurium	*S.* Derby	Other serovar
		% (95% CI)	**% (95% CI)**	**% (95% CI)**	**% (95% CI)**
Austria	617	2.0 (1.1-3.6)	0.7 (0.2-2)	0.3 (0.1-1.1)	1.1 (0.5-2.3)
Belgium	601	13.9 (9.8-19.3)	7.8 (5.3-11.5)	1.3 (0.4-3.6)	4.9 (3.0-7.9)
Bulgaria	176	16.7 (8.1-31.4)	1.8 (0.6-4.9)	4.9 (1.3-16.4)	10.1 (4.9-19.7)
Cyprus	359	12.4 (10.1-15.2)	1.0 (0.8-1.3)	0	11.5 (9.1-14.5)
Czech Republic	654	5.8 (3.8-8.9)	1.6 (0.8-3.3)	1.4 (0.5-4.1)	2.7 (1.6-4.5)
Denmark	998	7.7 (5.5-10.7)	4.5 (3.4-5.9)	1.3 (0.8-2.2)	2.0 (1.4-3.0)
Estonia	420	4.7 (2.3-9.4)	1.1 (0.6-2.1)	0	3.8 (1.7-8.3)
Finland	419	0	0	0	0
France	1163	18.1 (16-20.5)	7.1 (5.4-9.5)	6.5 (5.6-7.4)	4.5 (3.2-6.3)
Germany	2567	10.9 (8.8-13.5)	6.1 (4.7-7.8)	1.2 (0.8-1.8)	4.3 (3.4-5.5)
Greece	345	24.8 (18-33.2)	3.4 (1.6-7.1)	3.8 (1.6-8.8)	17.2 (11.7-24.6)
Hungary	658	9.3 (5.3-15.8)	2.9 (1.4-5.9)	1.5 (0.4-5.2)	4.7 (2.9-7.6)
Ireland	422	16.1 (15.6-16.7)	9.1 (9-9.2)	2.4 (2.3-2.5)	3.6 (2.0-6.4)
Italy	709	16.5 (14.1-19.1)	1.6 (0.9-2.6)	5.4 (3.8-7.7)	9.6 (7.7-12.1)
Latvia	392	5.6 (3.3-9.1)	0.3 (0.1-2)	1.9 (0.6-6)	3.4 (1.7-6.6)
Lithuania	461	1.8 (0.8-3.9)	1.3 (0.5-3.8)	0	0.5 (0.2-1.5)
Luxembourg	313	22.4 (12.7-36.4)	16.1 (8.8-27.6)	1.5 (0.7-2.8)	4.0 (1.6-9.6)
Poland	1176	5.1 (3.7-6.9)	1.4 (0.8-2.5)	0.1 (0-0.2)	3.5 (2.5-4.9)
Portugal	**658**	**23.4 (19.4-28)**	**8.4 (6.1-11.5)**	**2.5 (1.3-4.7)**	**12.1 (10.3-14.2)**
Slovakia	385	4.8 (2.6-8.9)	0.8 (0.3-2.1)	1.1 (0.4-2.7)	3.6 (1.8-6.8)
Slovenia	431	6.2 (4.2-9.1)	0.7 (0.2-2)	0.6 (0.1-2.6)	5.1 (3.4-7.5)
Spain	2619	29.0 (24.9-33.5)	10.6 (8.6-13.1)	2.8 (1.8-4.3)	16.1 (13.5-19.1)
Sweden	394	1.3 (1.2-1.5)	1.2 (0.5-2.7)	0	0.5 (0.3-0.5)
Netherlands	1087	8.5 (7.3-9.8)	4.9 (4.7-5)	1.3 (0.8-2.1)	2.1 (1.4-3.2)
United Kingdom	639	21.2 (17.8-25)	13.8 (11.9-15.8)	4.8 (3.6-6.3)	3.8 (2.5-5.5)
EU	**18663**	**10.3 (9.2-11.5)**	**4.7 (4.1-5.3)**	**2.1 (1.8-2.6)**	**5.0 (4.4-5.7)**
Norway	408	0.3 (0.04-1.6)	0.3 (0.04-1.6)	0	0

Table 4: Baseline study (EU Regulation 2160/2003) in breeding pigs in EU and Norway showing the prevalence of positive breeding holdings [37]

Country	N	*Salmonella* spp.	S. Typhimurium	S. Derby	Other serovar
		% (95% CI)	**% (95% CI)**	**% (95% CI)**	**% (95% CI)**
Austria	79	6.3 (3.2-13.2)	3.8 (1.8-10.0)	1.3 (0.4-6.0)	1.3 (0.4-6.0)
Belgium	16	18.8 (7.3-45.1)	12.5 (4.2-37.8)	6.3 (1.4-29.7)	6.3 (1.4-29.7)
Bulgaria	47	2.1 (1.6-8.2)	0 (0.0-4.9)	0 (0.0-4.9)	2.1 (1.6-8.2)
Cyprus	4	50.0 (15.0-85.0)	0 (0.0-60.4)	25.0 (1.3-78.1)	25.0 (1.3-78.1)
Czech Republic	106	10.4 (7.2-15.9)	3.8 (2.1-7.7)	0.9 (0.5-4.1)	5.7 (3.6-10.3)
Denmark	95	41.1 (34.4-48.9)	15.8 (11.3-22.6)	12.6 (9.1-18.8)	17.9 (13.4-24.7)
Estonia	6	0 (0.0-14.3)	0 (0.0-14.3)	0 (0.0-14.3)	0 (0.0-14.3)
Finland	50	0 (0.0-6.1)	0 (0.0-6.1)	0 (0.0-6.1)	0 (0.0-6.1)
France	157	50.3 (44.2-57.1)	7.0 (4.5-11.4)	25.5 (20.5-31.7)	26.8 (21.8-33.2)
Germany	46	28.3 (18.4-42.6)	8.7 (3.9-20.3)	10.9 (5.3-22.9)	6.5 (2.6-17.4)
Hungary	40	30.0 (17.1-46.7)	10.0 (3.3-24.6)	7.5 (1.9-21.5)	15.0 (6.2-30.5)
Ireland	40	52.5 (51.2-53.7)	17.5 (17.1-19.5)	20.0 (19.5-22.0)	17.5 (17.1-19.5)
Italy	43	51.2 (39.2-65.1)	7.0 (2.7-17.7)	16.3 (9.1-29.0)	16.3 (9.1-29.0)
Latvia	5	20.0 (14.3-42.9)	0 (0.0-28.6)	20.0 (14.3-42.9)	20.0 (14.3-42.9)
Lithuania	10	0 (0.0-9.1)	0 (0.0-9.1)	0 (0.0-9.1)	0 (0.0-9.1)
Luxembourg	3	33.3 (1.8-87.5)	0 (0.0-69.0)	0 (0.0-69.0)	33.3 (1.8-87.5)
Netherland	109	57.8 (50.0-66.2)	13.8 (9.3-20.9)	18.3 (12.9-26.1)	38.5 (31.3-47.2)
Poland	144	6.9 (3.9-12.3)	2.8 (1.1-6.9)	1.4 (0.4-4.9)	3.5 (1.6-7.9)
Portugal	**33**	**45.5 (38.5-53.8)**	**9.1 (7.7-17.9)**	**9.1 (7.7-17.9)**	**33.3 (28.2-43.6)**
Slovakia	96	11.5 (9.0-16.4)	2.1 (1.5-5.2)	3.1 (2.2-6.7)	6.3 (4.5-10.4)
Slovenia	27	0 (0.0-9.1)	0 (0.0-9.1)	0 (0.0-9.1)	0 (0.0-9.1)
Spain	150	64.0 (57.8-70.4)	14.0 (10.4-19.5)	10.0 (7.0-14.9)	53.3 (47.2-60.0)
Sweden	57	1.8 (1.3-6.3)	1.8 (1.3-6.3)	0 (0.0-3.8)	0 (0.0-3.8)
United Kingdom	67	52.2 (44.6-61.5)	19.4 (13.8-27.7)	14.9 (10.0-23.1)	29.9 (23.1-39.2)
EU	**1377**	**28.7 (26.3-31.0)**	**7.8 (6.1-9.5)**	**8.9 (7.4-10.5)**	**15.9 (14.2-17.6)**
Norway	108	0 (0.0-2.2)	0 (0.0-2.2)	0 (0.0-2.2)	0 (0.0-2.2)
Switzerland	71	15.5 (12.6-20.7)	4.2 (3.4-8.0)	1.4 (1.1-4.6)	8.5 (6.6-13.8)

Table 5: Baseline study (EU Regulation 2160/2003) in breeding pigs in EU and Norway showing the prevalence of positive production holdings [37]

Country	N	*Salmonella* spp. % (95% CI)	S. Typhimurium % (95% CI)	S. Derby % (95% CI)	Other serovar % (95% CI)
Austria	173	5.8 (3.2-10.3)	0 (0.0-2.1)	0.6 (0.1-3.2)	5.2 (2.8-9.6)
Belgium	209	36.4 (30.5-43.1)	11.0 (7.6-15.9)	10.0 (6.8-14.8)	21.5 (16.7-27.6)
Bulgaria	25	0 (0.0-13.5)	0 (0.0-13.5)	0 (0.0-13.5)	0 (0.0-13.5)
Cyprus	60	18.3 (13.8-26.4)	0 (0.0-4.6)	8.3 (5.7-14.9)	8.3 (5.7-14.9)
Czech Republic	161	15.5 (10.9-21.9)	2.5 (1.0-6.1)	3.7 (1.8-7.8)	11.2 (7.4-17.0)
Denmark	198	41.4 (35.2-48.4)	12.6 (8.9-17.9)	14.6 (10.6-20.2)	18.7 (14.1-24.7)
Estonia	28	3.6 (0.2-20.2)	0 (0.0-15.0)	0 (0.0-15.0)	0 (0.0-15.0)
Finland	157	0 (0.0-2.1)	0 (0.0-2.1)	0 (0.0-2.1)	0 (0.0-2.1)
France	186	38.7 (32.3-46.0)	3.2 (1.5-6.9)	20.4 (15.4-26.9)	19.9 (14.9-26.3)
Germany	155	20.6 (15.2-27.8)	3.2 (1.4-7.3)	8.4 (5.0-13.9)	9.0 (5.5-14.7)
Hungary	141	27.7 (22.1-34.6)	1.4 (0.6-4.5)	12.8 (8.9-18.6)	14.2 (10.1-20.2)
Ireland	149	47.7 (42.3-53.8)	17.4 (13.8-22.6)	13.4 (10.2-18.4)	26.2 (21.6-32.1)
Italy	171	43.9 (36.9-51.5)	5.8 (3.3-10.4)	12.3 (8.3-18.1)	11.7 (7.8-17.4)
Latvia	28	28.6 (20.5-41.0)	0 (0.0-7.7)	3.6 (2.6-12.8)	25.0 (17.9-38.5)
Lithuania	72	8.3 (7.1-12.9)	0 (0.0-2.4)	0 (0.0-2.4)	8.3 (7.1-12-9)
Luxembourg	41	22.0 (11.1-38.0)	2.4 (0.1-14.4)	17.1 (7.7-32.6)	7.3 (1.9-21.0)
Netherland	212	55.7 (49.4-62.2)	8.0 (5.2-12.4)	17.0 (12.8-22.5)	42.5 (36.4-49.2)
Poland	178	9.6 (6.1-14.8)	1.7 (0.6-4.8)	2.8 (1.2-6.4)	5.1 (2.7-9.4)
Portugal	**134**	**43.3 (35.6-52.0)**	**13.4 (8.8-20.3)**	**5.2 (2.6-10.4)**	**29.9 (23.0-38.2)**
Slovakia	96	18.8 (12.6-27.7)	3.1 (1.2-8.7)	4.2 (1.8-10.1)	13.5 (8.3-21.8)
Slovenia	87	10.3 (5.7-18.7)	0 (0.0-4.1)	1.1 (0.3-6.2)	10.3 (5.7-18.7)
Spain	209	53.1 (46.6-60.0)	12.4 (8.7-17.7)	6.7 (4.1-10.9)	42.6 (36.3-49.5)
Sweden	150	0 (0.0-2.4)	0 (0.0-2.4)	0 (0.0-2.4)	0 (0.0-2.4)
United Kingdom	191	44.0 (37.8-50.9)	9.9 (6.7-14.8)	11.0 (7.5-16.0)	31.9 (26.3-38.7)
EU	**3050**	**33.3 (30.9-35.7)**	**6.6 (5.3-7.9)**	**9.0 (7.6-10.5)**	**21.6 (19.5-23.6)**
Norway	143	0 (0.0-2.5)	0 (0.0-2.5)	0 (0.0-2.5)	0 (0.0-2.5)
Switzerland	154	11.7 (7.9-17.3)	1.9 (0.7-5.2)	1.9 (0.7-5.2)	7.8 (4.9-12.8)

1.2.5 Diagnostic methods

The two main diagnostic methods for the detection of *Salmonella spp.* infection are the detection of the immune response to the agent or detection of the agent itself.

1.2.5.1 Detection of the immune response

The detection of the immune response to the agent (serological methods), is mainly done using ELISA tests. These tests detect antibodies against *Salmonella*, and are used in most of the monitoring/surveillance programmes. They are quick and cheap. However they evaluate if the pig was exposed to the agent and not if the pig is shedding the agent [38]. Even a recent infection could test negative if there was not yet seroconversion [39]. A correlation between serology and shedding of *Salmonella* has been demonstrated in experimental studies [40] and field studies [41] conducted in the countries that developed these tests. All existing ELISAs are based on antigens of lipopolysaccharides (LPS). These are part of the cellular wall of many bacteria but are specific for each type of bacteria. In the case of *Salmonella* the LPS is very specific for each serovar, and in most of the ELISA tests, several antigens specific for different

serovars are included. Therefore it is advisable to do a microbiological survey before developing and using a ELISA test to monitor the infection to be sure that the serovars present in that country will be detected by the test [39].

For all these reasons the serological tests should be interpreted with some caution, taking into consideration the following factors:

a) The type of antigens that the test could detect.
b) Cut-off value – optical density (OD) cut-off value will influence the sensitivity and specificity of the test.
c) Status of the infection – there is a time lapse of 2 weeks (experimental infections) to 2 months (field conditions) between the peak of bacteriological shedding and the serological conversion.
d) Serovar – the immune response variation between serovars.
e) Passive immunity – in field conditions the piglets ingest their mother's colostrum, which could transmit passive immunity to the piglets if the mother is seropositive. The maternal antibodies remain for 8 to 10 weeks. Therefore, ELISA tests should only be used in piglets more than 10 weeks old.
f) Seroconversion failure – some individuals are not able to develop an immunological reaction to infection and do not seroconvert even after being infected by serovars that usually induce a serological response. Part of the explanation may be due to genetic resistance in some pigs [42, 43].
g) Specificity – the specificity of the ELISA test to *Salmonella* is considered high. However it is not recommended to use ELISA tests in low prevalence areas [39].

One of the more frequently used serological tests is the mixed ELISA (Danish mixed ELISA - DME). The DME was developed for the first time in Denmark based on the local antigens distribution for *Salmonella*, focussing on the serovars that are important for food safety in that country [40]. Since its development, it has been used in meat juice (drip fluid released from meat after freezing and thawing) and blood in several countries. The DME uses a combination of LPS of *Salmonella* Cholerasuis, and Typhimurium [40]. Other laboratories, with the aim of increasing their test sensitivity, developed indirect ELISAs based on the DME, using the same antigens or adding antigens for different serovars of *Salmonella* which are more prevalent in the country or region where the test is going to be used [44, 45]. Some companies sell several ELISA tests for swine, such as the kit Salmotype® (Salmotype Labordiagnostik, Leipzig, Germany) and the IDEXX (Herdchek *Salmonella*, IDEXX Laboratories, Schiphol-Rijk, Noord-Holland, Netherlands). These two tests when compared to faecal culture, show a sensitivity of 65% and specificity of 84% for a 25% OD cut-off for Salmotype, and a sensitivity of 59% and a specificity of 69% for a 9% OD cut-off for IDEXX [38].

The ELISA tests are considered useful tools for the detection of herds that are usually infected by *Salmonella*, but they do not provide definitive information about the infectious state of the animal (or group) at slaughter for instance [46, 47].

1.2.5.2 Detection of the agent

Culture

Culture of the agent is used to provide a good characterization of the different serovars present, to evaluate the extent of antimicrobial resistance, and to identify sources of infection in outbreaks with different foods [39].
Culture of the bacteria can be carried out using animal faeces, tissues or food. The results of the faecal culture will depend if the animal is shedding or not, which affects the test sensitivity. Culture allows the isolation and identification of the agent. Therefore, it is considered the perfect test in terms of specificity (no false positive results). However it is expensive, time-consuming and lacks sensitivity. The false negative results vary from 10 to 80%. Faecal culture is also prone to sampling errors if at the collection time the animal is not yet shedding [48-50]. However if used repeatedly at herd level (e.g. control programmes), this increases herd sensitivity. In these cases consecutive herd negative faecal cultures at one month intervals, indicate with some confidence that the herd is free from *Salmonella* [39].
Several studies have compared the microbiological techniques for isolation of *Salmonella* from different sources, including faecal samples. The diagnosis of subclinical shedding of *Salmonella* needs specialized culture methods with several steps of selective pre-enrichment. Compared to faeces, lymph nodes and meat have a lower level of competitive flora, and *Salmonella* will, even when present in low numbers, be more easily isolated from such materials. [39].
Standard methods for the isolation of *Salmonella,* e.g. ISO 6579, have been developed and evaluated in relation to the analysis of food and feed. As the matrix has considerable influence on the performance of the method (for example due to levels of competitive flora), methods developed for analysis of food cannot be assumed to be appropriate for analysis of other materials (e.g. faeces). In recent years, efforts have been made to develop and evaluate a standard bacteriologic method for the isolation of *Salmonella* from samples from primary animal production. These studies have resulted in the addition of an annex to the established ISO-method [39].
Comparison between immunologic and culture methods
Both methods have some advantages and disadvantages. The most important are: the bacteriological results express the actual infection status of the animal, including transmission

or recent contamination; it allows isolation of the agent, which enables further characterisation (e.g. serovar and antimicrobial resistance profiles). However, the analytical procedure is laborious and may lack sensitivity when compared to immunological methods. The immunological methods indicate previous exposure and infection from the host to the infectious agent expressed by the presence of detectable specific antibodies against *Salmonella*. Among the advantages, ELISA methods can identify carriers but does not differentiate them from previously infected animals which have already cleared the infection. It detects only those serogroups included in the test and therefore newly emerging serovars may not be detected. The method can be automated, and it is less laborious [39].

Molecular methods

There are several molecular methods that can be used to detect *Salmonella* and also to quantify the resistance profile. The main methods are the following:

- PCR (polymerase chain reaction) is based on the amplification of the specific DNA sequence of interest within a few hours. There are different types of PCR, such as multiplex PCR and real-time PCR [51-55].
- DNA-DNA hybridization (Southern blot). This method is suitable for identifying DNA sequences in bacterial food-borne pathogens [52].
- DNA fingerprinting, also referred to as genotyping. Genotyping methods are commonly based on identification of restriction fragments, e.g., by pulsed field gel electrophoresis (PFGE); by amplified fragment length polymorphism (AFLP) or repetitive sequence PCR (Rep-PCR); or DNA sequence, by multilocus sequence typing (MLST) [56, 57], variable number tandem repeat (VNTR) [58] and single-strand conformation polymorphism (SSCP) [59]. The PFGE has been used for subtyping human *Salmonella* isolates [52].

The described molecular diagnostic and subtyping methods have the potential to play a pivotal role in the epidemiological identification of food-borne pathogens at individual or population levels and to make the information exchange between human outbreaks of *Salmonella* and its source at herd level [56-58].

1.3 - Risk factors for Salmonella spp. pre-harvest and harvest level

Risk factors are variables that are associated with an increase of a disease/outcome. The factor and the disease/outcome can be causally associated either directly or indirectly.

1.3.1 Farm risk factors

There are several known risk factors for this agent which can be divided into categories:

a) humans as vectors [60, 61], biosecurity measures (like washing hands, changing clothes and boots before entering the herd, and others) when applied to the workers and visitants are associated with a decrease in the infection risk [35, 61-63];

b) floor type, certain floors decrease the contact between faecal material and the pigs which can reduce the faecal-oral transmission between pigs [64, 65];

c) contamination of buildings (*Salmonella* has the ability to survive 6 years in the environment, therefore disinfection and hygiene of buildings are very important to reduce contamination) [66, 67];

d) animal management (the all-in-all-out systems are frequently suggested for controlling *Salmonella* but there is no evidence that they are always associated with a decrease in the risk) [66, 68, 69];

e) transmission between sows and piglets [70];

f) vectors like insects, rodents, birds, and domestic and wild animals [65, 71, 72];

g) feed contamination (although the serovars isolated in feed are not the ones most often isolated from pig herds and pork meat) [73];

h) feed structure and components (dry, pellet, fermented) [66, 69, 74, 75];

i) acidification of feed with organic acids [65];

j) season of the year and environmental temperature (improper ventilation and stress due to high temperatures can explain the association between temperature and *Salmonella* prevalence);

k) animal density (increases the transmission between animals and decreases the immunity because of the stress) [61]; and

l) herd sanitary status (to PPRSV and parasitosis) [35, 63, 67, 76].

Table 6 summarizes several known risk factors associated with *Salmonella* spp. in pigs.

Table 6: Risk/Protection factors for Salmonella spp. infection, adapted from [77]

Risk factor			OR * 90% CI ** 95% CI	Reference
Biosecurity measures and equipment	Cleaning measures and 'empty and clean' period	Frequency of sow manure removal in farrowing pens during the lactation period lower than once a day	2.9 (1.2-6.7)*	[63]
	Hygiene and clothes	Lack of emptying pits below slatted floors after removal of previous sow batches	2.6 (1.1-6.4)*	[63] [78]
		Poor herd hygiene	39.7 – model	[73]
		High pressure washing and disinfection of the pens	0.7 (0.5-0.99)**	[67]
			0.9 (0.84-0.96)**	[66]
		Residual Salmonella contamination of the pen before loading of the following batch	3.1 (1.4-7.1)*	[63]
			1.9 (1.2-2.9)** - HR	[35]
		Duration of 'empty and clean' period lower than 6 days in farrowing pen	3.1 (1.7-5.5)*	[63]
		Duration of 'empty and clean' period lower than 7 days in post-weaning pens	3.2 (1.3-8.2)*	[78]
		Duration of 'empty and clean' period lower than 3 days in fattening pen	2.0 (1.1-3.5)*	[63]
		Detection of Salmonella on boots or environmental samples and/or lack of boot-dip at the entrance of the facilities	NA	[62, 79, 80]
		Increased washing and disinfection frequency with cold water between batches	1.4 (1.03-1.99)**	[75]
		Lack of hand hygiene before tending to pigs; lack of toilet	4.4 (1.6-11.6)**	[81]
			11.1 (1.8-70.2)*	[61]
		Use of specific clothes before entering buildings	0.5 (0.3-0.9)** - HR	[35]
	Infection through people or equipment	More than two humans present at a finisher site daily	4.8 (1.4-17.1)*	[61]
		Sharing equipment	NA	[60]
	Floors	Partially slatted floor versus fully slatted	8.9 (5.0-15.9)*	[64]
		Solid floors/straw on floors versus slatted floor	1.5 (1.4-1.6)*	[65]
	Biosecurity	Closed herds	0.19 (0.05-0.66)**	[75]
			0.92 (0.87-0.97)**	[66]
		Herd closed to exterior	0.4 (0.2-0.8)** - HR	[35]
		Snout contact between pens	1,7 (1,01-2,9)**	[69]
			1.7 (1.1-2.8)** - model	[82]
		No use of boot-dip	1.2 (1.1-1.3)**	[66]
Intercurrent diseases / Herds sanitary status	"Specific pathogens free herds"		0.7 (0.5-0.8)**	[83]
	Infections by *Lawsonia Intracelularis*		3.2 (1.4-7.2)*	[63]
	Infection by PRRSV (Porcine Reproductive and Respiratory Syndrome Virus)		3.0 (1.3-6.7)*	[63]
			1.6 (1.1-2.5)** - HR	[35]
	Diarrhoea in growing pigs		NA	[84]
	Infection by PRCV (Porcine Respiratory Coronavirus)		6.9 (2.2-21.6)*	[78, 85]
	Liver infestations by *Ascaris suum* with high level (>16%) of liver condemnation at slaughterhouse during meat inspection		2.1 (1.1-4.2)**	[67]

Legend: HR – hazard ratio, OR – odds ratio, CI – confidence interval, NA – value not available

Table 6: Risk/Protection factors for *Salmonella* spp. infection, adapted from [77] (cont.)

Risk factor			OR * 90% CI ** 95% CI	Reference
Feed and watering	Feed contamination		1.6 - model	[73]
	Feed acidification or fermented liquid feed	Use of dry or liquid feed in comparison with fermented liquid feed	5.0 (2.0-10.0)**	[67]
		Adding organic acids decreases prevalence	NA	[86, 87]
			0.7 (0.6-0.8)**	[65]
	Probiotics	Use of probiotics decreases intestinal adhesion to *Salmonella*	NA	[88]
	Feed source	Commercial feed	1.8 (1.6-2.0)**	[83]
	Dry feed	Dry feed versus wet feed	3.2 (1.4-7.1)*	[63]
			4.9 (1.9-12.7)*	[3]
			4.1 (1.4-12.2)*	[68]
			1.5 (1.3-1.8)**	[83]
	Pelleted feed	Pelleted ration vs. wet or dry non-pelleted ration	12.5 (2.2-100.0)**	[74]
			1.7 (1.1-2.8)**	[69]
		Pellet ration vs. wet feed	10 (1.4-100.0)**	[74]
			10.3 (1.7-61.6)**	[75]
			2.4 (1.5-4.0)**	[69]
			1.4 (1.4-1.5)**	[66]
	Do not give colostrum to piglets		2.6 (1.2-6.3)**	[69]
	Feeder design	Use of automated fermented feed	0.09 (0.005-0.4)**	[89]
		Mixture of pellet feed with water	4.1 (1.4-11.8)**	[89]
	Water	Adding chlorate to water has a beneficial effect in reducing faecal concentration of *Salmonella*	NA	[86]
		Bowl drinkers are associated with higher prevalence than nipple drinkers	8.0 (3.4-19.0)*	[3]
Herd management	Sow infection	The infection of sows at gestation/ maternity or contaminated environmental is associated with an increased seroprevalence on piglets	1.2 (1.1-1.4)*	[70]
		Introduction of sows/growers in a herd	NA	[90, 91]
	Herd size	More than 100 animals in fattening	1.1 (1.0-1.2)**	[75]
		Less than 800 animals in the herd	1.5 (1.1-1.9)**	[67]
	Herd type	Fattening vs. piglet production	38.2 (1.6 – 927.8)*	[92]
		Fattening vs. post weaning	4.2 (2.1-8.3)**	[81]
	Stocking density	Space allowance less than to $0.75 m^2$ per pig	4.5 (1.3-15.7)*	[61]
	Other breeding and contacts with domestic species or wild animals	Contact with rodents	NA	[71] [72] [79]
		Contact with birds	NA	[72]
		Poultry breeding on the farm	1.2 (1.1-1.3)*	[65]
		Other domestic species at the site or indirect contacts with other herds	NA	[72] [60]
			4.7 (1.2-18.0)*	[61]
		Controlling insects	0.4 (0.3-0.6)** - model	[82]
	Pig source	Recruitment of pigs from more than 3 different supplier herds	3.3 (1.6-6.8)**	[69]
	Mixing batches	Continuous production of pigs compared to all-in/all-out	3.7 (1.9-7.1)**	[69]
			1.4 (1.3-1.5)**	[66]
			3.9 (1.4-10.5)*	[68]
		Mixing piglets at post weaning (increases the social stress)	NA	[93]
		Mixing batches during the fattening period	1.5 (1.4-1.6)*	[65]

Legend: HR – hazard ratio, OR – odds ratio, CI – confidence interval, NA – value not available

Table 6: Risk/Protection factors for *Salmonella* spp. infection, adapted from [77] (cont.)

Risk factor			OR * 90% CI ** 95% CI	Reference
Herd management	Antibiotic	Combination of chlortetracycline, procaine penicillin and sulphamethazine supplemented ration versus approved growth promoter or probiotic	4.1 (1.8-9.2)**	[74]
		Using chlortetracycline as growth promoter during the fattening period	6.9 (2.8-17.1)**	[94]
		Preventive antibiotic treatment during fattening enhances serological prevalence	2.4 (1.7-3.4)** - HR	[35]
			1.5 (1.4-1.7)*	[65]
			5.6 – model	[73]
		Using tylosine as growth promoter at the end of the fattening period	1.6 (1.1-2.3)**	[67]

Legend: HR – hazard ratio, OR – odds ratio, CI – confidence interval, NA – value not available

1.3.2 Risk factors on transport, lairage and slaughter associated with meat contamination

When shipping the pigs to slaughter, the stress due to transport increases the transmission of the infection between animals from the same herd and from different herds [73]. In lairage the cross contamination happens again, allowing infected pigs to shed the agent in great quantities to the environment [95, 96]. Therefore the majority of slaughterhouses have highly contaminated environments which allow cross contamination between batches [97, 98]. The implementation of a Hazard Analysis Critical Control Points (HACCP) plan allows a reduction or elimination of the contamination in slaughter. Some of the known risk factors in transport, lairage and slaughter are shown in Table 7.

A research study [99] followed 60 animals/carcasses along the slaughter process. Reductions in the bacteria number in the scalding, singeing, and dehairing steps (reduction of 4.5 $\log_{10}$ cfu/m^2) were observed. The final washing increased the bacteria numbers in 3.6 to 3.8 $\log_{10}$ cfu/m^2, while chilling increased the counts in 4.5 to 4.7 $\log_{10}$ cfu/m^2. The prevalence of *Salmonella* in carcasses was 31% after bleeding, 1% after scalding, 7% after dehairing, 0% after singeing and polishing and 7% after evisceration [100].

Table 7: Transport, lairage and slaughter process risk factors.

Risk factor			OR * 90% CI ** 95% CI	Reference
Transport	Pig *Salmonella* positive sanitary status before loading		4.0 – model	[73]
	Poor hygiene		1.1 – model	[73]
	Stress during transport		1.9 - model	[73]
Lairage	Time in lairage	3-6h vs. <3h	3.3 (1.1-9.9)*	[95]
		>6h vs. <3h	13.1 (4.7-36.1)*	[95]
		>12h vs.<12h	2.83 (1.33-6.01)**	[101]
	Hygiene	Contaminated pens	NA	[96]
	Use of water spray on pigs	When environmental temperature is high	6.96 (3.24-14.95)**	[98]
Slaughter process	Polishing		3.74 (1.43-9.78)**	[97]
	Scalding and evisceration		3.63 (1.66-7.96)**	[97]
	Steam scalding		0.18 (0.05 – 0.69)**	[98]
	Time between slaughter and scalding (increased)		1.43 (0.88-2.34)**	[98]
	Routine evisceration vs. careful evisceration		11.8 (2.3-113.3)**	[102]
	Washing and disinfection of splitting machine	3 times/day	0.13 (0.017-0.97)**	[98]
	Chilling of carcass	2 days vs. 1 day	0.19 (0.045-0.813)*	[103]
Season	Summer vs. Autumn		11.9 (1.1-125.5)**	[97]
Slaughter duration	Along the slaughter process contamination increases	Comparison between the end and the beginning of the slaughter process	3.97 (2.51-6.27)**	[97]

Legend: OR – odds ratio, CI – confidence interval, NA – value not available

1.4 – Surveillance systems and control measures for Salmonella spp. in swine

1.4.1 Control/Prevention

In 1980, the World Health Organization (WHO) had already formulated three lines of defence against *Salmonella*, which still comprise valid strategic approaches to risk mitigation:

a) the first approach focuses on controlling *Salmonella* in the food-producing animal (pre-harvest control),

b) the second approach involves improving hygiene during the slaughter and further processing of the meat (harvest control),

c) the third approach targets the food final preparation by educating the food industry and consumers about good hygiene practices (post-harvest control).

Successful prevention of food-borne salmonellosis originating from animal production must involve all three lines of defence. Pre-harvest control of *Salmonella* at the farm

level has long been considered an important part of pathogen reduction schemes, because traditional meat inspection cannot control *Salmonella*-contaminated carcasses [4].

The focus of this revision is on pre-harvest although some risk mitigation options at harvest are also suggested.

1.4.1.1 Pre-harvest control

The World Organisation for Animal Health (OIE) concluded that *Salmonella* control programmes should follow the same general rules that have been successfully applied to other infectious diseases. It is fundamental that monitoring/surveillance programmes should be established to identify *Salmonella*-infected herds and animals and that efforts are made to find and control the sources of infection and prevent further spread. The ultimate objective is to produce *Salmonella*-free animals [4]. It should also be emphasised that *Salmonella* is a pathogen and not a ubiquitous bacteria or a normal inhabitant of the intestinal flora of domestic animals, as has sometimes been claimed previously [39].

Serovars to be controlled

Since any serovar, including those that infect animals or colonise their intestine, is a potential hazard to human health, measures to prevent food-borne salmonellosis must be directed at all serovars of *Salmonella*. However, a *Salmonella* reduction strategy which is limited to a few selected serovars should also have a preventive effect on most other serovars since most of the time the same control measures are applicable for any serovar. If such a strategy is implemented, a supporting surveillance programme will also be needed to detect the prevalence of zoonotic serovars and prevent their build-up in the production chain. If no interventions are made at this early stage, these serovars could later spread widely, perhaps reaching epidemic proportions [4, 39].

Live animals as source of infection

Salmonella-infected food-producing animals excrete *Salmonella* bacteria in large numbers, sometimes intermittently during their entire productive life [4]. During the acute phase of the disease, pigs will shed up to 10^6 - 10^7 *Salmonella* bacteria per gram of faeces and the disease-producing dose is of a magnitude of 10^8 to 10^{11} bacteria [39]. Excreted bacteria infect neighbouring animals on the farm and contamination of the environment takes place, with infections being transmitted to rodents and other wild fauna. When moved, the *Salmonella*-infected animals are effective at introducing the

infection into their new holdings. Therefore they must be at least subject to isolation (quarantine) as the stress linked to transport may reactivate shedding in carriers [4, 39]. In the absence of 'guaranteed *Salmonella*-free replacement animals', other methods must be used to limit the risk of introducing *Salmonella* with incoming animals. In general, animals should be introduced only from herds of the same or a higher health status [4]. An important measure to control the disease is to identify infected animals. Animals found to be infected may be temporarily raised under isolation and controlled conditions but all infected animals must be sent to slaughter [39].

Diagnosis and monitoring methods

A control programme also needs a supporting monitoring programme. Monitoring using bacteriological methods is needed to obtain a true picture of *Salmonella* status. Serological methods can be recommended, especially in medium- and high-prevalence countries, since they are cheap, fast and suitable for large-scale use, but their limitations should also be considered (e.g. they do not detect emerging serovars) [4].

Hygiene and husbandry

Optimal hygiene and management routines are of major importance in aiding animals to withstand exposure to *Salmonella*, and to minimise the possible subsequent spread of the agent on the farm. Improvements in hygiene and management are also effective against other infectious agents [4, 39].

Raising livestock in separate groups, without mixing animals from different sources and ages, has proved to be an effective health measure. The 'all-in, all-out' system, with careful cleaning and disinfection between batches, has long been essential in broiler production, and is now also routine in *Salmonella* control programmes for beef and swine production (this involves entirely emptying the pen of animals and cleaning it before any new ones are introduced, so that infection cannot be passed on to incoming livestock) [4, 39].

The occurrence of diseases like *Brachyspira hyodysenteriae*, Aujeszky's disease and Porcine Reproductive and Respiratory Syndrome (PRRS) are stressing factors that increase the susceptibility of swine to *Salmonella* exposure, and their control thus also contributes to the prevention or control of *Salmonella* infection in exposed herds [39].

Biosecurity prevents animals like rodents, birds, foxes, cats, dogs as well as other farm animals (that can be contaminated and infected with *Salmonella* and spread the agent) from coming into the herd, and pigs should be kept separate from other species of farm animals. Biosecurity also prevents visitors and equipment from becoming a source of infection [39].

The importance of hygienic management of animal effluents, including manure, is evident, especially when considering intensive production [39]. The temperature, storage time and interaction between these, influence the decrease of *Salmonella* in the slurry [104].

Husbandry systems where pigs are outdoor (pasture, free range etc.) are at an increased risk of becoming infected with *Salmonella.* Control under these circumstances will be very difficult as a result of the continuous exposure [39].

Feed

The control of *Salmonella* contamination of feed is essential and is an integrated part of the pre-harvest control of *Salmonella*. Good Manufacturing Practices (GMP) and HACCP guidelines are available for feed manufacturers. In all countries there is most likely a constant but varying risk for animals to be exposed to *Salmonella* through their feed. The documentation of the significance of this risk can be difficult to establish in countries with a relatively high prevalence of *Salmonella* without in-depth epidemiological studies. Under such circumstances it can be difficult to exclude sources other than feed as the source of infection. In countries with low prevalence of *Salmonella*, feed is considered as a major source of *Salmonella* infections in swine, in particular because of the great potential for spreading to a large number of farms [39, 105].

Some measures for the control of *Salmonella* in feed include the following basic elements [39, 106]:

a) monitor the raw materials which are used in feed;
b) use heat treatment: 80ºC for 30 to 45 seconds is enough. It is also important to prevent recontamination after heat treatment, in the cooling, transport or storage of the feed;
c) zero tolerance HACCP systems to *Salmonella* contamination;
d) relevant action has to be taken immediately in case of finding of *Salmonella* in the feed mill. The development of an efficient procedure for cleaning and disinfection can ensure that *Salmonella* is eliminated.

Feed composition

Fermenting feed or fermented feed components (fermented liquid feed) used as a wet feeding system were found to have a *Salmonella* reducing effect, although the temperature of the feed must be taken in consideration [107]. Adding organic acid (e.g. formic, acetic or lactic acid) to feed can also have a *Salmonella* reducing effect [39, 108]. In some studies the beneficial relationship was not proven [109].

Water

Adding acid to drinking water also showed beneficial results [39] in the reduction of *Salmonella* seroprevalence [110]. The acids most commonly used are lactic and formic acid, but the concentration required and the final water pH needs further research.

Antimicrobials

The use of antimicrobials to prevent suffering and economic losses in individual animals and herds can be justified but should always be combined with other *Salmonella* reduction actions. Antibiotics have sometimes also been used to prevent shedding of *Salmonella*, but the use of antibiotics in pigs with enterocolitis has not been found to reduce the prevalence, magnitude or duration of *Salmonella* shedding by sick or recovered animals. Preventive treatment of carrier pigs with enrofloxacin was not able to eliminate the infection. The use of antimicrobials for therapy or growth promoting also disrupt the gut flora which often increase the susceptibility of pigs to *Salmonella* infection. The use of antibiotics may thus act as a trigger for the spread of a *Salmonella* infection within a herd which would not have occurred if the animals were untreated. Also, the use of antimicrobials for *Salmonella* control in pigs should be discouraged due to public health risks associated with development, selection and spread of antimicrobial resistance [39, 111].

Vaccines

Vaccines for the control of *Salmonella* infections are in use all over the world, mainly evolving inactivated vaccines [112-115]. In recent years increasing numbers of live vaccines have been developed [112, 116]. Experience has shown that *Salmonella* vaccines, in association with other measures related to improvement of veterinary hygiene and good management, can perform outstandingly in the control of salmonellosis. Vaccination could thus very well play an important role in the intervention of *Salmonella* in high prevalence herds. Vaccination at an early stage of life (after weaning) would not interfere with serological detection of antibodies against *Salmonella* for monitoring purposes at the end of the finishing period [39]. A special serological test has been developed to distinguish between vaccinated and naturally infected animals [116]. A disadvantage of such vaccines is that they are serovar specific (mainly *S.* Typhimurium) and offer probably only limited cross protection to infection with *Salmonella* from the same serogroup and provide limited protection against infection with *Salmonella* belonging to other serogroups [39]. Vaccination alone cannot eliminate *Salmonella* spp. from a herd, and whether vaccination is a suitable option in a control programme or not, depends on the aim of the control

programme (reduction or eradication), the prevalence of *Salmonella*, the serovars involved, the detection methods used and the cost-benefit [39, 112].

Competitive exclusion

Competitive exclusion comprises excluding enteric pathogens from the alimentary tract by preferentially colonizing it with commensal or beneficial bacteria indigenous to a particular animal species. The use of competitive exclusion is a valuable part of *Salmonella* control in poultry. Positive results from the use of competitive exclusion are also reported from pigs [117, 118]. To maximize effectiveness, competitive exclusion should be administered before a potential exposure to *Salmonella* spp.. Wider studies are needed to fully quantify the effects of competitive exclusion in preventing *Salmonella* infections in pigs [39].

Summary of the current strategies for intervention at pre-harvest level

The main points are [39]:

a) Outsourced feed – based in GMP and HACCP at the supplier;
b) Hygiene and management routines – all-in/all-out systems with cleaning and disinfection between batches, supply of clean drinking water, fly and rodent control, no access of pets and birds, visitor hygiene as part of biosecurity, and no close contact to other production animals. Housing strategies such as slatted floors, pen separations, pig flow through the herd, feeding troughs and drinking bowls, feeding systems (wet or dry feed, pelleted or meal feed), herd biosecurity, introduction of new animals, outdoor access, and multiple site production systems;
c) Feed interventions – acidification of feed and water, fermented liquid feeds;
d) Depopulation and *Salmonella*-free replacement animals – only acceptable in low prevalence regions;
e) Serovars to be the subject of focus – any serovars could potentially infect humans, so all of them should be considered in a control programme;
f) Monitoring – the use of bacteriological methods is required in order to obtain a true picture of the *Salmonella* status. Serological methods are applicable especially in medium and high prevalence MS as they are fast and suitable for large scale usage at a low cost, but they require to be supplemented by a strategic use of bacteriological methods.
g) Breeding production – pigs are generally most susceptible to *Salmonella* exposure during the growing period when the circulation of pathogenic agents usually is most pronounced. This is a critical point to be considered and has to

involve also the *Salmonella* status of the breeding animals and piglets earlier in the production chain. However, piglets delivered from units where the *Salmonella* prevalence is successfully reduced will readily be infected and colonized following transfer to the finishing herds, if mixed with pigs from herds of a lower *Salmonella* status or by residual infection in the finishing herds. An intervention for *Salmonella* control focused only on piglet production, breeding and grower herds can therefore not be recommended;

h) Finisher production – the main exposure of the human population is the consequence of *Salmonella* presence in finishing pigs. Therefore it is reasonable to focus the interventions initially on finishing pigs because this would have a more direct influence on the subsequent steps of the food chain (harvest and post-harvest level) and on public health. Experience shows that an emphasis on control measures in the finisher phase leads to a larger and more rapid reduction in *Salmonella* prevalence in pigs and pork, than only emphasis on the sow/breeding level.

1.4.1.2 Control at harvest level

After being exposed to the agent a pig can be infected in 2 to 3 hours [27], which is compatible with the time spent in transport and in lairage. This means, that pigs could be infected before slaughter and quickly start shedding the agent.

Salmonella contamination at slaughter is mainly because of faecal contamination, direct or indirect, between live pigs or carcasses. If *Salmonella* carriers are entering the slaughterhouse the possibility of transmission to consumers will always exist. Although live animals can be infected at herd, transport and lairage, the carcasses can only be contaminated with *Salmonella* during the slaughter process, due to cross contamination by equipment and workers, mainly because of equipment failure or poor hygiene procedures [119].

Summary of mitigation options for transport and lairage

Some of the possible measures are [39]:

- Cleaning and disinfection of trucks;
- Avoiding mixing batches of pigs from different herds in the same truck;
- Optimizing the transport logistics to reduce the transport duration;
- Promoting transport under less stressful conditions and in accordance with the welfare rules;
- To limit the duration of the lairage in accordance with welfare needs and meat quality considerations;

- To limit the environmental contamination by avoiding faecal accumulation: improving cleaning and disinfection protocols or the adaptation of floor structure to promote faecal elimination.

Slaughter

Slaughter pigs carrying *Salmonella* are known to be a considerable risk for the contamination of the ultimate meat and meat products [120, 121]. Within groups of slaughter pigs, there is a strong correlation between the proportion of animals carrying *Salmonella* in the faeces and the proportion of contaminated carcasses [122]. Pigs with *Salmonella* spp. in their faeces are 3 to 4 times more likely to give rise to a positive carcass than non-carrier animals [39].

In a EU study [123] the extent of cross-contamination in the slaughterhouse was estimated by first investigating pigs slaughtered from one or more *Salmonella* positive herds and then investigating pigs from one or more *Salmonella* negative herds. By sampling the carcasses at several points during the slaughter process, the contamination of the carcasses from the negative herds, when measured, provided information on the degree of cross-contamination brought about by manual handling and processing. The results showed that not all pigs from the *Salmonella* negative herds remained *Salmonella*-negative during and after slaughter. The source of contamination may have been the lairage, since it was possible for faecal matter to pass between the pens holding the positive and the negative pigs. Another source of contamination of the carcasses was considered to be the slaughter equipment, especially the carcass splitter. Carcasses of pigs may be cross contaminated from either *Salmonella*-positive pigs slaughtered previously on the same day, or from contaminated slaughter equipment. Such equipment can also be contaminated from *Salmonella* positive pigs slaughtered on the same day, but the results strongly suggested, that residual and/or persistent contamination of the equipment is also an important source.

Some authors [121] considered that contamination of 30% of positive carcasses arose from cross-contamination from other infected pigs, and that up to 70% by cross contamination from the carrier animals themselves [120]. These figures will, however, vary depending on the *Salmonella* prevalence in different batches of slaughtered pigs [39].

Summary of mitigation options at harvest level

Hygiene of slaughtering – The most important mitigation option is to ensure that slaughter and carcass dressing are performed in an efficient manner to ensure that

faecal contamination of the carcass and offal is not a common event. In addition, specified actions are required to be taken when visible faecal contamination is seen. Guidelines for hygienic slaughter are available at both national and international level. These comprise recommendations on the hygienic design of establishments and facilities including their equipment, process control systems including GHP as well as HACCP based systems and codes of personal hygiene. Finally, regular monitoring and auditing of all phases of the hygiene programmes, for slaughter and carcass dressing including microbiological testing, allows the means of ensuring effective control of carcass and offal contamination with *Salmonella* during this phase [39].

Cooling – The fast cooling of carcasses to 7ºC can stop the multiplication of bacteria, but this cooling must be done in a way that meat quality is preserved [39].

Logistic slaughtering – Separate slaughtering of *Salmonella*-negative herds or slaughtering negative herds before positive herds has a positive impact on the incidence and extent of *Salmonella* contamination of pig carcasses in the slaughterhouse. Better results can be obtained if batches from different herds are also separated during transport, lairage and, later, carcass cooling [124]. The most efficient means of achieving separation is by slaughtering *Salmonella*-negative herds in different slaughterhouses than *Salmonella*-positive herds.

Modifications of the slaughter line operations – Possible modifications of technical aspects of individual operations of pig slaughter line should be aimed at improving microbial status of pork carcasses.

These would include the following [39]:

- replacing submersion-scalding with spray-scalding would be beneficial;
- reducing contamination that occurs in dehairing machines;
- avoiding the polishing step, or inverting the singeing-polishing order, or repeating the singeing step, could prevent recontamination in polishing;
- a careful evisceration, avoiding quick and unhygienic manipulations, the speed at such points could be slowed down through "branching" the line so to achieve multiple evisceration stations;
- inclusion of a final carcass decontamination step, alone or in combination, e.g. a post-evisceration hot wash could reduce the microbial load on final carcasses.

Decontamination treatments – The reason for considering meat decontamination is the fact that certain levels of microbial contamination of fresh meat surface (i.e. carcasses) inadvertently but regularly occur during the slaughter and dressing of animals. Presently and under commercial conditions, this risk cannot be fully eliminated solely by process hygiene means, no matter how carefully the various procedures are carried out [125]. However the disadvantages of meat decontamination are the

disproportionate reliance on the decontamination step and consequent reduction of the process hygiene; limited reduction rates achievable enabling positive selection for surviving resistant strains; stress-mediated increase of virulence of the surviving strains; subsequent enhanced growth of surviving pathogens due to elimination of background meat microflora, environmental pressure of the treatment chemicals, occupational health aspects, cost-benefit variability, labelling and potential consumer reactions [39]. Consequently, current legislation does not allow for carcass decontamination treatments apart from using water.

The treatments can be divided into following [39]:

- Heat treatments – temperatures of 80 to 85ºC of carcasses, either by hot water or steam can be used. The total microbial reductions under differing conditions and different meat species vary, and normally are within a 2.5-3.7 logs range for vegetative forms of the main food-borne pathogens;
- Irradiation treatments – doses of 1-3 kGy are used for non-carcass meats in some non-EU countries. Generally, the microbial reduction rates achieved are within a 2-3 logs range for vegetative forms of the main food-borne pathogens (e.g. *Salmonella*), but not with viruses or microbial toxin [126];
- Chemical treatments – a range of low-molecule organic acids (e.g. lactic, acetic, citric, fumaric) are used commercially for meat decontamination in some countries. Generally, the microbial reductions achieved are within a 2-3 logs range for vegetative forms of the main food-borne pathogens (e.g. *Salmonella, L. monocytogenes*). Other chemicals used for pig meat decontamination include chlorine and trisodium phosphate and, generally, the microbial reductions of vegetative forms of main food-borne pathogens (e.g. *Salmonella*, *E. coli* O157) achieved are 1-1.5 logs [127];
- Other treatments – high voltage pulsed field, high pressure, etc. but not yet applied to carcasses.

Microbiological monitoring of carcasses and surfaces

The different aims of monitoring are monitoring/surveillance of pathogens in pigs on-farm via testing of resulting carcasses at abattoir; monitoring/surveillance of pathogens in foods via carcass testing at abattoir; microbiological carcass testing in the context of HACCP verification i.e. for the process hygiene assessment purpose HACCP and for the evaluation of the microbiological criteria, that includes *Salmonella* in pork carcass, defined in the European Regulation (EC) N.º 2073/2005 [39, 100, 125].

The testing methods vary from carcass surface testing (using destructive or non-destructive methods) to testing meat juice.

1.4.2 Surveillance and monitoring systems

During the last 15 years animal disease has been a problem for meat trade worldwide, as for example avian influenza and foot and mouth disease. The disturbance in trade caused by animal disease affects the consumption patterns of meat, changes the prices, and imposes high costs to the animal industry worldwide. The prohibition of importation from infected zones, combined with rigorous inspections and testing at borders, has reduced income in recent years [155]. For example, EU experienced classic swine fever, where the affected countries were not major exporters worldwide. However some disease outbreaks such as foot and mouth disease in swine in China affected the global trade. These isolated outbreaks of animal diseases do not seem to cause great losses in trade at long term, because the reduction in production by one exporter country is compensated by another exporter country [155]. However the costs associated with animal diseases can be quite high, e.g. foot and mouth disease in UK in 2001 cost $9,204million, and avian influenza (serotype H7N7) in Netherlands in 2003 cost €150million [155]. Therefore animal disease can cause great economic losses due to outbreaks or endemic disease which pose barriers to trade.
Several countries have been implementing monitoring and surveillance systems to *Salmonella* in pigs. The sampling protocol varies with the country prevalence. Here are some examples:

1.4.2.1 Countries with low prevalence (e.g. Sweden and Norway)

Sweden began controlling *Salmonella* around 1950 after an epidemic which affected more than 9000 people and caused 90 deaths [39]. The objective of the control is to ensure that all animal products for human consumption are free from *Salmonella.* Therefore all the critical points of the production chain are monitored to ensure that no *Salmonella* contamination occurs. Any finding of *Salmonella*, irrespective of serovar, in animals, humans, feed and food is compulsorily notifiable, independent of reasons for sampling. All primary isolates are sero and phage typed and primary isolates from animals are tested for antibiotic resistance. All sanitary slaughtered animals are tested for *Salmonella*. When *Salmonella* is isolated, actions are taken to eliminate the bacteria (e.g., herds subjected to restrictions which include animal movement ban except for sanitary slaughter). *Salmonella* carriers are eventually slaughtered or destroyed followed by careful cleaning and disinfection. Restrictions are lifted following two negative samplings of the whole herd. Up and down stream epidemiological tracing is undertaken and followed up by similar actions. According to an EU approved scheme additional monitoring for *Salmonella* has been done on a statistical basis since 1995.

Annually, approximately 6,000 pigs at slaughter (five ileocaecal/intestinal lymph nodes per animal), and approximately 6,000 carcasses (swabbing of 1,400cm^2) are analysed for the presence of *Salmonella*. For elite breeding and multiplier herds 59 faecal samples are tested annually. For sow herds pooled samples are tested twice a year. For herds affiliated to a voluntary quality assurance programme (covers 60-65% of all slaughtered pigs) 10 faecal samples are collected annually. In accordance with the Swedish animal feed legislation feed must be *Salmonella* negative. Several of the early guidelines on how to control *Salmonella*, were developed as industry recommendations in collaboration with government experts. The HACCP approach has been employed in the control of feed mills, with critical control points being monitored weekly [39].

The Norwegian *Salmonella* surveillance and control programme (NSSCP) was launched in 1995 and has been approved by the EU (EFTA Surveillance Authority Decision No. 68/95/COL of 19 June 1995) as the background for accepting testing meat, meat products or live animals for *Salmonella* before it is allowed to enter Norway from EU member countries [128]. The programme covers activities directed towards both live animals (cattle, pig and poultry) and meat (cattle, pig, sheep and poultry) and is similar to the Swedish and Finnish *Salmonella* control programmes. The pig part of NSSCP was designed to provide reliable documentation of the prevalence of *Salmonella* in pork production and to detect any increased occurrence of infections with *Salmonella* among food production pigs in Norway. The program includes systematic sampling in the breeding herds and random sampling of carcasses at the abattoirs in order to identify infected carcasses originating from breeding herds, integrating herds and herds with finishing pigs. The sample size has been calculated so that a prevalence of 5% in any breeding herd and 0.1% in the total population can be detected, assuming a diagnostic test sensitivity of 100%. Herds with positive carcasses are subject to animal trade and slaughter restrictions and samples are collected approximately every second month until the herd is proven to be free twice. All the breeding herds are sampled (pooled samples of floor faecal material) once a year by taking samples from a representative number (if having more than a total of 20 pens) or all pens (if having below 20). All herds are surveyed by examining ileo-caecal lymph node samples from randomly chosen pig carcasses during slaughter. About 3,000 carcasses (approximately one per every 500 sows or finishing pig slaughtered) are sampled at the abattoirs every year. From each positive herd 59 individual samples (10 g of faeces collected from the rectum of different animals in the pen or from the pen floor) and pooled samples (10g faeces, 5–8 times) from all the pens with piglets and finishing pigs (to herds with more than 59 sows and boars) or up to 59 individual

samples and pooled samples from all the pens (to herds with less or 59 sows and boars) are tested [128].

1.4.2.2 Countries with medium or high prevalence (Denmark, United Kingdom)

Denmark has had a control and surveillance programme for *Salmonella* in pigs since the beginning of the 90s (1993) [129]. The programme is based on routine testing and classification of finishing herds and then pigs are slaughtered according to their risk classification status. The feed is also monitored including commercial feed and raw materials to be incorporated in feed. Pigs from breeding and multiplying herds are tested monthly by serologic testing of blood samples. If a specific cut-off level is reached, bacteriologic confirmatory testing is carried out. Fattening herds are monitored continuously by serologic testing of meat juice. The meat samples are collected at the slaughter line, and the sample size and frequency of sampling are determined by the size of the herd. Approximately 700,000 slaughter pigs are currently tested each year. Herds sending less than 200 pigs to slaughter each year are not tested, leaving 1.6% of the slaughter pigs outside the monitoring scheme. The herds are categorized in three levels based on the proportion of seropositive meat juice samples during the last three months. Producers in level 2 and 3 are encouraged to seek advice on how to reduce *Salmonella* in the herd (e.g., feeding, hygiene, and management). Furthermore, there are payment penalties from the slaughterhouse to these levels. Pigs from herds in levels 1 and 2 are slaughtered traditionally without any special precautions. Pigs from level 3 herds can only be slaughtered in special slaughterhouses under special hygienic precautions. Carcasses from level 3 herds are tested for *Salmonella* after slaughter, and if the level of contamination exceeds a certain threshold all carcasses from the particular herd have to undergo heat treatment or other risk-reducing process. All slaughterhouses carry out routine bacteriologic testing of carcasses according to a sampling plan, which ensures that testing is random and representative of the national swine production (>30,000 samples/year). Slaughterhouses that exceed a certain threshold level for *Salmonella* in the routine monitoring are obliged to investigate and reduce the contamination problem to an acceptable level [130, 131].

The British Zoonosis Action Plan *Salmonella* Programme (ZAP) was an industry-owned initiative that began in June 2002. In January 2003 ZAP was extended to producers in Northern Ireland [132]. Muscle samples were collected by Meat and Livestock Commission staff from 3 pigs for every Pig Movement Order received at the abattoir, with the objective that at least 15 samples were collected every 3 months. Samples were linked to their herd of origin via the recorded slap mark. An indirect

lipopolysaccharide (LPS) mix-*Salmonella* meat-juice Enzyme-Linked Immunosorbent Assay (MJE) was conducted by a commercial laboratory. Results from individual samples and the positive and negative controls were converted to a Sample to Positive Ratio (S/P Ratio) which was interpreted as negative if it was less than or equal to 0.25 and positive if it was greater than 0.25. From July 2003, all herds where at least 15 samples had been reported in the preceding 3 months were assigned a ZAP level according to the MJE results and expected to act as follows: ZAP level 3 – 85% prevalence; an action plan should have been developed and implemented to reduce to ZAP level 1 within 11 months; ZAP level 2 – 65% to 85% prevalence; an action plan should have been developed and implemented to reduce to ZAP level 1 within 17 months; ZAP level 1 – less than 65% prevalence; no action required. To been able to achieve a ZAP level 1 status it was necessary to have MJE prevalence below 65% in a three month period [39, 132]. ZAP introduced new criteria for allocation of ZAP scores in June 2006. ZAP was replaced by a new scheme – the Zoonosis National Control Plan (ZNCP) for *Salmonella* in pigs – in April 2008 [132]. The changes were: collection on average of four meat juice samples per herd monthly, and no herd categorization. The herds with less than 10% seroprevalence in the last one year were recognized as the ones with best practices. There was also the guideline to have an action plan against *Salmonella*, independent of the prevalence, which should have been revised annually to show improvements [132]. The ZNCP changed on 1st of July 2012, stopping the meat juice testing and replacing it with an on-farm risk-assessment tool (http://www.bpex-zncp.org.uk/zncp11/about/news.eb).

1.5. Economic analysis of salmonellosis costs

1.5.1 Cost analysis of human cases to society

The food-borne *Salmonella* infections are responsible for substantial economic losses. The infections can be potentially fatal in old aged and immunocompromised people. However in the majority of cases people fail to go to the doctor, so their infection is not reported. The high proportion of unreported cases makes it difficult to estimate the true incidence of the disease, which increases the range of the cost estimations [133]. The costs associated with the disease are the medical care (visiting a general physician, visiting a hospital, required hospitalization, premature death, medications), productivity loss (loss of work days due to own disease or attending family care), and others (diapers, money spend with physician, etc.). In USA the cost was estimated (based in

two different methods of estimation) between $464 and $2329 million per year [133]. In the United Kingdom (UK) for 23,000 cases that occurred in 1988 the cost was estimated in £18.1 million (this value included the cost of outbreak research, treatments, productivity loss and costs for the families affected) [134]. The UK global estimation for an average cost per case of *Salmonella* spp. was £131.79, with a total cost per year of £46.4 million [135]. In Netherlands the costs were estimated at €8.8 million per year (costs taking in consideration the disease incidence, general practice (GP) consultations, specialists' consultations, hospitalisation, drugs, rehabilitation, other medical services, patients travel costs, costs for additional diapers, informal care and co-payments by patients, production loss due to temporary absence from work, permanent or long-term disability and premature mortality). The concept of Disability Adjusted Life Year (DALY), was used to evaluate the disease burden [136]. In a European study, using data from 2003 to 2005, the DALY was estimated for several disease, including *Salmonella*, in a range of countries [137]. In Portugal a DALY of 46.8 was estimated. The highest results were for Germany (4248.7 DALY), Czech Republic (1946.7 DALY), UK (1163.9 DALY) and Poland (1142 DALY). In these countries the disease incidence is higher than for the rest of EU.

1.5.2 Cost-benefit analysis of controlling *Salmonella* in swine production

Some studies analysed the costs of different control options and their efficiency. In a Dutch study [138] the results show that the combination of articulated interventions both at pre-harvest and harvest allowed a better reduction on the prevalence of contaminated carcasses. However, the cost-benefit was reduced if all herds and abattoirs implemented the intervention practices to reduce *Salmonella*. The gross cost per slaughtered pig at pre-harvest and harvest was €2.99 and €1.47, respectively.
Another study in Denmark considered four methods to reduce prevalence, and analysed the cost-benefit of controlling *Salmonella* in pig production, the benefit of reduction of human incidence and subsequent reduction in human costs using that methods [139]. The methods considered were: hot water decontamination of all pigs slaughtered (reduces the *Salmonella* contaminated carcasses prevalence in 2 log units, when applied hot water at 80ºC for 14 to 16 seconds); sanitary slaughter of pigs from herds with high levels of *Salmonella* (in the Danish case from level 2 and 3); use of home-mixed feed in herds with slaughter pigs; and use of acidified feed for slaughter pigs. Only hot-water decontamination was socio-economically profitable. Hot-water decontamination had a net present value over 15 years of €3.5 million (Table 8).

Table 8: Results of a cost–benefit analysis of four different national strategies against Salmonella in Danish pork [139]

Mean values of discounted net benefits (€ million) for the time period 2005–2020	Hot-water decontamination	Sanitary slaughter	Home-mixed feed	Acidified feed
Consumers	45.7	3.5	4.3	6.4
National Authorities	7.5	0.6	0.7	1.0
Subtotal	53.2	4.1	5.0	7.4
Farmers	0	0	-265.8	-90.5
Abattoirs	-49.7	-47.7	-1.5	3.2
Subtotal pig sector	-49.7	-47.7	-267.3	-87.3
Total net present value	3.5	-43.6	-262.3	-79.9

As an example, the estimated costs of control and monitoring *Salmonella* in pigs in Denmark, in 2007, was around €3.5 million (Danish Agriculture and Food Council, data not published).

An EU study, requested by the DG SANCO concerning the cost and benefits of setting a target for the reduction of *Salmonella* in slaughter pigs [6], estimated €90 million annual human health losses due to *Salmonella* in pigs. For minimizing the risks to humans and to reduce the economic impact of the disease to society they estimated the cost benefit of several possible pre-harvest interventions. They incorporated in their analysis the cost of interventions in feed, breeding pig and replacement stock, farm level, transport, abattoir, monitoring and a support unit. They developed a deterministic model and based on the known impacts of the interventions on pre-slaughter *Salmonella* prevalence in pigs, four scenarios were developed and placed into the model to determine their costs. The scenarios varied from: small scale interventions of a support and monitoring unit relying on the existing structures of the pig industry and public sector; to a targeted selection of interventions prioritized on the basis of the Quantitative Microbiological Risk Assessment of Salmonella in Slaughter and Breeder Pigs [105]; and finally a wholesale level of interventions. The costs varied from €287 million for the smallest set of interventions up to €1,458 million for a most comprehensive programme [6].

The model was used to perform a cost-benefit analysis on four intervention scenarios:

1. An establishment of a support unit and some increased sampling (surveillance)
2. Scenario 1 plus improvement of:
    a. feed practices at feed mill and farm-level
    b. farm-level biosecurity
3. Scenario 1 plus targeted interventions according to country *Salmonella* levels
    a. High prevalence – countries with slaughter pig prevalences above the EU average:

i) Clean replacement pigs

b. Low prevalence – countries with slaughter pig prevalences below the EU average:

i) Feed control measures

4. Scenario 3 plus all transport and abattoir measures.

For scenario 2 and scenario 4, it was assumed they would achieve a reduction in Salmonella of 50% and 90% in slaughter pigs, respectively. The costs per scenario were estimated (discounted cost). Then, a benefit-cost ratio (BCR) was estimated for each scenario using the benefits to human health (reduction of the number of human cases and consequently the costs associated) or the benefits to human health and pig production (reduction of human and pig cases; the benefit of having a *Salmonella* free pig was taken to be €1.55 per pig). The results are shown in Table 9.

Table 9: Summary of cost-benefit analysis of the four scenarios [6]

Scenario	Description	Discounted cost (€ million)	BCR Human Health	BCR Human Health and pig production	Cost per slaughter pig (€)
1	Establish support unit and increased sampling (varying rate of reduction of human health losses)	287	0.44	0.66	0.11
1+	Scenario 1 (but constant rate of reduction in human health costs and increase in pig productivity of 6%)	287	0.66	1.07	0.11
2	Scenario 1 plus feed practices and farm-level biosecurity	1089	0.17	0.28	0.43
3	Scenario 1 plus targeted MS interventions, based on high and low prevalence	752	0.38	0.61	0.29
4	Scenario 3 plus transport and abattoir measures	1458	0.31	0.50	0.57

Legend: benefit-cost ratio (BCR)

The authors cautioned about the lack of precise data but concluded that the most economically preferable approach would be a gradual introduction of *Salmonella* control measures starting with the establishment of surveillance measures [6].

1.6. Infection Models

Infection models are simplified representations of the reality with the aim of simulating the dynamic of a disease, making possible the evaluation of the disease evolution in populations and the effect of control measures. The models can be of three types:

deterministic, stochastic or a mixture of the two. Deterministic models use point-values as inputs and therefore the outputs are also point values with an associated confidence interval. Stochastic models, however, incorporate uncertainty and/or variability into the model. Variability represents the true heterogeneity in a population, e.g. the weight of an individual pig will vary between pigs in one cohort and the fact that the same value cannot be assign to the weight of all the pigs in that cohort is not due to incomplete knowledge; it is inherent to the population. Uncertainty, on the other hand, reflects our lack of knowledge about the exact value of a parameter. For example, the inactivation of *Salmonella* when subjected to high temperatures may be modelled by an exponential decay, dependent on the time of exposure and on an inactivation parameter. It is hard to assign only one value to this inactivation parameter because several uncontrolled factors bring in heterogeneity upon the matrix where the parameter is being measured: therefore it cannot be quantified with precision. In a stochastic model variability and uncertainty can be modelled using statistical probability distributions, instead of fixed parameter values. Incorporating distributions into the model results in a distribution for the model output; hence providing more information compared to the deterministic approach *[105]*. A mixture of the two models is quite common as a way of incorporating variability/uncertainly in the model, and, at the same time, decreasing the computation time.

Salmonella spp. control was considered necessary by the European food-safety policy makers under the EC Regulation 2160/2003. In practice, however, the control of this agent has proved to be difficult and expensive at the farm level [5]. Consequently the evaluation of the efficiency of control strategies for this agent has become an important and stringent issue, as stated in recent reports [6]. Modelling the dynamics of *Salmonella* spp. in pigs can become useful when assessing alternative control strategies.

Susceptible – Infectious – Resistant (SIR) models are attractive tools to help in assessing the disease dynamics. The SIR model describes the dynamic of different states of individuals in the population in terms of a system of ordinary differential equations. The variables in the system are given by the three compartments: susceptible (S), infectious (I) and resistant/carrier (R). The mathematical models provide a description of the movement in and out of the three compartments, and the transitions between compartments are governed by transmission rates. If modelling is to be helpful in infectious disease control, it is crucial to have the best possible estimate of these rates.

Some infection models for *Salmonella* in pigs have been already described in the literature, simulating the food chain or part of the food chain. Table 10 shows some

models found in the literature. To construct such models a high number of parameters are needed, such as production parameters, infection parameters, risk factors and disease prevalence which are difficult to obtain. Therefore it is frequently necessary to call upon expert opinion to estimate the parameter value. This is not the ideal option to improve the quality and credibility of the model, but frequently it is a way of overcoming the lack of data to estimate parameters.

The infection models found in the literature (Table 10) were used to simulate the dynamics of the infection and test possible control measures in terms of *Salmonella* prevalence reduction. They were also used for cost-benefit analysis for different control measures.

Table 10: Infection models for Salmonella spp. infection in pigs found in the literature.

Reference	Model type	Which part of the food chain simulates	Time unit	Software used
[140]	Discrete stochastic	Maternity to Fattening	1 week	Scilab 4.0
[141]	Stochastic	Entrance of replacement gilts to slaughter	Reproductive cycle	Not stated
[142]	Discrete stochastic	Growing to Slaughter	1 day	Delphi5
[143]	Stochastic	Growing to Fattening	1 day	Risk4.5, Microsoft Excel, VBA
[144]	Stochastic	All food chain	Not stated	Risk software
[145]	Stochastic	Growing to Fattening (duration of 113 days)	1 day	Python programming language v.2.5.1, R
[105]	Discrete stochastic	Farm(piglets) to consumption	1 day	Matlab R2008b (© Mathworks Ltd, USA)

Some of the infection models simulate the entire food chain [105, 144], others only a part of the food chain: some simulate mainly the herd [140, 143, 145] others simulate from herd to slaughter [141, 142]. All of them were built with the idea of testing control measures and some [105, 140] have incorporated a production model link to an infection model to try to simulate what happens at farm level, to increase the accuracy of their results and at the same time simulate control measures linked to production aspects. The complexity of the mathematical models varies from simple [145], with median estimates as transmission parameters, to complex *[105]*, with distributions for the transmission parameters (some of them even with distributions for some parameters of their distribution). For the majority of the simulation studies published *[140-143, 145]* the transmission rates were estimates based on the best fit to *Salmonella* spp. prevalence in the country or expert opinion, due to the lack of longitudinal infection studies that allow the estimation of these parameters using experimental or field data.

Following cohorts of animals in order to determine the dynamics of *Salmonella* spp. in susceptible populations is a very expensive procedure. Therefore few longitudinal studies [146-149] regarding the dynamic of infection with *Salmonella* spp. have been published in the last few years. One of the explanations for few studies being available is that in most cases *Salmonella* causes subclinical infection with no apparent symptoms of disease in pigs, which makes it difficult to assess the infection status of individual pigs in an infected population without testing each animal several times. Another explanation is the difficulty in obtaining accurate estimates for the transmission parameters, which stems from the fact that the currently available bacteriological and serological tests used to assign the infection status are imperfect, bringing uncertainty when trying to classify each animal. An additional source of uncertainty comes from the fact that pigs, once infected, shed the agent intermittently and for different periods.

1.7. European Union policy for Salmonella spp. management

Several legal documents were produced with the aim of controlling this agent in EU pig productions systems. One of the most important is the Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of *Salmonella* and other specified food-borne zoonotic agents, which establish a schedule to control *Salmonella* spp. in the poultry and pig industry. The legal initiative includes the baseline studies to harmonize the prevalence of *Salmonella* spp. between MS and also a timeline to put reduction targets on those species, including breeding and finishing pigs. To help finance the baseline studies in pigs, the Commission Decision of 29 September 2006 and the Commission Decision of 20 December 2009 refer to the financial contribution from the Community towards a baseline survey on the prevalence of a) *Salmonella* in slaughter pigs and b) *Salmonella* spp. and Methicillin-resistant *Staphylococcus aureus* in herds of breeding pigs, respectively, to be carried out in the MS. In the near future a mandatory target reduction could be enforced in the EU regarding the *Salmonella* prevalence in pigs.

1.8. Overview of the pig sector in Portugal

1.8.1. The livestock and pig sector

World livestock production is a sector where the global demands are still increasing. Although a great part of this increase occurs in developing countries, it has a great influence in the industrialized world due to the global economy. Production in industrialized countries is forced by the competition in the global trade, which

decreases food prices and thus producers profit leading to a change to more intensive production systems. However the intensive production is not favoured by consumers in developed countries due to environmental, welfare and food safety issues. The changes to organic production and to a more specialised production are transforming animal production [150].

1.8.1.1 World and in Portugal

The major swine producers are the following countries (2011 data): Republic of China (49.5 million tons), EU (22.5 million tons), United States (USA) (10.3 million tons), and Brazil (3.2 million tons). The major pork meat exporters are USA (2.2 million tons), EU (2.0 million tons), Canada (1.1 million tons) and Brazil (582,000 tons) [151]. The Republic of China produces mainly for self-consumption changing the pattern between producers and exporters.

Portugal has a deficit in terms of pork meat trade [152], as it produce around 66.5% of the pork meat that is consumed (Table 11). This situation could be regarded as an opportunity by the Portuguese pork meat industry as they have potential for growth in the internal market.

Table 11: Pork meat market in Portugal (units in 1000tons)[153]

	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Pig population (1000heads)	2 389	2 344	2 249	2 348	2 344	2 295	2 374	2 340	2 325	2 145
Internal production	282	288	296	283	295	291	318	332	318	331
Import (Live animals)	65	72	66	65	65	81	75	86	90	83
Export (Live animals)	4	4	7	8	7	6	7	14	12	6
Net production	343	356	355	340	353	366	386	404	396	408
Import (pork meat)	122	124	122	123	120	138	157	150	161	125
Export (pork meat)	17	17	17	22	26	30	41	55	51	45
Consumption	447	454	459	445	448	467	492	504	508	498
Capitation (Kg/person/year)	43.4	43.8	44.0	42.4	42.4	44.1	46.4	47.4	47.8	46.8
Self-providing (%)	63.1	63.4	64.5	63.6	65.8	62.3	64.6	65.9	62.6	66.5

Pork meat has a high import volume, representing 42% of the total meat imported in the country. Spain is the main source, with 99.8% and 96.6% of live animals and pork meat imported, respectively [152].

Pork meat is the most consumed meat in the country: consumption comprises 41% pork, 31% poultry, 18% beef, 5% offal, 2% lamb and goat and 3% other meat [152].

In 2010, poultry production was the leading national livestock production with a weight of 39.3%, followed by pork production (38%) and beef production (10.9%) [152] (Table 12 and 13).

Table 12: Number of animals in Portugal (unit 1000heads)[153]

Type of production	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Beef Cattle	1 404	1 395	1 389	1 443	1 441	1 407	1 442	1 439	1 391	1 375
Dairy Cattle	338	341	328	338	324	307	306	301	289	275
Swine	2 389	2 344	2 249	2 348	2 344	2 295	2 374	2 340	2 325	2 145
Sheep	3 459	3 457	3 356	3 541	3 583	3 549	3 356	3 145	2 906	2 512
Goats	561	538	502	547	551	547	509	496	487	444

Table 13: Livestock production in Portugal (unit tons)[153]

Type of meat	2004	2005	2006	2007	2008	2009	2010
Beef	119 259	119 020	106 087	91 742	108 540	102 995	93 159
Lamb	21 994	21 990	23 356	24 235	21 503	17 895	18 279
Goat	1 574	1 363	1 563	1 733	1 495	1 551	1 517
Pork	340 279	352 998	365 869	385 864	404 153	395 970	407 808
Meat	221 181	229 449	237 815	250 812	262 700	257 380	265 076
Lard	119 098	123 549	128 054	135 052	141 453	138 590	142 732
Poultry	289 737	294 369	287 812	315 823	324 815	333 483	338 639
Broilers	215 711	215 925	209 549	230 839	239 077	251 546	253 091
Turkey	38 682	41 444	42 025	44 604	42 535	40 222	41 719
Eggs	131 683	118 148	119 119	121 592	123 515	124 184	131 123
For incubation	17 992	18 167	18 008	20 050	20 503	22 130	22 528

1.8.1.2 Type of production

Pig production in the industrialised countries is mainly intensive. The concentration of pigs in certain geographical areas leads to environmental questions, concerning the waste management and pollution of soils and water sources, and unpleasant smells. Welfare issues also are important in industrialized countries which have been increasing the legal requirements for animal keeping and management supported in legislation in Europe and North America.

Pig production is divided into breeding, maternity, post-weaning and finishing phases. These phases can occur in the same herd (farrowing-to-finish herds), or be divided into different specialised herds (breeding herds, piglet production, weaner to finishers, finishers). The approach of specialised herds is adopted more as the herds increase their size.

In intensive production biosecurity and health management are the major priorities. Most of the intensive herds have a high sanitary status, barriers and restricted access. However, controlling diseases is difficult in areas with high population density, and when disease appears it is difficult to control it without using methods such as partial or total depopulation or by use of vaccines. In the past the use of antibiotics in animals feed with a prophylactic or growth promoter goal has been common. However this has been forbidden by EU legislation since 2006 [154].

In the future, EU pig production may tend to outdoor units, to fulfil welfare and environmental legislation (Council Directive 2008/120/EC and Council Directive 2008/1/CE). Part of this legislation is already in place in the EU.

1.8.2 Characterisation of the pork food chain in Portugal

Pig herds in Portugal are mainly herds of low dimension (few animals per herd); although the majority of the total number of animals belongs to big herds (Table 14).

Table 14: Type of swine herds in 2005 (FPAS, unpublished)

Number of animals per herd	Herds (1000)	Total number of animals (1000)
1-2	66.8	110.2
3-9	20.5	89.1
10-19	5.7	60.4
20-49	2.7	59.8
50-99	1.0	53.7
100-199	0.7	84.7
200-399	0.5	115.2
400-999	0.6	279.6
1000 or more	0.7	1491.5
Total	99.2	2344.1

The majority of the pork meat produced comes from piglets (Centre Region) and finishing pigs (Lisbon and Tagus Valley Region) (Table 15 and 16).

Table 15: Distribution of the number of pigs per region in 2010 (1,000 animals) [153]

Regions	Total	<20Kg	20-50Kg	Finishing pigs (kg)				Boars	Sows
				Total	50-80	80-110	≥110		
North	79	18	17	33	17	11	11	1	10
Centre	427	149	93	117	72	42	42	2	65
Lisbon	853	268	198	288	169	112	112	2	97
Alentejo	476	127	111	177	83	77	77	2	58
Algarve	24	10	4	6	3	2	2	-	4
Madeira	42	11	10	16	12	3	3	-	5
Azores	17	5	5	5	2	2	2	-	2

Table 16: Distribution of the number of industrial herds (raising pigs for commercial purpose) by region in Portugal in 2009 (DGAV, unpublished)

Regions	Commercial herds	
	N	%
North	42	3
Centre	276	20
Lisbon and Tagus Valley	727	54
Alentejo	281	21
Algarve	27	2
Total	1353	100

Most of the herds are concentrated in Lisbon and Tagus Valley region followed by the Centre and Alentejo Region (Table 16) which corresponds also to the greatest number

of animals (Table 15). In terms of the number of pig abattoirs, the majority exist in the north region of the country, followed by Lisbon and Tagus Valley region (Table 17). However in terms of slaughter volume, the Lisbon and Tagus Valley Region slaughter more pigs followed by the North Region (Table 17). Even so the North Region slaughter more pigs than the number that are raised in that region. Therefore a high proportion of the pigs slaughtered travel from other regions of the country or from Spain (the major exporter for the national market) to be slaughtered in the north of the country. This has some health implications as it increases the time travelling to slaughter, which causes stress to the pigs and can promote shedding of *Salmonella* from carriers. In Portugal 5,965,601 pigs were slaughtered in 2010 [153].

Table 17: Number of abattoirs and volume of slaughter per region (between October 2010 and August 2011) [155].

Region	Abattoirs where pigs are slaughtered		Average weekly slaughter volume (pigs)	
	N	%	N	%
North	31	47.7	32697	36
Centre	12	18.5	8890	9.8
Lisbon and Tagus Valley	20	30.7	47016	51.7
Alentejo	2	3.1	2277	2.5
Total	65	100	90880	100

The Centre Region has a high number of slaughterhouses dedicated to the slaughter of weaned pigs (Table 18) which are commonly used in that region for traditional dishes.

Table 18: Distribution of abattoirs that slaughter piglets per region (between October 2010 and August 2011) [155].

Region	Abattoirs where piglets are slaughtered	
	N	%
North	1	2.5
Centre	30	75
Lisbon and Tagus Valley	9	22.5
Total	40	100

The regional distribution of cutting and processing plants and registered butchers is concentrated in the North, Centre and Lisbon and Tagus Valley Region, where the majority of the Portuguese population lives (Table 19).

Table 19: Distribution of cutting plants, minced meat and meat preparations plants, and butchers per region in 2009 (DGAV unpublished).

Regions	Cutting plants		Minced meat and meat preparations plants		Meat products plants		Butchers	
	N	%	N	%	N	%	N	%
North	45	23.8	14	14.1	160	21.4	2930	41
Centre	54	28.6	26	26.3	240	32	1681	24
Lisbon and Tagus Valley	59	31.2	48	48.5	134	17.9	1716	24
Alentejo	23	12.2.	6	6.1	115	15.3	431	6
Algarve	5	2.6	4	4	14	1.9	262	4
Azores	0	0	0	0	86	11.5	68	1
Madeira	3	1.6	1	1	0	0	43	1
Total	189	100	99	100	749	100	7131	100

1.9. Objectives of the PhD research

The importance of *Salmonella* as a zoonosis leaded the EU to impose future control programmes in the swine industry, mainly at herd level.

The main drivers to the development of this work were the lack of applied knowledge about the *Salmonella* situation in Portugal at herd level, regarding risk factors and cost-effective control measures. These factors and the fragile Portuguese economic situation, of which the pig sector is not excluded, allied with the experience from other European countries which shows how hard the control and eradication of this agent is at the level of pig production chain, also motivated this work.

The aim of this PhD is to improve the epidemiologic knowledge of *Salmonella* spp. disease dynamics in pig farms in Portugal, and to contribute to a better use of the available cost-effective control measures at farm level, taking into consideration the prevalence of the agent, the risk factors associated with *Salmonella* spp. and the Portuguese production system.

The overall aim has two objectives:

Objective 1: Risk characterisation at farm and abattoir level, which contributes to a better knowledge of the risk factors linked to the Portuguese production system.

Objective 2: Development of a simulation model that describes the Portuguese production system linked to the dynamics of *Salmonella* at farm level which in the future might allow the Portuguese Veterinary Authority or others to test different control measures in terms of efficiency for reducing the prevalence of disease.

Chapter 2 – Materials and Methods

This chapter describes the data, the statistical and mathematical methods used for Objective 1 and 2 that resulted in several manuscripts which compose the results section. For each objective of the PhD, the materials and methods are described.

2.1 Risk factors analyses for the farm and abattoir studies (Objective 1)

Salmonella has been reported as a frequent cause of food-borne disease in the European Union (EU) as described in Chapter 1. The EU Regulation (EU Regulation No 2160/2003) imposes to the Member States (MS) implementation of a control programme to reduce the prevalence in food production species including pigs. To set the reduction target each MS carried out baseline surveys to estimate the *Salmonella* spp. prevalence in some food production animals. The objective of the surveys was to obtain comparable data for all MS through harmonized sampling and testing schemes. In pigs the baseline study was done at abattoir level (collection of lymph nodes of pigs slaughtered) - Baseline Survey on *Salmonella* Prevalence in Slaughter Pigs [36], and at herd level (collection of pen faecal samples of breeding pigs) - Baseline Survey on the Prevalence of *Salmonella* in Breeding Pigs [37]. These cross-sectional studies also collected information regarding herd management practices and potential risk factors linked to this agent. The data used in this Objective was the EU Baselines studies in Portugal.

2.1.1 Slaughter Pigs risk factors study

A study to the data of Baseline Survey on *Salmonella* Prevalence in Slaughter Pigs in Portugal was done. The aims of the study were: 1) to search for potential risk factors for the presence of *Salmonella* sp. in the lymph nodes of pigs slaughtered in this country, and 2) to identify differences in the risk profile between groups of serotypes.

The sampling frame, the diagnostic testing methods, as well as the collection and reporting of data, and the timelines of the Baseline Survey in Slaughter Pigs were specified in the Commission Decision 2006/668/EC, Annex I. The sampling frame was the list of the slaughterhouses which together accounted for 80% of the pigs slaughtered within the Member State. The samples in Portugal were collected between January and September 2007. The sampling size was estimated by the Portuguese Veterinary Authorities (PVA) based in the Commission Decision 2006/668/EC. The minimum sample size for Portugal, according to this scheme, was 600 pigs, and an additional 10% was taken into account for non-response. The number of pigs sampled was stratified by slaughterhouse and was proportional to the slaughterhouse capacity. The sampling days for each slaughterhouse were selected at random. Each sample

was formed mainly by an aggregate of ileocaecal and sometimes jejunal lymph nodes to ensure that it had at least 15 grams of lymph nodes. The collection of the lymph nodes was done in an aseptic way to avoid external contamination. The lymph node samples were sent to the laboratory for microbiological detection of *Salmonella* according to the procedure defined by Annex D of ISO 6579. Each *Salmonella* isolate was serotyped in the National Reference Laboratory for *Salmonella* according to Kaulfmann-White scheme.

Along with the sample collection, information concerning the pig and the slaughterhouse was also collected using a structured questionnaire, to assess their potential influence in the presence of *Salmonella*. The variables collected were: transport of pigs from different herds to the slaughterhouse (yes or no); carcass approval for human consumption (total versus partial); detection of lesions in the lymph nodes (yes or no); sample collection time; month of the sample collection; time from the animal's arrival in the slaughterhouse until it was killed; weight of the carcass; weight of the lymph node sample; region of the slaughterhouse and annual capacity of the slaughterhouse. Questions about hygiene at lairage and slaughter were not collected.

As the data followed a multilevel structure, lymph nodes samples (first level) nested within slaughterhouses (second level), a two level hierarchical model was used.

To look for associations between the explanatory variables and the outcome variable for slaughtered pigs, a binomial Bayesian hierarchical model was used. Afterwards this analysis was refined to investigate if there were different associations for different serotypes. Because of the low number of cases per serotype, individual analysis of each *Salmonella* serotype was not feasible. Therefore the outcome variable was the isolation of *Salmonella* in each sample which was classified in three categories: i) no *Salmonella*, ii) serotype Typhimurium and S. Typhimurium-like strains with the antigenic formula: 1,4,5,12:i:-, and iii) other serotypes.

To model such an outcome variable, a categorical (Bayesian) hierarchical model was used. Monte Carlo Markov Chain (MCMC) estimation method was used and was implemented in the freely available software WinBUGS (BUGS project, http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/). The materials and methods are explained in detail in Results Chapter - Manuscript 1: "Risk factors for *Salmonella sp.* in pig lymph nodes in Portuguese abattoirs".

2.1.2 Breeding Pigs risk factors study

Two studies to the data of the Prevalence of *Salmonella* in Breeding Pigs on Portugal were done. The first study aim was to identify risk factors for the presence of *Salmonella* in herds with breeding pigs. The second study aim was to search for

potential risk factors for shedding from two different groups of *Salmonella* serotypes using pen faecal samples from herds with breeding pig representative of Portuguese reality. The two groups were *Salmonella* Typhimurium including *S.* Typhimurium-like strains with the antigenic formula: 1,4,5,12:i:-, and other serotypes.

The sampling frame, the diagnostic testing methods, the sample collection procedures and the timelines of the baseline study for *Salmonella* in Breeding Pigs were as specified in the Commission Decision 2008/55/EC. The target population was the holdings constituting at least 80% of the breeding pig population in the MS. In Portugal, the sampling frame was organized by the PVA. These holdings were stratified by the Regions of the National Veterinary Services structure. The sampling frame consisted of 4522 herds, with 204,584 breeding pigs and 1,827,533 pigs in total. The herd inclusion criteria for entering the sampling frame were: to have at least 50 breeding pigs, either for breeding or production purposes. The pig population included in the sampling frame represented 87% of the total registered pig population in Portugal in 2007.The sample size was calculated using the sampling criteria specified in the Commission Decision 2008/55/EC Annex I - expected herd prevalence of 50%, desired confidence level of 95%, accuracy of 7.5% and then a finite population correction factor was applied, with an increase of 10% for each group (breeding and production holdings) in case of non-response. A sample of 174 swine herds was randomly selected using probability proportional to the number of herds among the regions in Portugal. For each herd, only the pens with breeding pigs over six months of age were randomly selected. The breeding pigs that had been recently introduced into the herd and were in quarantine were not included in the survey. In each selected herd, faecal samples from 10 pens were taken representing a 95% probability of detecting at least one positive sample if the true prevalence of infected pigs in the population was 10% [21]. The number of pens sampled per breeding room in each herd was allocated proportionally according to the number of breeding pigs in the different stages of production. The age categories in the sampling were not predetermined. The specification was that at least 10 individual breeding pigs should be included in each pooled pen faecal sample otherwise no sample was collected.

The samples were collected between November 2008 and January 2009 by the herd veterinarian. The faecal samples were sent to the laboratory for microbiological detection of *Salmonella* according to the procedure defined by Annex D of ISO 6579. Each *Salmonella* isolate was serotyped in the National Reference Laboratory for *Salmonella* according to the Kaulfmann-White scheme.

Information about herd management and potential risk factors (at herd and pen level) was collected using a questionnaire along with the collection of the faecal samples. At

pen level: the type of housing (which sector it belonged, whether the animals in the pen had access to outside, whether it was an individual pen or a group pen); number of animals that contributed to the sample; whether diarrhoea was detected in the last three months; age (gilts, sows or mixture) and sex (females, males or mixture) of the pigs in the pen; production phase; floor type; whether sanitary gap (cleaning, disinfection and down time between batches) was applied before new breeding pigs enter the pen; feed type; source of the feed; use of organic acids or others (probiotics); use of antibiotics; and the approach used to collect the pooled sample (swab or individual pinches). At herd level: region of the country; production type (breeding or production holdings; intensive versus extensive; farrow-to-finish, farrow-to-weaners, farrow-to-growers holdings); number of breeding pigs; number of finishing pigs present; replacement management of breeding pigs (sows and boars); source of semen; and biosecurity measures (clothes for exclusive use in the herd, footbath, and control of pests).

As the data followed a multilevel structure, i.e. pen faecal samples (first level) nested within swine herds (second level), a two level hierarchical model was used.

To look for associations between the explanatory variables and the outcome variable for the breeding pigs a multilevel logistic regression model was fitted using the framework of generalized linear mixed model (GLMM) methods, implemented using the glmmPQL function of package MASS [156] of (free software) R (CRAN project, www. R-project.org). The materials and methods are explained in detail in Results Chapter - Manuscript 2: "Risk factors for *Salmonella* spp. in Portuguese breeding pigs using a multilevel analysis"

Afterwards this analysis was refined to investigate whether there were different associations for different serotypes. Because of the low number of cases per serotype, individual analysis of each *Salmonella* serotype was not feasible. Therefore the outcome variable was the isolation of *Salmonella* in each sample which was classified in three categories: i) no *Salmonella*, ii) serotype Typhimurium and S. Typhimurium-like strains with the antigenic formula: 1,4,5,12:i:-, and iii) other serotypes.

For this a Bayesian hierarchical categorical model was used. Monte Carlo Markov Chain (MCMC) was used for estimation and this was implemented in the freely available software WinBUGS (BUGS project, http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/). The materials and methods are explained in detail in Results Chapter - Manuscript 3: "Assessing risk profiles for *Salmonella* serotypes in breeding pig operations in Portugal using a Bayesian hierarchical model".

2.2 Production and infection model (Objective 2)

For achieving the aim of Objective 2, two studies were conducted and the results were organised in two manuscripts. The first manuscript describes the work done to estimates the transmission parameters for *Salmonella* Typhimurium and the second uses these estimates in a simulation model. The data and methods are described separately for each one of the studies.

Due to the lack of funding and time to perform a longitudinal study to estimate the transmission parameters for *Salmonella* spp. which were needed for the simulation model, it was decided to use the available data from a published study to estimate these parameters.

2.2.1 Transmission parameters estimation

Study herds, sampling, bacteriology and ELISA test

The data used was previously described by Kranker et al (2003)[146] and originated from three Danish pig herds known to be infected with *Salmonella* Typhimurium, but with different levels of infection density. The herds had moderate to high levels of *S.* Typhimurium infection. This implied that the within-herd prevalence was 40% or higher based on meat-juice samples collected over 3 months, evaluated by use of a cut-off of 20 optical density percentage (OD%). Two of the farms, with 650 and 440 sows, respectively, were two-site operations. The remaining farm was a three-site operation with 300 sows. The three herds were self-supplying. In each herd, 10 litters were randomly selected, and in each litter, the ears of six randomly selected piglets were tagged. To account for variations in *Salmonella* shedding over time, litters from each herd were divided into two groups of five litters, which were raised at approximately one month intervals. Thus, on each farm there were two cohorts consisting of 30 pigs each, yielding a total of 180 piglets at the start of the study. All ear-tagged pigs from a given cohort were supposed to be raised together for the entire observation period. The animals were followed longitudinally [146]. The animals were first tested at the age of 4 weeks and thereafter at 3 to 4 week intervals until the age of slaughter. At each testing occasion, sera and faeces from the animals were collected and tested for the presence of *Salmonella* spp. (at the age of 4 weeks only faeces were collected, because persistence of maternal antibodies could give a false positive result). An animal was considered serologically positive wherever the serological test revealed a result of OD% >20, and bacteriological positive if *Salmonella* was isolated from the faeces. The serological test used at this cut-off value is considered to have a sensitivity of 68% and

to be 100% specific [44]. The bacteriological test is considered to be 100% specific and the sensitivity ranges from 30 to 55% [157]. These test characteristics were incorporated into the statistical model.

Infection status of the pigs

The infection status of each pig was determined for every sampling period by both faecal shedding and by serology. Each animal was categorized as susceptible (S), infectious (I) or carrier (R). In the absence of reasonable sensitivity of the bacteriological culture method, serology offered an alternative and complementary way to assign the infection status of a pig.

Pigs were attributed status S when there was no presence of bacteria in the faecal samples and the OD% was below 20 OD%. Status I was assigned from the date when a pig was found bacteriologic-positive as well as over the next 4 weeks, assuming that a pig would shed *Salmonella* spp. within an average of 4 weeks. This average period was assumed from the data of the shedding period duration from experimental studies [40, 158]. Additionally, pigs were assigned to status I based on seroconversion. The beginning of the infectious period was set to 2 weeks prior to the recorded date of seroconversion [148, 158] and the duration was set to 4 weeks [40, 158]. Therefore for pig classification the information of both tests in parallel was used. Status I was followed by status R along the study, and the pigs could return to status I if they were found culture positive later on during the study period. We assumed that no pigs would return to the susceptible status after being infected because of the relative short life span of the finisher pigs.

Given that in the beginning of the follow-up piglets could only be tested by the use of bacteriology (which has low sensitivity), some piglets could have been erroneously classified as susceptible, whereas they could have been infected by the sow. Therefore, we began the analysis in each cohort at the time infected animals were found.

Transition between susceptible to infectious

To estimate the transmission rate parameter from susceptible to infectious (β) we considered a Binomial SIR-model for the transmission of *Salmonella* spp. between pigs. We assumed homogeneous mixing of pigs in each cohort, i.e. all pigs could come into contact with each other. At the beginning of the study, pigs were considered to be either in the S or I status depending on the test results. The following model (Binomial distribution with finite population size) was used in the estimation of β for each time interval:

$$C_{jt} \sim Binomial(S_{jt}, p_{jt})$$
$$\text{cloglog}(p_{jt}) = \log(\beta) + \log(I_{jt}) - \log(N_{jt}) + r_{1jt}$$

Where:

- C_{jt} denotes the number of new infectious animals in the cohort (j) at the end of the time interval (t),
- β is the transmission rate parameter for the transition between susceptible to infectious,
- S_{jt} is the number of susceptible animals in the cohort (j) at the beginning of the time interval (t),
- I_{jt} is the number of infectious animals in the cohort (j) at the beginning of the time interval (t),
- N_{jt} is the total number of animals in the cohort (j) at the beginning of the time interval (t), and
- r_{1jt} is the cohort time-dependent random effect,
- p_{jt} is the probability of a susceptible animal that becomes infectious in the cohort (j) and time interval (t).

The number of infectious pigs (I) was corrected taking into consideration the sensitivity of both tests used together in parallel. As the specificity was considered 100% in both tests, the parallel specificity was 1. In the correction, we simulated the number of infectious pigs that the tests were not able to detect (false negative pigs). When there was no infectious pig present at the beginning of a time interval, the rate was set equal to the baseline rate β plus the random effect. The infectious animals not detected (*Inob*) were simulated by sampling from the following Binomial distribution and assuming independence between the tests:

$Inob_{jt}$ ~ Binomial(N_{jt}, pND)

pND = (1-SenC)*(1-SenE)

Where:
- *SeC* is the sensitivity distribution of microbiological culture,
- *SeE* is the sensitivity distribution of the ELISA test,
- *pND* is the probability of not detecting infectious animals,
- $Inob_{jt}$ are the infectious animals not detected, and
- N_{jt} is the number of animals tested.

The infectious animals were added to the non-detected infectious animals to obtain (I).

Transition between infectious (I) to carrier (R)

This transition was modelled assuming a Binomial distribution:

$$Rnew_j \sim Binomial(I_j, pr_j)$$
$$\text{cloglog}(pr_j) = \log(\alpha) + r_{2j}$$

Where:

- $Rnew_j$ is the number of carrier animals which result from the transition between infectious and carrier in the cohort (j),
- I_j is the number of infectious animals at the start of the time step in the cohort (j),
- pr_j is the probability of transition between infectious to carrier in the cohort (j)
-α is the transmission rate parameter for the transition from infectious to carrier state, and
- r_{2j} is the cohort random effect.

Transition between carrier to infectious

A Poisson distribution was used to model the transition between R to I as the transition only happened three times in the entire study (in two cohorts). As this is a very rare event the Poisson distribution is appropriate as it approximates the Binomial distribution in the case where the probability of the event is very small. The transition was modelled as follows:

$$Inew_j \sim Poisson(\pi_{2j})$$
$$\log(\pi_{2j}) = \log(\nu) + \log(R_j) + r_{3j}$$

Where:

- $Inew_j$ is the number of new infectious animals which result from this transition in the cohort (j),
- π_{2j} is the mean number of carrier animals which became infectious in the cohort (j),
- R_j is the number of carrier animals at the start of the time step in the cohort (j),
-v is the transmission rate parameter for the transition from carrier to infectious state, and
- r_{3j} is the cohort random effect.

When there was no resistant pig present, the baseline rate v plus the random effect was used.

Cohort random effects

Random cohort effects r_j were incorporated into each transition step. For the transition between infectious to carrier and from carrier to infectious r_j was modelled as:

$$r_{kj} \sim Normal(0, \sigma_k^2), k = 2,3$$

Where:
- subscript j denotes cohorts per time interval and
- σ^2 is the variance of the unobserved cohorts effects.

The cohort random effects were different for each transition under the assumption that the unobserved cohort factors affected each transition in a different way.
For the transition between susceptible to infectious, the cohort random effects were assumed time-dependent and were modelled as:

$$r_{1j,t=1} \sim Normal(0, \sigma_1^2)$$
$$r_{1j,t} \sim Normal(r_{1j,t-1}, \sigma_1^2)$$

where the cohort random effect (r_{1jt}) for time t depends on the previous cohort random effect at time (t-1). With this cohort time-dependent random effect we capture the dependent structure of the spreading of infection within cohorts where the velocity of infection is dependent of the number of susceptible and infectious animals in the previous time step. For the transition from I to R we did not consider a cohort time-dependent random effect because this transition just depends on the shedding duration for *Salmonella* which was assumed that does not vary with time. The transition from R to I was so rare (just happened three times in the study) that we did not have adequate data to use a cohort time-dependent random effect.

Model settings
The time interval of our present study was chosen to be 2 weeks, which approximates the interval between different testing times. Since there was no previous information for informing prior distributions of log(β), log(α) and log(v), we used Normal distributions with zero mean and a variance of 100, which reflected prior ignorance while avoiding the use of improper distributions [159]. For the sensitivity of both serological and bacteriological tests a Beta distribution was used. Previous information about the sensitivity of both tests [44, 157] was utilised to inform those Beta distributions: a mean of 0.49 for faecal culture and a mean of 0.68 for Danish mix ELISA were used, so we specified SeC~Beta(48.5,50.5) and SeE~Beta (58.5, 27.5). Specificity was assumed to

be 100% in both tests. The precision of the Normal distribution of random effects was modelled with a Gamma (0.5, 0.005) distribution.
The final model was implemented in WinBUGS [160] and this was run long enough (100,000 iterations) with sufficient burn-in (5,000 iterations) to ensure convergence to the posterior distribution of the parameters [161]. Convergence was assessed by visual means (inspection of time-series plots) but also more formally using the Raftery and Lewis diagnostic, and the Gelman-Rubin R-hat diagnostic which should be sufficiently close to 1 if convergence was achieved [162, 163]. The chains were thinned by only collecting 1 in 10 consecutive samples and this eliminated autocorrelation in posterior samples (the R package "coda" [164] was used). Two MCMC chains were run with dispersed initial values, which is good practice to ensure convergence and mixing. Mixing in the chains was assessed by comparing the Markov Chain (MC) error with the standard deviation, for each parameter. Ideally the MC error for each parameter should be less than 5% of the standard deviation [165] for good mixing.

Calculations of the basic reproduction ratio (R_0)
Subsequently, R_0 was estimated by use of the following formula [166]:

$$R_0 = \beta / \alpha$$

Where:
- α is the transition parameter from I to R, and
- β is the transition parameter from S to I.
Results Chapter - Manuscript 4: "Transmission parameters estimated for *Salmonella* Typhimurium in swine using susceptible-infectious-resistant models and a Bayesian approach" - shows the results of this work and describes with more detail the methods used.

2.2.2 Production and infection model

The aim of this study was to construct a production and infection model that simulates the spread of *Salmonella* within a farrow-to-finish herd.

Description of the production part of the model
The model simulates a farrowing-to-finish herd in which batch farrowing was applied to sows, leading to batch management of pigs. This type of management is usual in countries like France and Portugal. In these herds the complete life cycle of sows is

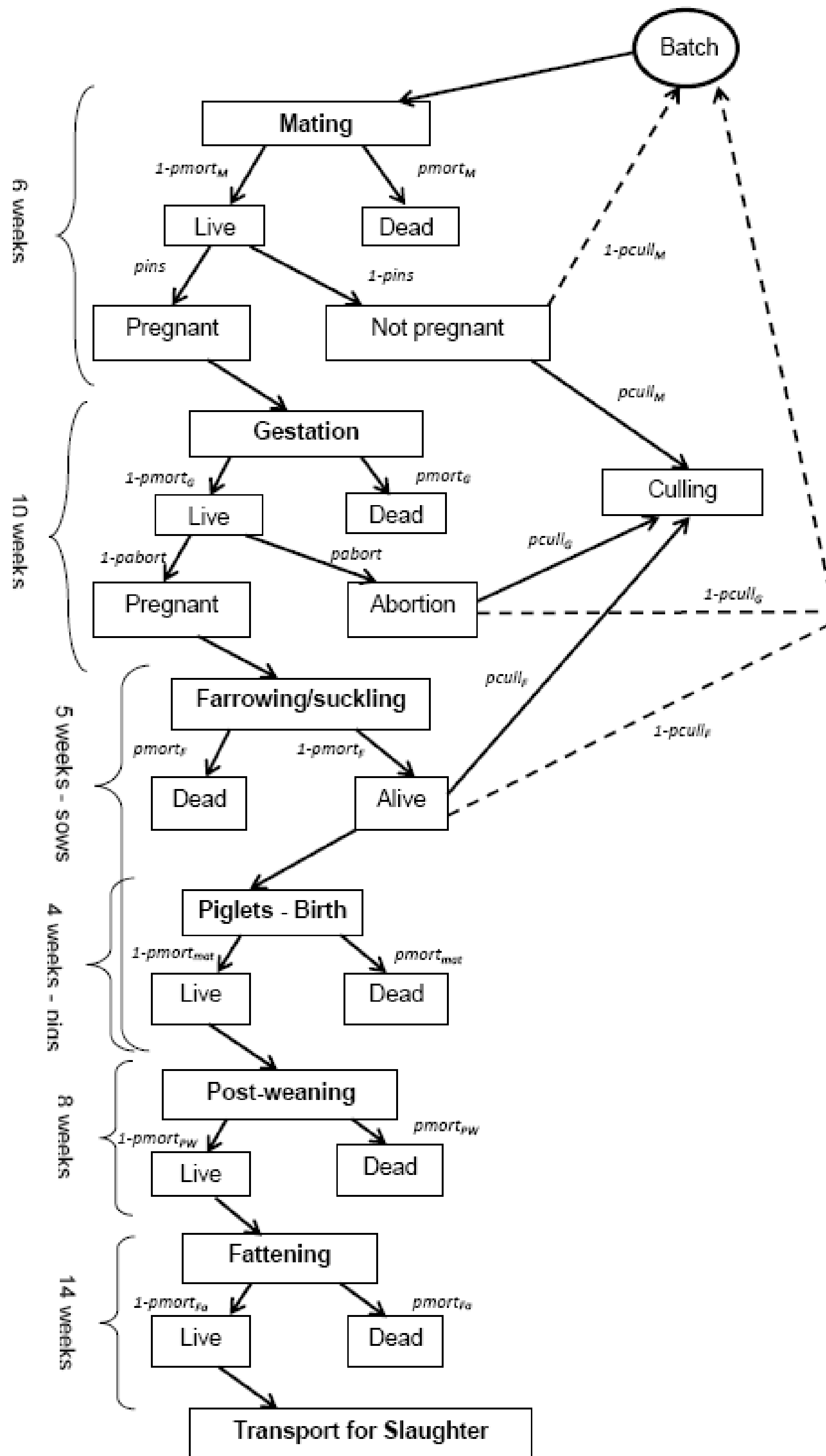
considered, from recruitment until culling/dying, and similarly for pigs, from birth till slaughter. The duration of the sow reproduction cycle depends of the weaning time of the piglets and this was fixed at 4 weeks (28 days). The pig growth period was fixed at 26 weeks (average age at slaughter in Portugal). The modelling unit was the batch (for both sows and pigs). This unit is useful because it simulates the interaction within sows and pigs which is important for infectious diseases, such as *Salmonella*, which are transmitted by close-contact between animals and by the batch environment, e.g. floors, feed, water, etc., so the exposure within batch is effectively uniform.

In the model, batches of sows were groups of sows (the same number per batch) that were mated at the same time. One week interval between two successive batch mating was assumed. The average Portuguese farrow-to-finish herd has approximately 264 sows. Therefore using the one week batch system during the year there were 22 batches entering mating (taking into account that each sow would have 2.5 litters per year) with 12 sows per batch. The model comprises two compartments: the reproductive phase (sow-compartment) and the pig growth (pig-compartment). The reproduction cycle was divided in three stages (mating period, gestation period and farrowing/suckling period) corresponding to the occupation of three different types of rooms. Each batch of sows was composed of gilts and sows although we did not differentiate between them in the model. Each batch of pigs was composed by the litters from the batch of sows. The pig growth was divided in three stages (sucking period, post-weaning period and fattening period) corresponding to the occupation of three different types of rooms. All animals simultaneously left the room they occupied except for those sows which aborted at gestation.

This production model describes the evolution of the number of animals within each batch, the time step was one week. The model has a stochastic element in the sense that it simulates the variability associated with biological processes such as mortality, culling, insemination failure, abortion and litter size. The model output is expressed in terms of probability distributions which in turn express: 1) the aforementioned variability, 2) the propagated uncertainty from having to estimate transmission parameters, and 3) the natural variability or randomness inherent in the behaviour of the disease (specifically, the binomial distribution was used to generate the number of animals in each production process).

The duration of the reproductive and growing stage, and therefore the duration in each room were kept fixed. The maximum capacity in each room was fixed for the maximum expected size of each batch and the pen capacity varied between batches depending on the number of animals per batch. Figure 1 shows a scheme of the production model.

Figure 1: Scheme of the production model



Legend: pmort is the mortality probability (M – mating, G – gestation, F – farrowing, mat – piglets, PW – post-weaning, Fa - fattening), pins is the probability of success by the artificial insemination, pcull is the culling probability (different for each room), pabort is the probability of abortion

Reproduction cycle of the sows

The sow-compartment comprises three stages of the reproduction cycle, which take place in three different rooms:

- The mating room where the sows remain from weaning until pregnancy testing (6 weeks);
- The gestation room where the sows remain almost until the end of pregnancy (10 weeks);
- The farrowing room, in which the sows are placed 1 week before farrowing and stay until the weaning of the piglets (5 weeks).

During the reproductive cycle, the sows were subject to the following processes: mortality, artificial insemination success, abortion, culling and gilt recruitment. The probability of mortality was "applied" at each time step and was constant in time. However, it varied between the stages of reproduction reflecting the variability that exists between the different stages. Each week the sows from a new batch entered the mating room and were inseminated. Individual pens were used at mating. At the end of the sixth week of mating, the artificial insemination success rate was used, to simulate pregnancy numbers. To represent the variability that exists between batches, the artificial insemination success rate was separately generated for each batch from a Weibull distribution. The square root of the simulated value for each batch corresponds to the probability of the artificial insemination success that was used to generate the number of sows that get pregnant and were moved to the next stage (gestation). The sows that failed to get pregnant were then either culled or moved to the following batch that has entered the mating room and be re-inseminated. The culling rate was different for mating, gestation and farrowing. In the gestation room the sows remained together in pens with a maximum of 4 sows per pen. Abortion could occur throughout the gestation period and the probability of abortion was kept constant for each week. After abortion, the sows were culled or moved to a following batch where they were going to be re-inseminated. In the farrowing room the sows were placed in individual pens (maternities). After weaning, some sows were voluntarily culled to allow renovation of the herd and the ones not culled entered in a new batch that was going to be re-inseminated in the mating room. To compensate for the mortality and culling in each batch, new gilts were introduced to ensure that the number of sows per batch was always 12.

Each batch of sows gave birth to a batch of pigs. The average litter size for each sow was drawn from a normal distribution of mean 10.45 and standard deviation of 0.87.

For mating the equations were:

$$Malive_t \sim Binomial(Malive_{t-1},(1-pmort_M))$$
$$Mpreg_{t=f} \sim Binomial(Malive_{t=f-1}, pins)$$
$$Mreturn \sim Binomial((1-Mpreg_{t=f}),(1-pcull_M)),\ f \text{ is the last week on mating room.}$$

For gestation the equations were:

$$Galive_t \sim Binomial(Galive_{t-1},(1-pmort_G))$$
$$Gpreg_t \sim Binomial(Galive_t,(1-pabort))$$
$$Greturn_t \sim Binomial((1-Gpreg_t),(1-pcull_G))$$

For farrowing (sows) the equations were:

$$Falive_t \sim Binomial(Falive_{t-1},(1-pmort_F))$$
$$Freturn \sim Binomial(Falive_{t=i},(1-pcull_F)),\ i \text{ is the last week on farrowing room.}$$

Where *pmort* is the mortality probability (different for each room), *pins* is the probability of success by the artificial insemination, *pcull* is the culling probability (different for each room), *pabort* is the probability of abortion, *alive* refers to the alive animals, *preg* refers to the pregnant sows, *return* refers to the sows that enter a new batch for mating (due to failure to get pregnant, abortion or after farrowing).

Pig Growth

The pig-compartment comprises three different stages, which take place in three different rooms:

- farrowing/maternity room (where they stay for 4 weeks until weaning),
- post-weaning room (where they stay 8 weeks), and
- fattening room (where they stay 14 weeks).

The mortality probability was different between rooms. The number of pigs that had died in each time step was simulated using a binomial distribution.

The maximum number of pigs per pen varies between rooms. In the farrowing room the number of piglets per pen was made similar taking in consideration the litters' size to simulate the mixing of piglets that occurs in the farms with the aim of improving the quality of the batch (to develop the milk production of gilts and also to allow piglets to have access to functional teats). In the post-weaning room the maximum number of pigs per pen was 20 (with a maximum number of 6 post-weaning pens per batch) and for fattening pens this value was reduced to 15 (with a maximum number of 12 fattening pens per batch). This means that the pigs were allocated to the pens in a way

that does not exceed that maximum number. These numbers were used taking into consideration the average Portuguese pen size per room, taken from an unpublished survey results [167].
For maternity (piglets) the equations were:

$$Npiglets_j \sim Normal(10.45, 0.87),\ j = \text{ number of sows per batch}$$
$$Pigalive_t \sim Binomial(Pigalive_{t-1}, (1 - pmort_{mat}))$$

For post-weaning (PW) and fattening (FA) the equations were:

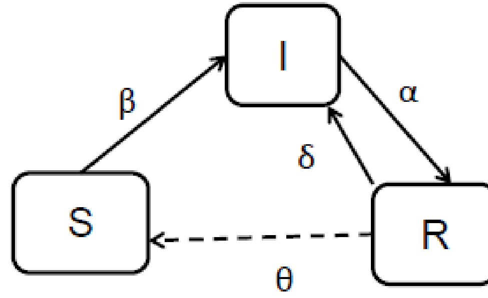
$$PWalive_t \sim Binomial(PWalive_{t-1}, (1 - pmort_{PW}))$$
$$FAalive_t \sim Binomial(FAalive_{t-1}, (1 - pmort_{FA}))$$

Where *Npiglets* refers to the born alive piglets per sow (this is drawn for each sow in each batch), *pmort* is the mortality probability (different for each room), and *alive* is the number of animals alive in each room at each time step.
The values of the production parameters are shown in more detail in Results Chapter - Manuscript 5: "Simulation model for *Salmonella* Typhimurium on a farrow-to-finish herd".

Infection model specification

The infection model was based on a Susceptible-Infectious-Resistant/Carrier model for *Salmonella* Typhimurium. Direct transmission between the pigs in a batch was assumed but also indirect transmission via contaminated floor, rodents, etc.
The transition steps considered between the states are shown in Figure 2.
For the sows, as they have a longer life span, it was assumed that they could experience all the states and transitions shown in Figure 2. For pigs, due to their short life span, it was assumed that they could not experience the transition from carrier to susceptible.
The mathematical model for these transitions and the transmission parameters used in this simulation model were described and estimated in Manuscript 4 ("Transmission parameters estimated for *Salmonella* Typhimurium in swine using susceptible-infectious-resistant models and a Bayesian approach"). The estimates were obtained using field data from a longitudinal study which followed infected cohorts [146], and were adjusted to the time step of one week (the time step in the field data was two weeks).

Figure 2: SIR model and its transition between states



Legend: S – susceptible, I – Infectious, R – resistant/carrier, β – transmission parameter for the transition from S to I, α – transmission parameter for the transition from I to R, δ – transmission parameter for the transition from R to I, θ – transmission parameters for the transition between R to S.

The binomial distribution was used to simulate the transition between susceptible and infectious state and from infectious to carrier state. For the transition between carrier state and infectious, and carrier state and susceptible, Poisson distributions were used. The transition between susceptible and infectious varied with time by parameterising it using a time-dependent cohort random effect. This random effect was used to capture the temporal structure of the spreading of infection within cohorts where the velocity of infection is dependent on the number of susceptible and infectious animals in the previous time step.
The equations used in the infection model were the following:

$$Inf_{j,t} \sim Binomial(S_{j,t-1}, p_{j,t})$$
$$\text{cloglog}(p_{j,t}) = \log(\beta) + \log(I_{j,t-1}) - \log(N_{j,t-1}) + r_{1j,t}$$
$$Rnew_{j,t} \sim Binomial(I_{j,t-1}, pr_{j,t})$$
$$\text{cloglog}(pr_{j,t}) = \log(\alpha) + r_{2j}$$
$$Inew_{j,t} \sim Poisson(\pi_{1j,t})$$
$$\log(\pi_{1j,t}) = \log(\nu) + \log(R_{j,t-1}) + r_{3j}$$
$$Snew_{j,t} \sim Poisson(\pi_{2j,t})$$
$$\log(\pi_{2j,t}) = \log(\theta) + \log(R_{j,t-1})$$
$$r_{kj} \sim Normal(0, \sigma_k^2), k = 2,3$$
$$r_{1j,t=1} \sim Normal(0, \sigma_1^2)$$
$$r_{1j,t} \sim Normal(r_{j,t-1}, \sigma_1^2)$$

Where *Inf* is the number of susceptible animals that became infectious, *S* is the number of susceptible animals at the beginning of each time interval, *p* is the probability of transition between susceptible to infectious, β is the transmission parameter between susceptible and infectious, I is the number of infectious animals at the beginning of the time interval, *N* is the total number of animals at the beginning of the time interval, r_{jt} is the cohort (j)-time (t) dependent random effects, *Rnew* is the number of animals that became carriers in each time step, *pr* is the probability for the transition between infectious and carriers, α is the transmission parameter from infectious to carriers, *Inew* is the number of carriers that became infectious in each time step, π_1 is the average number of carriers that became infectious, *v* is the transmission parameter between carrier to infectious, *R* is the number of carrier animals at the beginning of each time interval, *Snew* is the number of carrier animals that became susceptible (this step in the model only happens for sows), π_2 is the average number of carriers that became susceptible, θ is the transmission parameter between carrier to susceptible, and σ^2_k is the variance of the random effects.
So for each time step and for each pen, the number of sows in each state would be:

$$S_{jt} = S_{j,t-1} - Inf_{jt} + Snew_{jt}$$
$$I_{jt} = I_{j,t-1} + Inf_{j,t} - Rnew_{j,t} + Inew_{j,t}$$
$$R_{j,t} = R_{j,t-1} + Rnew_{j,t} - Inew_{j,t} - Snew_{j,t}$$

While for the pigs it would be:

$$S_{jt} = S_{j,t-1} - Inf_{jt}$$
$$I_{jt} = I_{j,t-1} + Inf_{j,t} - Rnew_{j,t} + Inew_{j,t}$$
$$R_{j,t} = R_{j,t-1} + Rnew_{j,t} - Inew_{j,t}$$

At the maternity stage, since the litter is in contact with the sow (mother), the sow health state was considered for the litter disease dynamics, but a protective factor (pf) for decreasing the transmission rate was taken into consideration due to the sow's milk protective antibodies. This protective factor was included in the model while the piglets were at the maternity stage for each batch. The study of Beloeil *et al.* (2003) [148] estimated the complete loss of maternal immunity between 61 and less than 80 days. We have assumed 70 days (10 weeks) to calculate the protective factor.
The transmission rate from carrier to susceptible was calculated, taking into consideration that pigs need around 68 days to clear *S.* Typhimurium from their organs after being infected ([40, 145] and another 42 days to lose the protective immunity against *Salmonella* [40, 145]: a total of 110 days – 15.7 weeks - to return to susceptible

state again. This value was used to calculate the transmission parameter between carrier and susceptible (1/15.7).

Data used

The transmission parameters used in this simulation model were the ones estimated in Manuscript 4 and have already been described.

For the production model, data from different sources was used: unpublished surveys, expert opinion and data collected from a commercial software company about breeding pigs.

The unpublished survey contained information from the region of Lisbon and Tagus Valley, which is the Portuguese Region with a high concentration of herds and pigs. The herds chosen to be sampled were randomly selected and the aim of the survey was to evaluate the biosecurity measures implemented at herd level and to associated them with *Salmonella* presence [167]. They have collected information regarding mortality, number of pigs per pen and number of animals per herd that were used in this simulation model.

Data collected from 200 commercial herds spread all over Portugal (2004 to 2006) was also made available to the authors by a software company. This data was used to estimate the average litter size, the piglet mortality, the duration of weaning, the number of breeding pigs per herd, and the insemination rate used in the simulation model. For the litter size and insemination rate data, the selected distribution was chosen from several known distributions (e.g. normal, lognormal, Weibull) using the lower maximum likelihood as criterion for chosen the ones with best fit. For the litter size the normal distribution was chosen and in the simulation model rounding was used to convert to integer value. For the insemination rate, the squared root of a Weibull distribution was chosen to simulate the probability of being pregnant at mating.

Expert opinion was used when no other source of information could be used to estimate values for the remaining parameters.

Model settings

The model was built and implemented in R (CRAN project, www. R-project.org). To ensure convergence of the final results (i.e. reduce sampling uncertainty) a long run (500,000 iterations) was conducted. Before running the model it was necessary to allocate an initial state to the sows/gilts, at mating in the first batch. The allocation was 50% of susceptible, 25% of infectious and 25% of carriers.

For each model run at the end of each room the following results were saved to be analysed: the proportion of sows alive in each room, the proportion of sows pregnant at

the end of mating and gestation rooms, and the proportion of sows/pigs in the infection states.

The proportion was selected in preference to the number (counts) because the total number of animals per room varied as function of the mortality and litter size (for pigs) making comparison between runs less straightforward. The proportions on the other hand can be directly compared.

The distributions of the results were tabled for the sows and plotted for the pigs. In the plots we have used the median as the central tendency measure because, unlike the mean, it is less affected by extreme values.

The validity of the model was assessed by comparing the predicted results from the model with observed (epidemiological) results in the population of interest: the Portuguese pig population. The results from the EU Baseline Survey on *Salmonella* Prevalence in Slaughter Pigs [36] and the EU Baseline Survey on the Prevalence of *Salmonella* in Breeding Pigs [37] were used as observed epidemiological results. The comparison was done by relating the magnitude of the predicted and observed value, and qualitatively assessing the degree of agreement/disagreement, as suggested by other authors [105, 145].

Sensitivity analysis

In the sensitivity analysis of the model, all the production parameters and infection parameters were perturbed, i.e. increased and decreased by 50%, and the results were compared with results from the unperturbed parameters. For the piglets' protective factor (pf) we ran several extra simulations (an increase of 250%, 500%, 750% and 1000% of the value, corresponding to the values of 0.25, 0.50, 0.75 and 1, respectively) due to the original value of the parameter being low. The lower the pf value is, the higher the protection against infection (e.g. a pf value of 0.25 means that the transmission parameter in piglets from susceptible to infectious will be reduced to 25% of it value, and in this way translating into a protective effect of 75%). For the infection state of replacement gilts, several combinations were tried. These combinations allowed to test the effect of high and low proportions for each infection state, considered more plausible by the authors.

For the transmission parameters from S to I (β), from I to R (α), from R to I (δ) and from R to S (θ) extra simulations were run to test the simultaneous effects of increasing or decreasing each parameter in different rooms. When the perturbation is applied to all the sows' rooms, we used the suffix "sows" (i.e. β sows, α sows, δ sows and θ sows). When the perturbation occurs in the maternity for sows and piglets at the same time (only for β parameter), we used the suffix "maternity" (β maternity). When the

perturbation is applied to all the pigs' rooms, we used "pigs" as a suffix (i.e. β pigs, α pigs, and δ pigs).

The perturbed and original parameters were compared by looking at the percentage change in their means – relative effect (RE):

$$RE = \frac{Mean.prop_{perturbed} - Mean.prop_{original}}{Mean.prop_{original}}$$

If RE is positive/negative the change in the parameter has increased/decreased the results (i.e. the means of the proportions). However if RE is equal to zero, the parameter change has no effect. The greater the magnitude of RE is, the greater the influence of the particular parameter.

To test if these REs were statistically significant it was used the fact that the results are in the form of samples from the distributions of the output, e.g. samples from the distribution of the proportion of sows pregnant at the end of mating. This is a direct consequence of the fact that the models used to estimate the transition parameters were Bayesian. Samples from the posterior distributions of the estimated transmission parameters from the Bayesian model were used in the simulated model to propagate the estimation uncertainty to the output from the simulated model, rather than ignoring it. Ultimately, it can be considered the output from the simulation model as samples from posterior distributions, e.g. 500,000 samples from the posterior distribution of the proportion of sows pregnant at the end of mating. It was the means of these distributions that were compared.

Therefore for each output of interest (e.g. proportion of sows pregnant at the end of mating) it was simulated a random sample from their posterior distribution. For instance, sample 1000 values from the posterior distribution of the proportion of sows pregnant at the end of mating, which means to randomly sampling 1000 values with replacement from the 500,000 samples of the output. If this procedure is done many times, e.g. 10,000 times, and calculated the mean each time, the final result is a sample of 10,000 values for the distribution of the mean. As the interest was to compare means from the original and perturbed parameter output, the differences in the mean samples was taken to obtain a sample from the distribution of the difference. e.g. suppose μ_{1i} and μ_{2i} for i=1,…,10,000 were samples from two means distributions, then $D_i = \mu_{1i} - \mu_{2i}$ was a sample from the distribution of their difference and it was tested whether zero was a likely value from this distribution. More formally, if zero was not included in the 95% credible interval of D_I, it can be argued that the value of the two means was significantly different.

The EU legislation (Council Directive 2008/120/EC of 18 December 2008 laying down minimum standards for the protection of pigs) banned the use of sow stalls starting in January 2013, which means that the individual pens have to be adapted (the pen size has to be increased). The model was changed to ensure that this welfare legislation could be met if sows were housed in groups at mating. We opted to change the pen used in the mating rooms the same as in gestation rooms (i.e. four pens per batch). This change was also included in the sensitivity analysis.

Sensitivity analysis was also used as a way of evaluating the uncertainty linked with some parameters, as for instance the piglet's protective factor and the infection state of replacement gilts.

Considering the results statistical significant, for each outcome the parameters which influenced it most (more than 5%), were plotted in a modified spider plot, where the percentage of change in the parameter was the x axis and the percentage of change in the outcome was the y axis.

When a variation from -50% to +50% is applied to the model the outcome varies changing positively or negatively as the parameter increases. The range of the change in the outcome due to the parameter change (from -50% to 50%) was tabled for the transmission parameters and piglets' protective factor in two separated tables, one for sows one for pigs. The criterion followed for the inclusion of each parameter in the table was to have at least a 5% variation either positive or negative. In the table a positive number means positive effect on the outcome when a variation from -50% to +50% is applied to the model (the outcome increases with the increase of the parameter); and a negative number means a negative effect on the outcome when a variation increment from -50% to +50% is applied to the model (the outcome decreases with the increase of the parameter).

Results Chapter - Manuscript 5: "Simulation model for *Salmonella* Typhimurium infection on a farrow-to-finish pig herd" - describes the Materials and Methods in more detail and shows the results of this work.

Chapter 3 – Results

Manuscript 1

“Risk factors for *Salmonella* sp. in pig lymph nodes in Portuguese abattoirs”

Revue Méd. Vét. 2013, 164: 4, 212-218

Risk factors for *Salmonella sp.* in pig lymph nodes in Portuguese abattoirs

C. CORREIA-GOMES[1,2*], D. MENDONÇA[1,2], M. VIEIRA-PINTO[3], J. NIZA-RIBEIRO[1,2]

[1]*Instituto de Ciências Biomédicas Abel Salazar (ICBAS-UP), Largo Prof. Abel Salazar, 2, 4099-003 Porto, PORTUGAL*
[2]*Instituto de Saúde Pública da Universidade do Porto (ISPUP), Rua das Taipas, 135, 4050-600 Porto, PORTUGAL*
[3]*Universidade de Trás-os-Montes e Alto Douro (UTAD), Veterinary Sciences Department, CECAV, Apartado 1013, 5001-801 Vila Real, PORTUGAL*

*Corresponding author: *carla.gomes@sruc.ac.uk*

SUMMARY

Salmonella is one of the major causes of food borne disease in the European Union (EU). Some of the human cases are related to pork products. An EU baseline survey to assess the *Salmonella* pork prevalence was performed. Mesenteric lymph nodes were cultured and *Salmonella sp.* isolates were serotyped. Data concerning the animal and the slaughterhouse was also collected. The aim of the present study was to search for potential risk factors to the presence of *Salmonella sp.* in pigs lymph nodes in Portugal and to search for differences in the risk profile between groups of serotypes. The data was analysed using a Bayesian approach to incorporate the hierarchical structure of the data (samples nested in slaughterhouses). Two models were analysed: a binomial (presence/absence of *Salmonella sp.*) and categorical model (absence of *Salmonella sp.*, serotype Typhimurium or serotype 1,4,[5],12:i:-, other serotypes). A total number of 659 samples were tested, belonging to 36 slaughterhouses. Around 23.7% of the samples were positive for *Salmonella sp.*. In the binomial model a significant association was found for region of the slaughterhouse - Lisbon and Tagus Valley Region with lower risk compared to the Centre Region (OR=0.36). In the categorical model a significant association for category Typhimurium or 1,4,[5],12:i:- was found for the variable hour when the sample was taken – afternoon with lower risk compared to morning (OR=0.20). The association found for the slaughterhouse region should be a matter of furthers studies to evaluate the hygiene practices in the slaughterhouses of that region.

Keywords: *Salmonella,* lymph nodes, risk associations

RESUME

Facteurs de risque de *Salmonella sp.* **dans les ganglions lymphatiques de porc dans les abattoirs portugais**

La salmonelle est une des principales causes de maladies d'origine alimentaire dans l'Union Européenne. Certains cas sont liés à des produits du porc. Une enquête a été faite pour évaluer la prévalence de *Salmonella* chez le porc au Portugal. Les ganglions lymphatiques des carcasses ont été cultivés et si *Salmonella* était présent, cela était sérotypée. Alors, des données concernant l'animal et l'abattoir étaient également recueillis. L'objectif de cette étude était de rechercher des facteurs de risque pour la présence de *Salmonella* ganglions lymphatiques des carcasses. Ces données ont été analysées en utilisant un modèle linéaire généralisé mixte pour prendre en incorporer la structure hiérarchique des données. Deux modèles ont été réalisés: un modèle binomial (présence/absence de *Salmonella sp.*) et un modèle catégorique (absence de *Salmonella sp*, sérotype Typhimurium ou sérotype 1,4,[5],12:i:-, autres sérotypes). Un total de 659 échantillons ont été testés, ceux-ci provenant de 36 abattoirs. La prévalence de *Salmonella sp.* est de 23.7%. Un risque significatif a été trouvé pour la région des abattoirs - Lisbonne et dans la Vallée du Tage (OR = 0,38) avec moins de risque par rapport à la région du Centre. Dans le cas du modèle catégorique, les résultats significatifs furent obtenus uniquement pour la catégorie Typhimurium ou 1,4,[5],12:i:- pour la variable le temps de collecte d'échantillons - l'après-midi avec moins de risque que le matin (OR=0.20). Les résultats obtenus devraient initier de prochaines études sur les conditions d'hygiène dans les abattoirs des régions les plus fortement touchées.

Mots-clés: *Salmonella,* ganglions lymphatiques, les associations des risques

INTRODUCTION

Salmonella is one of the major causes of food borne disease in the world. Pork products are related with some of the human cases. Because of the health impact of this agent the industrialized countries are engaged in controlling this agent. For that the European Union (EU) approved legislation (EU Regulation No 2160/2003) that imposed a reduction on the prevalence of this agent in food production animals, such as pigs. To set the target of this reduction for each country, at EU level, it was decided to carry out baseline surveys to estimate the prevalence of *Salmonella sp.* in some food production animals. The objective of the surveys was to obtain comparable data for all Member States through harmonized sampling schemes. In this context, a baseline survey in pigs at the slaughterhouse was done. Slaughterhouses are an important control stage for this agent as in some cases when there is a poor hygiene at lairage and during the slaughter process the initial contamination of the infected pigs can be disseminated through the slaughterline. The risk factors known at this stage are: poor hygiene and stress during the transport [3], hygiene and time at lairage [2, 13], hygiene of slaughter equipment and of the slaughter process [4, 7, 11], season [11] and duration of the slaughter [11]. This dataset refer to the Baseline Survey on *Salmonella* Prevalence in Slaughter Pigs in Portugal. The aims of the study were: 1) to search for potential risk factors for the presence of *Salmonella sp.* in the lymph nodes of pigs slaughtered in this country, and 2) to identify differences in the risk profile between groups of serotypes.

MATERIAL AND METHODS

SAMPLING FRAME AND SAMPLE COLLECTION

The objectives, the sampling frame, the diagnostic testing methods, as well as the collection and reporting of data, and the timelines of the Baseline Survey in Slaughter Pigs were specified in the Commission Decision 2006/668/EC, Annex I. The sampling frame was the list of the slaughterhouses that together accounted for 80% of the pigs slaughtered within the Member State. The samples in Portugal were collected between January and September 2007. The sampling size was estimated by the Portuguese Veterinary Authorities (PVA) based in the Commission Decision 2006/668/EC, which took in consideration an estimated prevalence of 50%, considered an infinite population, a significance level of $\alpha=0.05$ and 4% precision error. The minimum sample size for Portugal, according to this scheme, was 600 pigs, and an extra of 10% was taken to account for non-response. The number of pigs to sample was stratified by slaughterhouse and was proportional to slaughterhouse capacity. The sampling days for each slaughterhouse were selected at random. Written procedures were given to the local veterinary services to assure that sampling fulfilled the guidelines recommended by the Decision. Each sample was formed mainly by an aggregate of ileocaecal and sometimes jejunal lymph nodes to assure that it had at least 15 grams of lymph nodes. The collection of the lymph nodes was done in an aseptic way to avoid external contamination. The lymph node samples were sent to the laboratory for microbiological detection of *Salmonella* according to the procedure defined by Annex D of ISO 6579. Each *Salmonella* isolate was serotyped in the National Reference Laboratory for *Salmonella* according to Kaulfmann-White scheme.

DATA COLLECTION

Along with the sample collection, information concerning the pig and the slaughterhouse was also collected, to assess their potential influence to the presence of *Salmonella*. The variables collected were: transport of pigs from different herds to the slaughterhouse (yes or no); carcass approval for human consumption (totally versus partially); detection of lesions in the lymph nodes (yes or no); sample collection time; month the sample was collected; time since the animal arrived the slaughterhouse until it was killed; weight of the carcass; weight of the lymph node sample; region of the slaughterhouse and annual capacity of the slaughterhouse. Questions about hygiene at lairage and slaughter were not collected.

DATA ANALYSIS

Some quantitative variables were aggregated into categories, such as annual capacity of the slaughterhouse (less than 30.000 pigs slaughtered/year, between 30.000 and 100.000 pigs, and more than 100.000), sample collection time (8:01a.m to 12a.m, 12:01a.m. to 8p.m., and 8:01p.m to 8a.m), and time between arrival to slaughterhouse till slaughter of the animal (less than 12h, 12h to 24h, more than 24h). Descriptive statistics were calculated for the continuous variables that were not categorized. Other categorical variables were aggregated in few categories such as month when the sample was collected (January till March, April to June, and July till September).

The data has a "natural" multilevel structure; pigs which provide the lymph node samples (first level) were nested in slaughterhouses (second level). The data was analysed using a Bayesian hierarchical model. Monte Carlo Markov Chain (MCMC) was used for estimation and implemented in WinBUGS software (BUGS project, http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/), open source software.

The analysis consisted of two models with two different outcome variables: 1) a binomial model for the presence/absence of *Salmonella sp.*; 2) a categorical model for different groups of *Salmonella* serotypes. In this second model, besides the reference category "no *Salmonella*", the positive samples for *Salmonella* were divided in two groups: i) serotype Typhimurium or serotype 1,4,[5],12:i:-, ii) other *Salmonella* serotypes. These groups were formed because of the relevance of serotype Typhimurium to human cases [9]. Serotype 1,4,[5],12:i:- was added to Typhimurium group because they share similar characteristics in terms of genetic similarity, virulence and antimicrobial resistance [1]. The different serotypes were not analysed individually because of the low number of cases per serotype (Table 1). This approach intended to identify and explore differences in risk factors between the categories of serotypes and the "no *Salmonella*" category. In the binomial model a logit link function was used. In the categorical model it was used a logit conditional link function. The random effects were assessed for the slaughterhouse level. As not all slaughterhouses in the country were sampled having in the model a random slaughterhouse effect allows inferring information from the sample to all slaughterhouses population. The probability for each category of the categorical outcome is modelled using the same explanatory variables but different slope parameters to assess whether these variables affect each category in a different way. A preliminary univariable analysis to investigate the variables to be included in the multivariable model was performed. The variables with a $P<0.30$ were considered to enter into the multivariable model. A $\alpha=0.05$ was considered in the final model.

The model was implemented in WinBUGS and it ran long enough with sufficient burn-in to ensure convergence to the posterior distribution of the parameters. Convergence was assessed by visual means (inspection of time-series plots) but also more formally using the Raftery and Lewis diagnostic, and the Gelman-Rubin R-hat diagnostic [10, 12]. R-hat should be arbitrarily close to 1 for convergence. The chains were thinned by only collecting 1 in 10 consecutive samples and this eliminated autocorrelation in posterior samples

Serotype	Number of samples typed	Percentage of samples typed
S. Typhimurium	57	36.5
*S.*Rissen	22	14.1
S. 1,4,[5],12:i:-	17	10.9
*S.*Derby	17	10.9
S. Enteritidis	9	5.8
*S.*Give	7	4.5
*S.*Newport	7	4.5
*S.*Anatum	6	3.8
*S.*Agona	5	3.2
*S.*Bovismorbificans	2	1.3
*S.*Gaminara	1	0.64
*S.*Havana	1	0.64
*S.*Mbandaka	1	0.64
*S.*Ohio	1	0.64
*S.*Eboko	1	0.64
*S.*Panama	1	0.64
*S.*Infantis	1	0.64
Total	156	100

Table I: Number and percentage of samples typed for each serotype

(using the R-CODA package [5], in R). Mixing in the chains was assessed by comparing the MC (Markov Chain) error with the standard deviation, for each parameter. Ideally, the MC error should be less than 5% of the standard deviations for good mixing [6] and this was true for all parameters here. The presence of confounding was investigated by analyzing the correlation matrix of the joint posterior distribution for all model parameters but especially the slope parameters.

Priors for fixed effects were expressed as a normal distribution with zero mean and 10^2 variance. For random effects the prior was expressed as a normal distribution with mean zero and τ variance. The τ variance was expressed as a gamma distribution (0.5,0.05). As the median is not affected by the asymmetry of the distributions we used it as central tendency measure. The posterior median results were then converted to odds ratio (OR) to easy interpretation and also the 95% OR posterior credible interval (CrI) was calculated.

RESULTS

A total number of 659 samples from 36 slaughterhouses were tested. *Salmonella sp.* was isolated from 156 samples (23.7% of prevalence). Table 1 shows the results for each serotype. Most of the positive samples were identified as *Salmonella* Typhimurium followed by *Salmonella* Rissen.

After grouping the serotypes for the categorical model, the group serotype Typhimurium or serotype 1,4,[5],12:i:- had 74 samples (47.4% of the positive samples) and the group other serotypes had 82 samples (52.6% of positive samples).

The descriptive analysis of categorical variables is shown in Table 2. The dataset presented some missing cases as reported in Table 2. Table 3 shows the descriptive statistic for the continuous variables. It is not evident any difference between the groups of serotypes and the group with no *Salmonella*.

Results of the binomial analysis of the data (no *Salmonella* versus *Salmonella* presence) are shown in Table 4. A significant association with the presence of *Salmonella* was found for the region of the slaughterhouse: Lisbon and Tagus Valley Region with lower risk (OR=0.38) compared with the Centre Region, adjusted for the month of sample collection, sample collection time and annual capacity of the slaughterhouse.

For the categorical model the following variables were selected to enter in the multivariable model, based in the results of the univariable analyses: region of the slaughterhouse, slaughterhouse annual slaughter volume, month when the sample was collected, and sample collection time. For the final multivariable mode two variables were selected to enter: region of the slaughterhouse and sample collection time. Table 5 shows the final adjusted model results. In this model a significant association with the presence of serotype Typhimurium or serotype 1,4,[5],12:i:- (compared to "no *Salmonella*") was found for sample collection time: collecting the sample at afternoon had a lower risk (OR=0.2) compared to morning, and adjusted for the region of the herd. This result has a wide 95% credible interval (0.03-0.77).

DISCUSSION

The detection of *Salmonella sp.* in the lymph nodes of slaughter pigs is an indicator of the infection status of pigs to *Salmonella sp.*. To define a reduction target for this agent and consequently a control programme it is important to have information concerning the country prevalence and risk factors present, hence justifying the present study.

Variables		Samples positive to *Salmonella sp.*			Negative samples
		Samples positive to S. Typhimurium or serotype 1,4,[5],12:i:-	Samples positive to other serotypes	Total positive	
Transport of pigs from different herds to the slaughterhouse					
	No	58	67	125	392
	Yes	16	14	30	104
	Missing cases	1	1	2	8
Lesions in the lymph nodes					
	No	64	69	133	434
	Yes	11	12	23	70
	Missing cases		1	1	
Partial rejection of the carcass					
	No	74	82	156	500
	Yes	1		1	4
Hours since the animal arrived the slaughterhouse and was killed					
	< 12h	14	23	37	119
	12-24h	52	50	102	320
	>24h	8	9	17	61
	Missing cases	1		1	4
Month when the sample was collected (2007)					
	January to March	19	25	44	136
	April to June	21	23	44	199
	July to September	35	34	69	169
Sample collection time					
	8:01a.m. to 12a.m.	61	68	129	397
	12:01 to 8p.m.	2	6	8	42
	8:01p.m. to 8a.m.	12	8	20	65
Region of the slaughterhouse					
	Centre	12	12	24	45
	North	23	23	26	166
	Alentejo	2	2	4	11
	Lisbon and Tagus Valley	38	45	83	282
Slaughterhouse annual slaughter volume					
	< 30 000 carcass	2	2	4	24
	30 000 – 100 000	29	39	68	219
	> 100 000	44	41	85	261

TABLE II: Descriptive of explanatory variables concerning positive samples to *Salmonella* (S. Typhiumurium or serotype 1,4,[5],12:i:- versus other serotypes) and negative samples.

Variable	Presence of *Salmonella*	Mean	Minimum	Percentile 25	Median	Percentile 75	Maximum
Carcass weight (Kg)	No	80.5	57.6	74.2	80	85	167.7
	All samples positive	80.8	57	74	79.1	84.9	169.6
	Typhimurium or 1,4,[5],12:i:-	78.6	57	73.4	77.5	82	108.3
	Other serotype	82.9	64	75	80.3	87.1	169.6
Lymph nodes sample weight (g)	No	17	15	15	16	18	32.1
	All samples positive	17	14	15	16	18	28.4
	Typhimurium or 1,4,[5],12:i:-	17.4	14	15.1	16.4	18	28.4
	Other serotype	16.7	15	15	15.7	18	22.6

TABLE III: Descriptive measures of continuous variables for Salmonella presence by groups of serotypes

To improve the randomization and consistency of the samples collected the national authorities organized a training session for all the involved parties in the baseline study, before the beginning of the study. Also this data was validated by EFSA [8].

In this study we used hierarchical models that are naturally handled in the Bayesian framework because of the conditional independence assumed between each level in the hierarchy (lymph node samples in the first level and slaughterhouses in the second level). In conjunction with WinBUGS, freely available software, the methodology

Variable	Univariable		Multivariable adjusted model		
	Posterior median	Posterior SD	Posterior median	Posterior SD	OR (95%CrI)
Transport of pigs from different herds to the slaughterhouse					
No	0.0				
Yes	<-0.01	0.25			
Days in transport	-0.31	0.34			
Carcass weight					
Lesions in the lymph nodes					
No	0.0				
Yes	-0.20	0.33			
Partial rejection of the carcass					
No	0.0				
Yes	-0.44	1.22			
Hours since the animal arrive the slaughterhouse and is killed					
< 12h	0.0				
12-24h	0.12	0.24			
>24h	-0.03	0.36			
Month when the sample was collected* (in 2007)					
January to March	0.0		0.0		1.00
April to June	-0.36	0.25	-0.37	0.25	0.69 (0.42-1.12)
July to September	0.23	0.24	0.22	0.24	1.24 (0.77-1.99)
Sample collection time*					
8:01 a.m. to 12a.m.	0.0		0.0		1.00
12:01 to 8p.m.	-0.57	0.41	-0.60	0.43	0.55 (0.22-1.22)
8:01p.m. to 8a.m.	0.02	0.34	-0.27	0.37	0.76 (0.36-1.54)
Region of the slaughterhouse*					
Centre	0.0		0,0		1.00
North	-0.53	0.41	-0.72	0.42	0.48 (0.22-1.13)
Alentejo	-0.31	0.82	-0.77	0.83	0.46 (0.09-2.21)
Lisbon and Tagus Valley	-0.63	0.40	-1.01	0.41	0.36 (0.16-0.80)
Slaughterhouse annual slaughter volume*					
< 30 000 carcass	0.0		0.0		1.00
30 000 – 100 000	0.56	0.64	0.79	0.68	2.04 (0.66-9.64)
> 100 000	0.67	0.66	0.98	0.71	2.67 (0.76-12.34)

* variables that were selected to enter in the final multivariable mode

Table IV: Binomial multilevel model univariable and multivariable results showing posterior median, posterior standard deviation (SD), odds ratio (OR) and 95% credible interval (CrI).

presented in the paper provides a general modelling tool which allows to incorporate expert knowledge in the specification of the priors or to restrict the priors taking into account the lack of information in the response variable.

The results of the Baseline Survey to *Salmonella* in Slaughter Pigs in Portugal showed a high prevalence of *Salmonella sp.* (23.7%) in the country. The authors decided to do two different models (a binomial and categorical model) to explore and identify differences in risk factors for the infection of carcass between *Salmonella* serotypes. Control programmes will be implemented to control all serotypes of *Salmonella sp.*, but it is possible to have different risk profiles. The knowledge of differences in risk may help to improve the economics and efficiency of the control programmes. As the data has a relative small number of samples was not possible to perform an analysis for each one of the serotype found. Then it was decided to create three major groups: no *Salmonella sp.* (reference group), serotype Typhimurium or serotype 1,4,[5],12:i:-, and other serotypes. In the binomial model the possible risk association found was region of the slaughterhouse: Centre Region had a higher risk (OR=2.6) of presence of *Salmonella sp.* than in the Lisbon and Tagus Valley Region, when adjusted for the others variables in the model. This result should be a matter of further studies to evaluate if this association is due to slaughterhouse management practices, as is suggested by other studies [4,7,11], or due to infected herds.

In the categorical model the variable found to be significant at the multivariable model was the sample collection time (afternoon compared to morning) for the category of serotype Typhimurium or serotype 1,4,[5],12:i:- as a protective association, although it had a wide credible interval. A possible cause for this wide credible interval is that we have a relative

Variable	Multivariable model					
	Typhimurium or 1,4,[5],12:i:-			Other serotype		
	Posterior median	Posterior SD	OR (95%CrI)	Posterior median	Posterior SD	OR (95%CrI)
Sample collection time						
8:01 a.m. to 12a.m.	0.0		1.00	0.0		1.00
12:01 to 8p.m.	-1.63	0.86	0.20 (0.03-0.77)	-0.36	0.48	0.70 (0.26-1.69)
8:01p.m. to 8a.m.	0.05	0.44	1.05 (0.42-2.44)	-0.51	0.52	0.60 (0.20-1.55)
Region of the slaughterhouse						
Centre	0.0		1.00	0.0		1.00
North	-0.67	0.57	0.51 (0.17-1.61)	-0.68	0.51	0.51 (0.19-1.40)
Alentejo	-0.52	1.14	0.60 (0.05-5.19)	-0.68	1.09	0.50 (0.05-3.66)
Lisbon and Tagus Valley	-0.87	0.54	0.42 (0.15-1.25)	-0.73	0.49	0.48 (0.19-1.28)

Table V: Final categorical multilevel multivariable model results showing posterior median, posterior standard deviation (SD), odds ratio (OR) and 95% credible interval (95%CrI) for category 1 (Typhimurium or 1,4,[5],12:i:-) and for category 2 (other serotype).

small number of samples, although it is considered a protective factor. A biological explanation for this association could be that the animals slaughtered in the afternoon have spent less time at all in the lairage because they enter the slaughterhouse early morning to be culled in that same day . Because the transmission of this type of *Salmonella* is strongly associated to transmission between live animals, the reduction in the contact between pig from different origins at the lairage could play an important role in explaining this finding. This data are representative of Portuguese slaughter pigs and contribute with valuable information for assessing risk factors. However the data collected did not evaluate the hygiene of the transport and lairage, known risk factors in various studies which could enlighten the slaughterhouse risk factors in this country.

CONCLUSION

These results show an association between the region of the slaughterhouse and the lymph node *Salmonella sp.* positivity, which could be explained by different management practices in the slaughterhouses. As this study did not evaluate hygiene measures and management practices at each slaughterhouse it is necessary to perform such studies to enlighten these results. Also the results show a protective association for sample collection time for the group Typhimurium. The statistical methodology used in the study proved to be useful when we have small dataset and a multilevel structure of data, and it could be used in similar studies.

Acknowledgments

We would like to thank FCT for the PhD scholarship (SFRH/BD/40932/2007) and the Portuguese official veterinary authority (DGV) for the data.

REFERENCES

1. (BIOHAZ) E.P.o.B.H.: Scientific Opinion on monitoring and assessment of the public health risk of "*Salmonella* Typhimurium-like" strains. EFSA Journal, 2010, 8, 48. DOI: 10.2903/j.efsa.2010.1826.
2. BELOEIL P.A., CHAUVIN C., PROUX K., MADEC F., FRAVALO P., ALIOUM A.: Impact of the *Salmonella* status of market-age pigs and the pre-slaughter process on *Salmonella* caecal contamination at slaughter. *Vet Res*, 2004 35, 13-30.
3. BERENDS B.R., URLINGS H.A., SNIJDERS J.M., VAN KNAPEN F.: Identification and quantification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs. *Int J Food Microbiol*, 1996, 30, 37-53.
4. BERENDS B.R., VAN KNAPEN F., SNIJDERS J.M.: Suggestions for the construction, analysis and use of descriptive epidemiological models for the modernization of meat inspection. I*nt J Food Microbiol*, 1996, 30, 27-36.
5. BEST N.G., COWLES M.K., VINES S.K.: CODA Manual Version 0.30, MRC Biostatistics Unit, Cambridge, UK, 1995.
6. CONGDON P.D.: Applied Bayesian hierarchical methods, 565 pages, Chapman&Hall/ CRC, US. 2010.
7. DELHALLE L., DE SADELEER L., BOLLAERTS K., FARNIR F., SAEGERMAN C., KORSAK N., DEWULF J., DE ZUTTER L., DAUBE G.: Risk factors for *Salmonella* and hygiene indicators in the 10 largest Belgian pig slaughterhouses. J Food Prot, 2008, 71, 1320-9.
8. EFSA: Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline survey on the prevalence of *Salmonella* in slaughter pigs, Part B. The EFSA Journal, 2008, 206, 111.
9. EFSA.: The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and

Food-borne Outbreaks in 2009. EFSA Journal, 2011, 9, 2090. DOI: 10.2903/j.efsa.2011.2090.

10. GELMAN A., RUBIN D.B.: Inference from iterative simulation using multiple sequences. *Stat. Sci.*, 1992, 7, 457–511.
11. HALD T., WINGSTRAND A., SWANENBURG M., VON ALTROCK A., THORBERG B.M.: The occurrence and epidemiology of *Salmonella* in European pig slaughterhouses. *Epidemiol Infect*, 2003, 131, 1187-203.
12. KOSTOULAS P., LEONTIDES L., ENØE C., BILLINIS C., FLOROU M., SOFIA M.: Bayesian estimation of sensitivity and specificity of serum ELISA and faecal culture for diagnosis of paratuberculosis in Greek dairy sheep and goats. Preventive Veterinary Medicine, 2006, 76, 56-73. DOI: 10.1016/j.prevetmed.2006.04.006.
13. ROSTAGNO M.H., HURD H.S., MCKEAN J.D., ZIEMER C.J., GAILEY J.K., LEITE R.C.: Preslaughter holding environment in pork plants is highly contaminated with *Salmonella* enterica. *Appl Environ Microbiol*, 2003, 69, 4489-94.

Manuscript 2

“Risk factors for *Salmonella* spp in Portuguese breeding pigs using a multilevel analysis”

Preventive Veterinary Medicine 2013, 108, 159– 166

Preventive Veterinary Medicine 108 (2013) 159–166

Contents lists available at SciVerse ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Risk factors for *Salmonella spp* in Portuguese breeding pigs using a multilevel analysis

C. Correia-Gomes[a,b,*], D. Mendonça[a,b], M. Vieira-Pinto[c], J. Niza-Ribeiro[a,b]

[a] *Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Largo Prof. Abel Salazar, 2, 4099-003 Porto, Portugal*
[b] *Instituto de Saúde Pública da Universidade do Porto (ISPUP), Universidade do Porto, Rua das Taipas, 135, 4050-600 Porto, Portugal*
[c] *Departamento de Ciências Veterinárias, Universidade de Trás-os-Montes e Alto Douro (UTAD), Edif. Clínicas Veterinária, Quinta dos Prados, Apartado 1013, 5001-801 Vila Real, Portugal*

ARTICLE INFO

Article history:
Received 30 September 2011
Received in revised form 24 July 2012
Accepted 29 July 2012

Keywords:
Salmonella spp
Multilevel analysis
Risk factors
Pigs
Logistic regression

ABSTRACT

Salmonella is the second most frequent cause of foodborne illness in the European Union (EU), so EU enforced legislation to achieve a reduction in *Salmonella* prevalence in the swine sector. To set the reduction target each country carried out a baseline survey to estimate *Salmonella* prevalence. The aim of our study was to identify risk factors for the presence of *Salmonella* in breeding pigs based on the data of the Baseline Study for *Salmonella* in Breeding Pigs in Portugal. In total, 1670 pen fecal samples from 167 herds were tested by culture and 170 samples tested positive. Along with the collection of the samples a survey was applied to collect information about the herd management and potential risk factors. Multilevel analysis was applied to the data using generalized linear mixed models and a logit link function. The outcome variable was the presence/absence of *Salmonella* in the pen fecal samples. The first level was assigned to the pen fecal samples and the second level to the herds. The results showed significant associations between *Salmonella* occurrence and the factors ($p < 0.05$): maternity pens versus mating pens (OR = 0.39, 95%CI: 0.24–0.63), feed from external or mixed source versus home source (OR = 2.81, 95%CI: 1.19–6.61), more than 10 animals per pen versus 10 animals per pen (OR = 2.02, 95%CI: 1.19–3.43), North Region versus Alentejo Region (OR = 3.86, 95%CI: 1.08–13.75), rodents control (OR = 0.23, 95%CI: 0.090–0.59), more than 90% of boars homebred or no boars versus more than 90% of boars from an external source (OR = 0.54, 95%CI: 0.3–0.97), semen from another herd versus semen from insemination centers (OR = 4.47, 95%CI: 1.38–14.43) and herds with a size of 170 or more sows (OR = 1.82, 95%CI: 1.04–3.19). This study offers very relevant information for both the Portuguese veterinary authorities and the pig farmers currently developing control programmes for *Salmonella*. This is the first study providing evidence for semen and boars source as risk factors for *Salmonella* in breeding pigs.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Salmonella has been reported as the second most frequent cause of foodborne illness in the European Union (EU) in the past ten years (EFSA, 2010). The contribution of pork products to the total burden of human salmonellosis cases varies between countries but it is estimated to be around 10% (Pires et al., 2010). The EU Regulation (EU Regulation No 2160/2003) imposes to the Member States (MS) implementation of a control programme to reduce the prevalence in food production species including pigs. To set the reduction target each MS carried out baseline surveys to estimate the *Salmonella spp.* prevalence in some food production animals. The objective of the surveys was to obtain comparable data for all MS through harmonized sampling and testing schemes. In pigs the baseline study

* Corresponding author at: Epidemiology Research Unit - SAC, Drummondhill, Stratherrick Road, Inverness, IV2 4JZ, UK. Tel.: +441463 717832.
E-mail addresses: cgomes@icbas.up.pt, carlasofiagomes@gmail.com (C. Correia-Gomes).

0167-5877/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.prevetmed.2012.07.013

was done at abattoir level (collection of lymph nodes of pigs slaughtered) and at herd level (collection of pen fecal samples of breeding pigs). These cross-sectional studies also collected information regarding herd management practices and potential risk factors linked to this agent. After the specification of the reduction target each MS will have the responsibility to establish an effective national control programme adjusted for the country-specific characteristics, such as the risk factors, the disease prevalence and the financial implications for stakeholders.

It was expected that the baseline surveys supplied enough data to enable the identification and quantification of potential risk factors to be used in the development of programmes and procedures that reduces *Salmonella* shedding in pig herds economically and effectively. It is important that this information is available before *Salmonella* reduction programmes are implemented at the herd level to enable farmers to make informed choices, enhance public health and avoid unnecessary costs (Bahnson et al., 2006).

Some of the known risk factors already identified were linked to: (1) biosecurity measures (Baptista et al., 2010) such as potential biological vectors (as rodents) (Letellier et al., 1999; Meerburg and Kijlstra, 2007; Skov et al., 2008), hygiene of hands, equipment and facilities (Lo Fo Wong et al., 2004), purchase of animals from different suppliers (Lo Fo Wong et al., 2004), (2) herd management, such as herd size (Poljak et al., 2008), batch production system (Funk and Gebreyes, 2004), housing – type of floor (partial slatted floor) (Nollet et al., 2004; Rossel et al., 2006), type of pen separations (Lo Fo Wong et al., 2004), (3) feeding practices such as dry feed (Bahnson et al., 2006), purchase of feed (Benschop et al., 2008), adding organic acids to feed (Funk and Gebreyes, 2004), (4) the use of antibiotics (Beloeil et al., 2007; Funk et al., 2007), parasite infestations (van der Wolf et al., 2001; Beloeil et al., 2004), and health status of the herd (Funk and Gebreyes, 2004) among others (Fosse et al., 2009).

The data used in the present study were collected as part of the Baseline Survey on the Prevalence of *Salmonella* in Breeding Pigs in Portugal. The aim of the study was to identify risk factors for the presence of *Salmonella* in herds with breeding pigs.

2. Materials and methods

2.1. Sampling and samples collection and analysis

The data used in this study were transferred to the authors by the Portuguese Veterinary Authorities (PVA) and they are derived from the baseline study for *Salmonella* in Breeding Pigs in Portugal. This study was carried out by the PVA in the context of the Commission Decision 2008/55/EC. The authors were not involved in the baseline study and the data collection methodology described below is of the entire responsibility of the PVA.

The sampling frame, the diagnostic testing methods, the sample collection procedures and the timelines of the baseline study for *Salmonella* in Breeding Pigs were specified in the Commission Decision 2008/55/EC.

The target population was the holdings constituting at least 80% of the breeding pig population in the MS.

In Portugal, the sampling frame was organized by the PVA. These holdings were stratified by the Regions of the National Veterinary Services structure. There are currently five regions NUT II based in the Continental Portugal. In the sampling frame there were 4522 herds with a total of 1,827,533 pigs, of which 204,584 were breeding pigs. In each region, herds with 50 or more breeding pigs were included. The sampling frame used in this study contained 87% of the total number of pigs reported in 2007 in Portugal. The required sample size was estimated based on an expected prevalence of 50%, a desired confidence level of 95%, an accuracy of 7.5%, then applied a finite population correction factor, with an increase of 10% for each group (breeding and production holdings), to account for non-response, as specified by the Commission Decision 2008/55/EC Annex I. The sample size used by PVA was 174 swine herds. The choice of the herds to sample was random and proportional to the region, to take in consideration the difference in the number of herds in each region. The samples were collected between November 2008 and January 2009 by the herd veterinarian. Pooled fecal samples from 10 pens were collected in each herd. The pens were proportionally allocated to represent the number of breeding pigs in the different stages of production. The collection and composition of each pool was performed following the guidelines outlined in the Commission Decision 2008/55/EC. At least 10 individual breeding pigs had to contribute to one fecal pool. This procedure was estimated to provide 95% certainty of detecting at least one positive sample in a herd, if the true prevalence of infected pigs in the population was 10% (Anonymous, 2007). Before the sample collection the PVA conducted clarification meetings with all herd veterinarians involved in the study. The fecal samples were sent to the laboratory for microbiological detection of *Salmonella* according to the procedure defined by Annex D of ISO 6579. Each *Salmonella* isolate was serotyped in the National Reference Laboratory for *Salmonella* according to Kaulfmann-White scheme. The sensitivity of this culture method is around 80% and the specificity is 100% (Hoorfar and Mortensen, 2000; Arnold et al., 2005).

2.2. Data collection

Information about herd management and potential risk factors was collected using a questionnaire along with the collection of the fecal samples.

At herd level, the variables of the following theme categories were included: identification of the region of origin, the categorization of the holding production type (three variables), quantity and types of animals present (five variables), biosecurity measures and animal purchasing policy (eight variables). For detailed description of these variables see Table 1.

At pen level, the variables intended to characterise the type of housing (two variables), the number and type of animals in the pen (four variables), the clinical heath of pen (two variables); the floor type, the type of sanitary measures adopted in the holding before new breeding pigs

Table 1
Herd variables distribution and univariable analyses to *Salmonella spp.* using data from the Baseline Survey on the Prevalence of *Salmonella* in Breeding Pigs in Portugal.

Variables	Herds with ≥1 positive pen sample	Herds with no positive pen sample	Univariable analyses	
			OR estimate	*p*-Value
System type				
Outdoor	1	2	–	
Indoor	64	75	2.72	0.41
Missing cases	11	14		
Herd type				
Selection and multiplication unit	15	18	–	
Production unit	61	72	0.87	0.68
Region of the herd				
Alentejo	11	14	–	
Centre	17	15	1.63	0.31
Lisbon and Tagus Valley	42	58	0.96	0.93
North	6	4	2.72	0.11
Production type				
Farrow-to-weaners	12	7	–	
Farrow-to-growers	10	17	0.40	0.20
Farrow-to-finish	39	49	0.58	0.27
Missing cases	15	18		
Number of boars				
<3	31	45	–	
≥3	45	46	1.80	0.03
Number of sows				
<170	33	50	–	
≥170	43	41	1.53	0.13
Number of gilts				
<22	32	46	–	
≥22	44	45	1.45	0.18
Total number of breeding pigs				
<203	33	50	–	
≥203	43	41	1.53	0.13
Number of finishers pigs/herd				
<100	8	19	–	
≥100	67	71	1.98	0.09
Missing cases	1	1		
Management of breeding sows				
More than 90% purchased	25	28	–	
>90% homebred	38	54	0.83	0.55
10–90% homebred	13	9	1.62	0.27
Management of breeding boars				
More than 90% purchased	42	28	–	
Without boars or >90% homebred	26	53	0.40	<0.01
10–90% purchased or homebred	8	10	0.77	0.55
Source of replacement pigs				
All homebred	23	41	–	
Others sources	52	50	1.56	0.13
Missing cases	1	0		
Source of semen				
Insemination centre – CI	18	34	–	
Own boar + CI	40	43	2.09	0.02
Boar from another herd	14	11	5.28	<0.01
Missing cases	4	3		
Good herd replacement policy				
Yes	60	60	–	
No	16	31	1.76	0.08
Rodents control				
No	9	17	–	
Yes	67	74	0.49	0.08
Control of birds				
No	20	15	–	
Yes	56	76	1.45	0.27
Use of foot bath				
No	22	31	–	
Yes	54	60	0.77	0.38
Clothes for exclusive use in the herd				
Yes	74	85	–	
No	2	6	0.35	0.18
Good biosecurity measures				
Yes	34	40	–	
No	42	51	0.86	0.60

Table 2
Pen variable distribution to *Salmonella spp.* and univariable analyses in pen fecal samples using data from the Baseline Survey on the Prevalence of *Salmonella* in Breeding Pigs in Portugal.

Variable	Positive pen samples	Negative pen samples	Univariable analyses	
			OR estimate	*p*-Value
Number of animals per pen				
=10	128	1284	–	
>10	42	216	2.60	<0.01
The pen has direct access to outside				
No	122	1146	–	
Yes	48	354	1.48	0.15
Individual pen				
No	29	306	–	
Yes	139	1194	1.05	0.80
Missing cases	2	0		
Diarrhoea in the last 3 months				
No	163	1445	–	
Yes	3	33	1.44	0.57
Missing cases	4	22		
Age of the breeding sows				
Only gilts or mixed age	111	874	–	
Without gilts	59	626	0.73	0.11
Sex of the breeding pigs				
Only sows	158	1430	–	
Boars or/and sows	12	70	2.13	0.04
Breeding sector room				
Mating	31	219	–	
Gestation	88	789	0.79	0.20
Mixture of animals of different sectors	15	58	1.68	0.22
Maternity	29	390	0.43	<0.01
Replacement breeders	7	44	0.83	0.64
Floor				
Fully slatted	14	137	–	
Others	146	1353	0.96	0.89
Sanitary gap before new breeders in the pen				
No	107	874	–	
Yes	63	626	1.77	<0.01
Feed				
Dry pellet	34	229	–	
Dry non pellet	133	1230	0.87	0.72
Wet	3	41	0.32	0.23
Source of feed				
Exclusively own	16	199	–	
Purchased + mixture	154	1301	1.52	0.33
Potential *Salmonella* control substances added to water				
No	149	1291	–	
Yes	21	209	1.08	0.84
Use of antibiotics in the last 4 weeks in breeders				
No	148	1229	–	
Yes	22	271	0.50	0.01
Approach used to collect the pooled sample				
Individual pinches	158	1379	–	
Swab	12	121	0.49	0.03

entered the pen were also characterized along with feeding management policy (three variables). The method used to collect the fecal samples, swab or individual pinches, was also recorded in the questionnaire. For detailed description of these variables see Table 2.

2.3. Statistical analysis

To perform the present study the authors created a database. After entering the data in the database, the variables and their categories were recoded or aggregated to fewer categories as necessary to avoid sparse data problems, and two new binary variables were created: Good herd replacement policy and Good biosecurity measures.

The variable “Good herd replacement policy” groups the questions about management and source of replacement breeding pigs; it was coded as ‘Yes’ if more than 90% of the breeding sows and boars were homebred or without boars, and if the semen did not come from another herd, and as ‘No’ otherwise. The variable “Good biosecurity measures” groups the questions about biosecurity measures was coded as ‘Yes’ for herds which controlled rodents and birds access to barns, had a foot bath and had clothes exclusively for use in the herd, and as ‘No’ otherwise. Tables 1 and 2 summarise the variables.

For continuous variables basic description statistics including mean, median and percentiles were derived (Table 3). These results were used to give information on how to categorise the continuous variables.

Table 3
Descriptive measures of continuous variables for the presence of *Salmonella spp* in pen fecal samples using data from the Baseline Survey on the Prevalence of *Salmonella* in Breeding Pigs in Portugal.

Variable	Presence of *Salmonella*	Mean	Minimum	Percentile 25	Median	Percentile 75	Maximum
Number of boars	Yes	4.4	0	2	3	5	28
	No	3.5	0	2	3	4	18
Number of sows	Yes	245.2	8	100	200	325	1077
	No	210.9	35	90	136	250	1074
Number of gilts	Yes	35.6	0	15	25.5	40	187
	No	32.7	0	10	21	38	300
Number of reproductive pigs	Yes	285.2	43	130	224.5	370	1186
	No	248.2	41	103	182	293	1214
Number of animals in the pen	Yes	12.8	10	10	10	10	90
	No	11.5	10	10	10	10	130

To identify the risk factors for the presence of *Salmonella* in breeding pigs, the response variable was the presence of *Salmonella* in each fecal sample and it was classified as positive when *Salmonella* was detected and negative otherwise. As the data follow a multilevel structure, pen fecal samples (first level) nested within swine herds (second level), a two level hierarchical logistic regression model was fitted using the framework of generalized linear mixed model (GLMM) methods implemented in the glmmPQL procedure of package MASS (Venables and Ripley, 2002) of R free software (CRAN project, www.R-project.org). The fixed effects were estimated by a second order penalized quasi-likelihood (PQL) using the Breslow and Clayton's algorithm (Breslow and Clayton, 1993). The algorithm iterates between a series of iterated weighted least squares iterations to update the fixed effects and a single Fisher scoring iteration to update the standard deviation of the random effects.

The data were modeled in the following way:

$$Y = \begin{cases} 0 & (\text{no Salmonella}) \\ 1 & (\text{Salmonella}) \end{cases}$$

where Y is the response variable.

$$Pr(Y) = p_{ih}, i = 1, \ldots, 1670 \text{ and } h = 1, \ldots, 167$$

The generic model used:

$$\begin{aligned} \log it(p_{ih}) = a &+ \beta_k \text{herd variables}_h + \beta_k \text{pen variables}_{hi} \\ &+ \beta_k \text{herd variables}_h * \text{herd variables}_h \\ &+ \beta_k \text{pen variables}_{hi} * \text{pen variables}_{hi} \\ &+ \beta_k \text{herd variables}_h * \text{pen variables}_{hi} + b_h \end{aligned}$$

When modeling dichotomous data the lowest-level residual variance is not in the model equation because it is part of the specification of the error distribution (Hox, 2002; Goldstein, 2011). The second level random effect is given by $b_h \sim N(0, \sigma^2)$ where σ^2 is the variance of the random effects at herd level.

The logit link function was used to model the probability of occurrence of *Salmonella*. The random effects are in the form of a random intercept and this allows for the fact that the observations are nested in herds. Treating the herd effect as random, also allows for the fact that the number of herds (167) is a sample of all existing herds and not the whole population.

2.4. Univariable analyses

Candidate variables for the multivariable model were screened with univariable analysis. A relaxed significance level of $\alpha = 0.15$ was used to select variables to enter in the multivariable model.

As the variables were all categorised, association between the independent variables were tested using a chi-square test. The existence of significant associations between the independent variables was tested before adding them into the final multivariable model. It was expected the existence of association between variables like "Good herd replacement" or "Good biosecurity measures" and the variables that were used to create them. When association between variables was present, it was allowed to enter in the multivariable model just one variable at each time. The selection between which candidate variable would be included into the final model was decided by testing both variables and selecting the one presenting the smallest p-value.

2.5. Multivariable analysis

Stepwise procedures were used to select the statistically significant variables to enter/remove in the final multivariable model. At each step, the independent variable not in the model that had the smallest p-value was entered, and variables already in the model were removed if their p-value became larger than the significance level of $\alpha = 0.05$. The model was terminated when no more variables were eligible for inclusion or removal.

Two-way interaction between variables of the same level (herd or pen) and also cross-level interactions were analysed. Interactions between variables with biological meaning (e.g. source of semen and management of breeding boars, number of sows and number of animals per pen) were manually tested at both levels and retained if the $p < 0.05$. Confounding was assessed through the examination of the changes in the magnitude of the coefficients and looking at their biological significance and the regression coefficients were converted to odds ratio (OR) and the respectively 95% OR confidence interval (CI) were estimated. The relevance of the herd random effects was tested by looking at the variance estimate; the interpretation was that when this estimate it is close to zero it gives an indication that the herd effect does not contribute to the

dispersion of the outcome variable and a simpler model (without random effects) could be chosen (Twisk, 2006).

3. Results

A total of 1670 fecal pen samples (level 1) belonging to 167 herds (level 2), that responded to the questionnaire, were tested. Among the samples tested 170 from 76 herds were positive to *Salmonella*. *Salmonella* Typhimurium, followed by *Salmonella* Rissen were the most frequent serotypes found in the positive samples.

In the 167 herds there were 33 breeding holdings (45.45% had at least one sample positive to *Salmonella*, CI: 37.9–53.1%) and 134 productions holdings (45.45% had at least one sample positive to *Salmonella*, CI: 28.5–62.4%).

Tables 1 and 2 describe the different variables taking into consideration the presence of *Salmonella* in the pen fecal samples. Table 3 shows the descriptive statistics of the herd and pen continuous variables.

There was information missing in 15% of the herds for the variables system type and production type nevertheless these variables at univariable analyses did not meet the criterion to enter in the multivariable model.

The results of the univariable analyses are shown in Tables 1 and 2. The variables region of the herd, number of boars, number of sows, total number of breeding pigs, number of finishers pigs/herd, management of breeding boars, source of replacement pigs, source of semen, good herd replacement policy, rodents control, number of animals per pen, pens with access to outside, age of breeding sows, sex of the breeding pigs, breeding sector room, sanitary gap before new breeders in the pen, source of feed, use of antibiotics in the last 4 weeks in breeders and approach used to collect the pooled sample were selected to enter the multivariable model. Although the variable source of feed had a p value higher than 0.15 in the univariable analysis, it was forced to enter in the multivariable model, because this variable has been described as a risk factor in several previous studies (Lo Fo Wong et al., 2004; Benschop et al., 2008). To avoid collinearity problems, the variables number of sows and number of boars rather than total number of breeding pigs, and the variables management of breeding boars and source of semen rather than Good herd replacement policy were selected to enter the multivariable model. No significant association was found between the remaining variables. In the final multivariable model just the variables with $p < 0.05$ were selected to remain (Table 4). The OR for each variable is adjusted for the remaining variables in the model. There was not any significant interaction between the variables that were kept in the final multivariable model.

The significant results were: (1) region of the herd: samples from herds in the North Region had higher odds of being positive to *Salmonella* than samples from herds in the Alentejo Region; (2) rodents control: samples from herds with rodents control showed lower odds of being positive; (3) number of sows: herds with 170 and more sows presented higher odds of being infected; (4) source of semen: use of semen from another herd was a risk factor; (5) management of breeding boars: herds without boars or with 90% homebred boars showed lower odds of being positive; (6) breeding sector room: samples collected by the PVA at the maternity pens had lower odds of being positive than samples from mating pens; (7) source of feed: the samples where the source of feed was not exclusively from own herd had higher odds of being positive; and (8) number of animals per pen: having more than 10 animals in the pen showed higher odds of being positive. The variance of the random effect (σ^2) at herd level was estimated to be 1.5 which given the small standard error associated was interpreted as the variance being different from zero (Table 4).

4. Discussion

In this study a representative sample of the herds with breeding pigs in Portugal was used. The herds sampled were obtained using a sampling frame assembled by the PVA. The sample was representative of the country and took into consideration the different number of herds per region. The herds were randomly allocated to the study. The risk factors were assessed using data from a questionnaire filled by the herd veterinarian which were also responsible for the collection of the feces samples. The majority of the questions were closed; only a few were semi-open or open, such as the type and source of feed, soil type, the use of antimicrobial substances added to water or feed, and which antibiotic was used in breeders in the last four weeks before sample collection. To minimize the bias that could be introduced by having different people collecting the data, clarification meetings coordinated by the PVA were entertained with the herds veterinarians before the sample collection took place and the questionnaire had clear filling out instructions attached. Our judgment is that the validity of the data is quite robust given the care taken in the collection of the information and in the *Salmonella* isolation procedure.

Sampling the pen as a unit allows overcoming the problem of individual low sensitivity of the fecal culture, partly due to the intermittent shedding that infected pigs show. After the study conducted by Arnold and Cook (2009) it was demonstrated that the use of pooled fecal samples collected according to guidelines outlined in Commission Decision 2008/55/EC increases the likelihood of detecting pens where there is at least one pig infected with *Salmonella*. Therefore the overall sensitivity and ability to detect infected pens was increased in this study. As the specificity is 100% we are sure about the presence of *Salmonella* in positive samples.

Concerning the statistical data analysis it was decided to use a multilevel model because of the “natural” structure of data: the pen fecal samples (level 1) were nested in herds (level 2). Using this model the data structure is taken into consideration and the relationship of all variables, measured at herd or pen level is preserved and accounted for. This model also increases the power of the analysis and at the same time evaluates the variability associated with herd. The random effects are applied to models when is believed that the variance at group level is higher than zero. The variance (σ^2) of the random effect at herd level (b_h) was estimated to be 1.5, which means that a relatively large variability in the data was due to herd effect and the

Table 4
Final multivariable model for the presence of *Salmonella spp* in pen fecal samples using the data from the Baseline Survey on the Prevalence of *Salmonella* in Breeding Pigs in Portugal (in bold $p < 0.05$).

Variable	Multivariable analysis		
	OR		
	Estimate	95% CI	*p*-Value
Herd variables			
Region of the herd			
Alentejo	1.00		
Centre	1.97	0.75–5.22	0.17
Lisbon and Tagus Valley	1.40	0.61–3.20	0.43
North	3.86	1.08–13.75	**0.04**
Number of sows			
<170	1.00		
$\geq$170	1.82	1.04–3.19	**0.04**
Management of breeding boars			
More than 90% purchased	1.00		
Without boars or > 90% homebred	0.54	0.30–0.97	**0.04**
10–90% purchased or homebred	0.93	0.38–2.30	0.88
Source of semen			
Insemination centre – CI	1.00		
Own boar + CI	1.84	0.97–3.46	0.06
Boar from another herd	4.47	1.38–14.43	**0.01**
Rodents control			
No	1.00		
Yes	0.23	0.09–0.59	< **0.01**
Sample variables			
Number of animals per pen			
=10	1.00		
$\geq$10	2.02	1.19–3.43	< **0.01**
Breeding sector room			
Mating	1.00		
Gestation	0.78	0.53–1.15	0.21
Mixture of animals of different sectors	1.55	0.62–3.89	0.35
Maternity	0.39	0.24–0.63	< **0.01**
Replacement breeders	0.81	0.26–1.81	0.61
Source of feed			
Exclusively own	1.00		
Not exclusively own	2.81	1.19–6.61	**0.02**
Random effects[a]	Variance	Standard deviation	
At herd level	1.50	0.75	

OR: odds ratio, CI: confidence interval for odds ratio.
[a] Variance at pen fecal level constrained to be 1 (binomial variance).

use of multilevel model was an adequate choice. The multilevel methodology provides a solid approach and could be considered when the data follows a multilevel structure to allow the incorporation of group effect.

In the final multivariable model several significant risk associations were found. The pens where the feed was purchased had a higher risk of being *Salmonella* positive, similar to what has been found in another study (Benschop et al., 2008). Feed is a source of potential transmission of *Salmonella* and this hazard should be controlled by feed producers. The role of rodents in the transmission of this agent was also highlighted in other studies (Meerburg and Kijlstra, 2007; Skov et al., 2008). A protective association for the herds that control rodents was also found in this study. Rodents are biological vectors of *Salmonella* and if not controlled could play an important role in the transmission of the agent within herds and between nearby herds. The number of sows in a herd is a measure of the size of the herd and in this study herds with 170 and more sows had higher risk of being positive. This type of association was already found in the literature for finishers herds (Poljak et al., 2008) and it is mainly associated with practices of mixture of pigs which happens commonly in big herds. The mating pens had a higher risk when compared to maternity pens. This result is similar to the result found in a longitudinal study (Nollet et al., 2005) where it was detected more sows shedding *Salmonella* at mating than in the other sectors, and it was justified by the hormonal changes in the sow at mating which contribute to a higher shedding of the bacteria. The results concerning the region (North with higher risk than the South) was surprising and need further investigation with spatial analysis to see if factors not collected in this study may influence this result. The use of semen from another herd was a risk factor when compared to the use of semen from insemination centres, where the quality and safety of semen is controlled and tested. This association was not previously found in literature probably because in the majority of the countries the semen comes from insemination centres. This risk factor highlights the need to change this practice in Portugal. The management of breeding boars (used either for heat detection and or for breeding purposes) was also a risk factor and using

homebred boars was safer than using purchased boars. This could be explained because only 20% of the herds with more than 90% purchased boars used semen from insemination centres, while in the herds without boars or with more than 90% homebred around 48% used semen from insemination centres. The fact that semen and boars are controlled practices in many countries preclude the assessment of these variables as risk factors when statistical analyses are carried out using datasets from these countries. However it is important to keep in mind that controlling these sources is of high importance in every system to effectively prevent *Salmonella* new infection of the herd.

So far in Portugal only a few studies about herd risk factors have been done (Baptista et al., 2010), therefore our results are pertinent and useful. Furthermore as the pig sector in Portugal has a similar structure to those in France, Ireland and Italy among other countries (VLA-DTU-RIVM, 2010) these results may contribute to the knowledge of risk factors in these countries.

5. Conclusion

The risks highlighted in this study are epidemiologically and biologically consistent and they are representative of the breeding pigs system currently used in Portugal. It is noticeable the identification of risks associated with semen and boars purchasing: this reinforces that attention should be paid to these factors when conceiving herd biosecurity programmes; also noticeable and important is the fact that these risk factors have not been highlighted before. Our findings are of high relevance to the Portuguese Veterinary Authorities and also to pig farmers which are currently facing the lack of country adapted information to elaborate the control programmes for *Salmonella*. To achieve prevalence reduction, control programmes have to be implemented and the measures of the future control programmes should be cost-effective and adapted to country features. In this context this study gives valuable information to be incorporated in the near future control programme for *Salmonella* in breeding pigs in Portugal.

Acknowledgments

We would like to thank FCT for the PhD scholarship (SFRH/BD/40932/2007) and the Portuguese official veterinary authority (DGV) for supplying the data from the Baseline Survey on the Prevalence of *Salmonella* in Breeding Pigs.

References

Anonymous, 2007. Report of the Task Force on Zoonoses Data Collection on a proposal for technical specifications for a baseline survey on the prevalence of Salmonella in breeding pigs. EFSA J. 99, 1–28.

Arnold, M.E., Cook, A., Davies, R., 2005. A modelling approach to estimate the sensitivity of pooled faecal samples for isolation of Salmonella in pigs. J. R. Soc. Interface 2, 365–372.

Arnold, M.E., Cook, A.J., 2009. Estimation of sample sizes for pooled faecal sampling for detection of Salmonella in pigs. Epidemiol. Infect., 1–8.

Bahnson, P.B., Fedorka-Cray, P.J., Ladely, S.R., Mateus-Pinilla, N.E., 2006. Herd-level risk factors for Salmonella enterica subsp. enterica in U.S. market pigs. Prev. Vet. Med. 76, 249–262.

Baptista, F.M., Alban, L., Nielsen, L.R., Domingos, I., Pomba, C., Almeida, V., 2010. Use of herd information for predicting Salmonella status in pig herds. Zoonoses Public Health 57, 49–59.

Beloeil, P.A., Chauvin, C., Proux, K., Fablet, C., Madec, F., Alioum, A., 2007. Risk factors for Salmonella seroconversion of fattening pigs in farrow-to-finish herds. Vet. Res. 38, 835–848.

Beloeil, P.A., Fravalo, P., Fablet, C., Jolly, J.P., Eveno, E., Hascoet, Y., Chauvin, C., Salvat, G., Madec, F., 2004. Risk factors for Salmonella enterica subsp. enterica shedding by market-age pigs in French farrow-to-finish herds. Prev. Vet. Med. 63, 103–120.

Benschop, J., Stevenson, M.A., Dahl, J., French, N.P., 2008. Towards incorporating spatial risk analysis for Salmonella sero-positivity into the Danish swine surveillance programme. Prev. Vet. Med. 83, 347–359.

Breslow, N.E., Clayton, D.G., 1993. Approximate inference in generalized linear mixed models. JASA 88, 9–25.

EFSA, 2010. The community summary report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks in the European Union 2008. EFSA J. 1496, 288.

Fosse, J., Seegers, H., Magras, C., 2009. Prevalence and risk factors for bacterial food-borne zoonotic hazards in slaughter pigs: a review. Zoonoses Public Health 56, 429–454.

Funk, J., Gebreyes, W.A., 2004. Risk factors associated with Salmonella prevalence on swine farms. J. Swine Health Prod. 12, 246–251.

Funk, J., Wittum, T.E., LeJeune, J.T., Rajala-Schultz, P.J., Bowman, A., Mack, A., 2007. Evaluation of stocking density and subtherapeutic chlortetracycline on Salmonella enterica subsp enterica shedding in growing swine. Vet. Microbiol. 124, 202–208.

Goldstein, H., 2011. Multilevel Statistical Models. John Wiley & Sons.

Hoorfar, J., Mortensen, A.V., 2000. Improved culture methods for isolation of Salmonella organisms from swine feces. Am. J. Vet. Res. 61, 1426–1429.

Hox, J.J., 2002. Multilevel Analysis: Techniques and Applications. Lawrence Erlbaum Associates.

Letellier, A., Messier, S., Paré, J., Ménard, J., Quessy, S., 1999. Distribution of Salmonella in swine herds in Québec. Vet. Microbiol. 67, 299–306.

Lo Fo Wong, D.M., Dahl, J., Stege, H., van der Wolf, P.J., Leontides, L., von Altrock, A., Thorberg, B.M., 2004. Herd-level risk factors for subclinical Salmonella infection in European finishing-pig herds. Prev. Vet. Med. 62, 253–266.

Meerburg, B.G., Kijlstra, A., 2007. Role of rodents in transmission of Salmonella and Campylobacter. J. Sci. Food Agric. 87, 2774–2781.

Nollet, N., Houf, K., Dewulf, J., De Kruif, A., De Zutter, L., Maes, D., 2005. Salmonella in sows: a longitudinal study in farrow-to-finish pig herds. Vet. Res. 36, 645–656.

Nollet, N., Maes, D., De Zutter, L., Duchateau, L., Houf, K., Huysmans, K., Imberechts, H., Geers, R., de Kruif, A., Van Hoof, J., 2004. Risk factors for the herd-level bacteriologic prevalence of Salmonella in Belgian slaughter pigs. Prev. Vet. Med. 65, 63–75.

Pires, S.M., Vigre, H., Makela, P., Hald, T., 2010. Using outbreak data for source attribution of human salmonellosis and campylobacteriosis in Europe. Foodborne Pathog. Dis. 7, 1351–1361.

Poljak, Z., Dewey, C.E., Friendship, R.M., Martin, S.W., Christensen, J., 2008. Multilevel analysis of risk factors for Salmonella shedding in Ontario finishing pigs. Epidemiol. Infect. 136, 1388–1400.

Rossel, R., Rouillier, J., Beloeil, P.A., Chauvin, C., Basta, F., Crabos, J.P., Theau-Audin, S., 2006. Salmonella en élevage de porcs du Sud-Ouest de la France: séroprévalence en fin d'engraissement et facteurs de risque associés. Journées de la Recherche Porcine 38, 371–378.

Skov, M.N., Madsen, J.J., Rahbek, C., Lodal, J., Jespersen, J.B., Jørgensen, J.C., Dietz, H.H., Chriél, M., Baggesen, D.L., 2008. Transmission of Salmonella between wildlife and meat-production animals in Denmark. J. Appl. Microbiol. 105, 1558–1568.

Twisk, J.W.R. (Ed.), 2006. Applied Multilevel Analysis – A Pratical Guide for Medical Researchers. Cambridge University Press, Cambridge.

van der Wolf, P.J., Wolbers, W.B., Elbers, A.R.W., van der Heijden, H.M.J.F., Koppen, J.M.C.C., Hunneman, W.A., van Schie, F.W., Tielen, M.J.M., 2001. Herd level husbandry factors associated with the serological Salmonella prevalence in finishing pig herds in The Netherlands. Vet. Microbiol. 78, 205–219.

Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S. Springer, New York.

VLA-DTU-RIVM, 2010. EFSA Quantitative Microbiological Risk Assessment on Salmonella in Slaughter and Breeder Pigs: Final Report. Question No EFSA-Q-2007-00245. Grant number CFP/EFSA/BIOHAZ/2007/01. Veterinary Laboratories Agency., p. 437.

´´Manuscript 3

"Assessing risk profiles for *Salmonella* serotypes in breeding pig operations in Portugal using a Bayesian hierarchical model"

BMC Veterinary Research 2012, 8:226
http://www.biomedcentral.com/1746-6148/8/226

Correia-Gomes et al. BMC Veterinary Research 2012, 8:226
http://www.biomedcentral.com/1746-6148/8/226

BMC Veterinary Research

RESEARCH ARTICLE Open Access

Assessing risk profiles for *Salmonella* serotypes in breeding pig operations in Portugal using a Bayesian hierarchical model

Carla Correia-Gomes[1,2*], Theodoros Economou[3], Denisa Mendonça[1,2], Madalena Vieira-Pinto[4] and João Niza-Ribeiro[1,2]

Abstract

Background: The EU Regulation No 2160/2003 imposes a reduction in the prevalence of *Salmonella* in pigs. The efficiency of control programmes for *Salmonella* in pigs, reported among the EU Member States, varies and definitive eradication seems very difficult. Control measures currently recommended for *Salmonella* are not serotype-specific. Is it possible that the risk factors for different *Salmonella* serotypes are different? The aim of this study was to investigate potential risk factors for two groups of *Salmonella* sp serotypes using pen faecal samples from breeding pig holdings representative of the Portuguese pig sector.

Methods: The data used come from the Baseline Survey for the Prevalence of *Salmonella* in breeding pigs in Portugal. A total of 1670 pen faecal samples from 167 herds were tested, and 170 samples were positive for *Salmonella*. The presence of *Salmonella* in each sample (outcome variable) was classified in three categories: i) no *Salmonella*, ii) *Salmonella* Typhimurium or *S*. Typhimurium-like strains with the antigenic formula: 1,4,5,12:i:-, , and iii) other serotypes. Along with the sample collection, a questionnaire concerning herd management and potential risk factors was utilised. The data have a "natural" hierarchical structure so a categorical multilevel analysis of the dataset was carried out using a Bayesian hierarchical model. The model was estimated using Markov Chain Monte Carlo methods, implemented in the software WinBUGS.

Results: The significant associations found (when compared to category "no *Salmonella*"), for category "serotype Typhimurium or *S*. Typhimurium-like strains with the antigenic formula: 1,4,5,12:i:-" were: age of breeding sows, size of the herd, number of pigs/pen and source of semen. For the category "other serotypes" the significant associations found were: control of rodents, region of the country, source of semen, breeding sector room and source of feed.

Conclusions: The risk factors significantly associated with *Salmonella* shedding from the category "serotype Typhimurium or serotype 1,4,5,12:i:-" were more related to animal factors, whereas those associated with "other serotypes" were more related to environmental factors. Our findings suggest that different control measures could be used to control different *Salmonella* serotypes in breeding pigs.

* Correspondence: carla.gomes@sruc.ac.uk
[1]Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto (ICBAS-UP), Population Studies Department, Largo Prof. Abel Salazar, Porto 2, 4099-003, PORTUGAL
[2]Instituto de Saúde Pública da Universidade do Porto (ISPUP), Rua das Taipas, Porto 135, 4050-600, PORTUGAL
Full list of author information is available at the end of the article

BioMed Central

© 2012 Correia-Gomes et al.; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background

Salmonella has been one of the major causes of food-borne disease in the European Union (EU) in the past years [1]. A considerable proportion of human cases are related to pork products [2]. The EU approved legislation (EU Regulation No 2160/2003) imposes a reduction on the prevalence of this agent in food production animals, such as pigs. To set the target for this reduction per country, baseline surveys were carried out in the EU to estimate the prevalence of *Salmonella* sp. in some food production animals. The objective of the surveys was to obtain comparable data for all Member States (MS) through a harmonized approach. These studies showed that the prevalence of *Salmonella* in holdings with breeding pigs was 31.8% (28.7% for breeding holdings and 33.3% for production holdings) [3] and also that there are different profiles in terms of serotype prevalence among different countries. In Portugal for instance, 9.1% of the breeding holdings were positive to *Salmonella* Typhimurium and 33.3% were positive to other serotypes than Typhimurium and Derby, while in Ireland these numbers were 17.5% for both cases [3]. Another important issue is that control programmes already being carried out in several MS have different efficiencies and so far, none seems to be able to reduce the level of *Salmonella* sp. to reach an eradication stage [4]. Control programmes should target all serotypes of *Salmonella* sp., since all of them have the potential to be pathogenic for humans. To improve the efficiency of control programmes, potential differences in serotypes prevalence which allows for differences in risk factors between serotypes should be taken in consideration. Some of the known risk factors in the literature are linked to: 1) biosecurity measures [5] especially those aimed at potential biological vectors (rodents) [6-8], hand, equipment and facility hygiene [9] and also purchase of animals from different suppliers [9]; 2) herd management - such as herd size [10], batch production system [11], housing - type of floor (partial slatted floor) [12,13] and type of pen [9]; 3) feeding practices such as dry feed [14], source of feed [15] and adding organic acids to feed [11]; 4) health disorders such as use of antibiotics [16,17], parasite infestations [18,19], and health status of the herd [11] among others. However, none of the aforementioned studies have taken into consideration whether risk factors differ between serotypes. To the best of our knowledge only one study compared the differences between risk factors for *Salmonella* serotypes with or without antimicrobial resistance [20]. The data for this paper were collected by the Portuguese Veterinary Authority (PVA) when the Baseline Survey on the Prevalence of *Salmonella* in breeding pigs was conducted in Portugal. The aim was to search for potential risk factors for shedding from two different groups of *Salmonella* serotypes using pen faecal samples from herds with breeding pig representative of Portuguese reality. The two groups were *Salmonella* Typhimurium including *S*. Typhimurium-like strains with the antigenic formula: 1,4,5,12:i:-, and other serotypes.

Methods

Herd selection

The objectives, the sampling frame, the diagnostic testing methods as well as the collection and reporting of data, and the timelines of the Baseline Survey on the Prevalence of *Salmonella* in breeding pigs were specified in the Commission Decision 2008/55/EC. The target population are holdings that constitute at least 80% of the breeding pig population in the Member State.

The sample size was calculated by the PVA and considered the number of swine herds existing in April of 2007, stratified by Region. The sampling frame consisted of 4522 herds, with 204,584 breeding pigs and 1,827,533 pigs in total. The herd inclusion criteria for entering the sampling frame were: to have at least 50 breeding pigs, either for breeding or production purposes. The pig population included in the sampling frame represented 87% of the total registered pig population in Portugal in 2007.The sample size was calculated using the sampling criteria specified in the Commission Decision 2008/55/EC Annex I - expected herd prevalence of 50%, desired confidence level of 95%, accuracy of 7.5% and then apply a finite population correction factor, with an increase of 10% for each group (breeding and production holdings) in case of non-response. A sample of 174 swine herds was randomly selected using probability proportional to the number of herds among the regions in Portugal.

Pen selection

In each herd only the pens with breeding pigs over six months of age were randomly selected. The breeding pigs that have been recently introduced into the herd and were in quarantine were not included in the survey. In each selected herd, faecal samples from 10 pens were taken representing a 95% probability of detecting at least one positive sample if the true prevalence of infected pigs in the population was 10% [21]. The number of pens sampled per breeding room in each herd was allocated proportionally according to the number of breeding pigs in the different stages of production. The age categories in the sampling were not predetermined. The specification was that at least 10 individual breeding pigs should be included in each pooled pen faecal sample otherwise no sample was collected.

Faecal samples collection

The faecal samples were collected and pooled together by the herd veterinary assistant and then sent to

laboratory for detection of *Salmonella.* The material consisted of freshly voided faeces. Each pooled sample should weigh at least 25g and two approaches were employed to collect these pooled faeces samples: 1) where there was an accumulation of mixed faeces within an area of a pen or yard, a large swab was used to pass through the faecal mass, ensuring that at least 25g of mixed material was collected; 2) where there was no such accumulation (e.g. field, large yard, farrowing house, pens or other accommodation with low numbers of pigs per group) then individual pinches were selected from individual fresh faecal masses or places with a minimum of 10 individuals contributing to the final volume of at least 25g. The sites from which these pinches

Table 1 Herd variables assessed by the questionnaire and distribution of the pen samples by the categories of the outcome variable

	Number of pen samples by the categories of the outcome variable				Number of pen samples by the categories of the outcome variable		
	1	2	3		1	2	3
HERD VARIABLES							
Type of system				*Management of breeding sows*			
Open air	29	0	1	more than 90% external source	*835*	*17*	*68*
Intensive	1242	38	110	>90% home raised	189	10	21
Missing observations	229	8	13	10-90% home raised	476	19	35
Type of herd				*Control of rodents*			
Selection and Multiplication Unit	292	8	30	No	1254	42	114
Production Unit	1208	38	94	Yes	*246*	*4*	*10*
Region of the herd				*Source of semen*			
Alentejo	229	8	13	Insemination centre – IC	491	11	18
Centre	278	8	34	Own boar + IC	869	23	88
Lisbon and Tagus Valley	*914*	*27*	*59*	Boar from another herd	*79*	*9*	*12*
North	79	3	18	Missing observations	*61*	*3*	*6*
Type of production				*Source of replacement pigs*			
Farrow-to-weaners	*164*	*5*	*21*	Just own herd	585	14	41
Farrow-to-growers	250	7	13	Others sources	906	31	83
Farrow-to-finish	794	26	60	Missing observations	9	1	0
Missing observations	292	8	30	Number of finishers pigs/herd			
Number of boars				<100	*263*	*3*	*14*
<3	*715*	*22*	*33*	≥100	1221	42	107
≥3	*785*	*24*	*91*	Missing observations	16	1	3
Number of sows				*Control of birds*			
<170	759	16	55	No	1192	34	94
≥170	741	30	69	Yes	*308*	*12*	*30*
Number of gilts				*Use of foot bath*			
<22	713	20	47	No	1017	38	85
≥22	787	26	77	Yes	483	8	39
Size of the herd (number of breeding pigs)				*Clothes for exclusive use in the herd*			
<203	759	16	55	Yes	1423	46	121
≥203	741	30	69	No	77	0	3
Management of breeding boars				*Herd replacement policy*			
more than 90% external source	606	24	70	Good	434	7	29
without boars or >90% home raised	735	15	40	Bad	1066	39	95
				Biosecurity measures			
10-90% external source or home raised	159	7	14	Yes	828	30	72
				No	672	16	52

Legend: 1 (no *Salmonella*), 2 (serotype Typhimurium or serotype 1,4,5,12:i:-), 3 (other serotypes).

were collected were distributed in a representative manner across the area concerned. In approach 1) at least 10 individual pigs contributed to each sample taken, otherwise approach 2) was applied (Commission Decision 2008/55/EC).

Salmonella isolation

At the laboratory, the isolation of *Salmonella* was done using the method described by Annex D of ISO 6579. The *Salmonella* strains isolated in the positive pen faecal samples were serotyped in the National Reference Laboratory for *Salmonella* according to Kaulfmann-White scheme. The sensitivity of cultured pooled faecal samples according to the described method varied around 80% and the specificity is 100% [22,23].

Data collection

A questionnaire was used to collect information about the herd management and potential risk factors for *Salmonella* sp. shedding. This was filled by the herd veterinary who also collected the faecal samples (both tasks were conducted the same day). The questionnaire was designed by the PVA following the guidelines of Commission Decision 2008/55/EC. To minimize the bias that could be introduced by having different people collecting the data, the following procedures were taken: the majority of the questions were closed, the questionnaire had clear filling instructions attached and clarification meetings were held between the PVA and the field veterinarians before the sample collection took place. All the variables in the questionnaire are shown in Tables 1 and 2.

Table 2 Pen variables assessed by the questionnaire and distribution of the pen samples by the categories of the outcome variable

	Number of pen samples by the categories of the outcome variable				Number of pen samples by the categories of the outcome variable		
	1	2	3		1	2	3
PEN VARIABLES							
The pen has direct access to outside				*Sanitary gap before breeders entering*			
No	1146	30	92	No	*626*	*19*	*44*
Yes	354	16	32	Yes	874	27	80
Individual pen				*Feed*			
No	1194	41	98	Dry pellet	229	7	27
Yes	306	5	24	Dry non pellet	*1230*	*36*	*97*
Missing observations	*0*	*0*	*2*	Wet	41	3	0
Diarrhoea in the last 3 months				*Floor*			
No	1445	45	118	Fully slatted	139	5	10
Yes	*33*	*1*	*2*	Others	1361	41	114
Missing observations	22	0	4	*Source of feed*			
Age of the breeding sows				Exclusively own	199	8	8
Only gilts or gilts and others	874	38	73	Bought + Mixture	*1301*	*38*	*116*
Without gilts	626	8	51	*Potential Salmonella control substances added to water*			
Sex of the breeding pigs				No	1291	38	111
Only females	1430	44	114	Yes	*209*	*8*	*13*
Males and females	*70*	*2*	*10*	*Use of antibiotics in the last 4 weeks in breeders*			
Breeding sector				No	1229	45	103
Mating room	210	10	21	Yes	*271*	*1*	*21*
Gestation room	789	26	62	*Way how was collected the sample*			
Mixture of room	58	1	14	Compose sample	121	1	11
Farrowing room	*390*	*7*	*22*	Swab	*1379*	*45*	*113*
Replacement breeders	*44*	*2*	*5*	Number of pigs in the pen			
				=10	1284	34	94
				>10	216	12	30

Legend:1 (no *Salmonella*), 2 (serotype Typhimurium or serotype 1,4,5,12:i:-), 3 (other serotypes).

Data analysis

From the information gathered in the questionnaires, two new binary variables were created. The first variable groups the questions regarding management of replacing breeding pigs and their source, and was codified as Good if more than 90% of the breeding sows and boars were homebred (also included herds with no boars) and if the semen was not from another herd otherwise it was codified as Bad. The second variable combines the questions about biosecurity measures and was codified as Yes when controls for rodents and birds were implemented, and also if herds had provisions for foot bathing and clothe changing before entering the herd and No otherwise. The variables and their categories were recoded or aggregated to fewer categories as necessary to avoid sparse data problems as shown in Tables 1 and 2.

The continuous variables were transformed into categorical using the median values as the cut-off points defining the categories. Their summary statistics are shown in Table 3.

Because of the low number of cases per serotype (Table 4), individual analysis of each *Salmonella* serotype was prohibitive. Therefore the outcome variable was the isolation of *Salmonella* in each sample and was classified in three categories: i) no *Salmonella*, ii) serotype Typhimurium and *S.* Typhimurium-like strains with the antigenic formula: 1,4,5,12:i:-, and iii) other serotypes. For the calculation of apparent herd prevalence, a herd was considered positive if it had at least one positive pen faecal sample. The percentage of positive *Salmonella sp.* pen faecal samples was 27% *Salmonella* Typhimurium or *S.* Typhimurium-like strains with the antigenic formula: 1,4,5,12:i:-, and 73% other serotypes.

The data have a "natural" multilevel structure: pen faecal samples (first level) nested in herds (second level) and were analysed using a Bayesian hierarchical model with a categorical response variable (three categories). Monte Carlo Markov Chain (MCMC) was used for estimation and this was implemented in the freely available software WinBUGS (BUGS project, http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/). Hierarchical models are naturally handled in the Bayesian framework because of the conditional independence assumed between each level in the hierarchy. In conjunction with the open-source software WinBUGS, this provides a general framework for implementing hierarchical models in similar applications.

Random effects were included at the herd level to account for the fact that the observations are 'nested' in herds. Treating the herd effect as random, also allows for the fact that the number of herds here (167) is a sample of all existing herds. All prior distributions were chosen to be as uninformative as possible. A more detailed description of the model is given in Additional file 1.

To decide which variables should be included in this multivariable model, an exploratory analysis was performed by fitting univariable models and considering as candidates for the multivariable model, all variables significant at the 0.15 significance level. Associations between the explanatory variables were tested using a chi-square test and if a significant association ($p < 0.05$) was found, only the variables with more biological justification were allowed to enter the model.

The final multivariable model was built using a forward selection process until all variables with a significant 95% credible interval were included. The significance level was set at 0.05.

The model ran long enough with sufficient burn-in (5000 iterations) to ensure convergence to the posterior distribution of the parameters. Convergence was assessed by visual inspection of the means in time-series plots but also more formally using the Raftery and Lewis, and the Gelman-Rubin R-hat diagnostics [24,25]. R-hat should be arbitrarily close to 1 for convergence. The chains were thinned by only collecting 1 in 10 consecutive samples and this eliminated autocorrelation in posterior samples (using the CODA package [26] in R). Mixing in the chains was assessed by comparing the MC (Markov Chain) error with the standard deviation, for each parameter. Ideally, the MC error should be less than 5% of the standard deviations for good mixing [27] and this was true for all parameters here. Two MCMC chains ran with dispersed initial values which is good practice to ensure convergence and mixing. WinBUGS code for implementing the model is given in Additional file 2.

The presence of confounding was investigated by analysing the correlation matrix of the joint posterior

Table 3 Distribution of the continuous variables (at herd and pen level)

Variable	Mean	Standard deviation	Minimum	Percentile 25	Median	Percentile 75	Maximum
Number of boars	3.9	3.9	0	2	3	4	28
Number of sows	226.5	192.9	8	98	170	300	1077
Number of gilts	34.0	38.3	0	12	22	40	300
Size of the herd (number of breeding pigs)	265.0	216.9	41	109	203	355	1214
Number of pigs per pen*	11.6	8.0	10	10	10	10	130

* 258 pens had more that 10 pigs per pen.

Table 4 Percentage of serotypes isolated in the study

Serotype	Percentage of isolates (n)			Percentage of herds that have at least one pen sample positive to the serotype (n)		
	Breeding holdings	Production holding	All Holdings	Breeding holdings	Production holding	All holdings
Typhimurium	15.8 (6)	25 (33)	23 (39)	13.6 (3)	25.6 (20)	13.2 (23)
Rissen	18.4 (7)	19.7 (26)	19 (35)	22.7 (5)	19.2 (15)	12.0 (20)
London	21 (8)	13.6 (18)	15 (26)	13.6 (3)	11.5 (9)	7.2 (12)
Derby	15.8 (6)	9.1 (12)	11 (18)	13.6 (3)	8.9 (7)	6.0 (10)
Give	13.1 (5)	5.3 (7)	7 (12)	9.1(2)	5.1 (4)	4.0 (6)
Brandenburg	0 (0)	6.1 (8)	5 (8)	0 (0)	2.6 (2)	1.8 (2)
1,3,19:-:-	2.6 (1)	4.5 (6)	4 (7)	4.5 (1)	6.4 (5)	3.6 (6)
1,4,5,12:i:-	5.3 (2)	3.8 (5)	4 (7)	9.1 (2)	3.8 (3)	3.0 (5)
Bovismorbificans	0 (0)	3 (4)	2 (4)	0 (0)	2.6 (2)	1.2 (2)
Gloucester	0 (0)	2.3 (3)	2 (3)	0 (0)	2.6 (2)	1.2 (2)
Muenchen	2.6 (1)	2.3 (3)	2 (4)	4.5 (1)	3.8 (3)	2.4 (4)
Anatum	0 (0)	1.5 (2)	1 (2)	0 (0)	2.6 (2)	1.2 (2)
Bredeney	0 (0)	0.8 (1)	1 (1)	0 (0)	1.3 (1)	0.6 (1)
Goldcoast	0 (0)	1.5 (2)	1 (2)	0 (0)	1.3 (1)	0.6 (1)
Livingstone	2.6 (1)	0 (0)	1 (1)	4.5 (1)	0 (0)	0.6 (1)
Mbandaka	2.6 (1)	0.8 (1)	1 (2)	4.5 (1)	1.3 (1)	1.2 (2)
Senftenberg	0 (0)	0.8 (1)	1 (1)	0 (0)	1.3 (1)	0.6 (1)

distribution for all model parameters but especially the slope parameters. Correlation values higher than 0.5 where takes to indicate significant correlation.

Posterior predictive simulation was used for model checking as described by Gilks et al.[28]. This technique is effectively testing whether the observed data are extreme in relation to the predictive distribution (fitted model). Model deviance was the measure adopted for comparison. The technique involves the estimation of a p-value which should not be extreme (close to 0 or 1) for good model fit.

Results

A total 167 herds (33 breeding and 134 production holdings) responded to the questionnaire and were tested: 76 herds were positive to *Salmonella* sp. (apparent prevalence of 45.5%, CI: 37.9% - 53.1%). Of these, 15 breeding holdings (apparent prevalence of 45.5%, CI: 28.5% - 62.4%), and 61 production holdings (apparent prevalence of 45.5%, CI: 37.1% - 53.9%) were positive to *Salmonella* sp. Among the 1670 faecal samples collected, 170 were positive (10.1%) and seventeen different serotypes were found (Table 4). There was no simultaneous occurrence of the two groups of serotypes in any of the positive samples. *Salmonella* Typhimurium was found in 23% of the positive isolates (15.8% in breeding and 25% in production holdings), followed by *Salmonella* Rissen (19%) (Table 4). The proportion of the different serotypes by type of holding is detailed in Table 4. Considering the distribution of serotypes groups through the herds, it was observed that 13.8% of the herds had at least one sample positive to serotype Typhimurium or *S.* Typhimurium-like strains with the antigenic formula: 1,4,5,12:i:-, and 31.7% of the herds had at least one positive sample to other serotypes. A significant association was found between number of sows and number of breeding pigs and for this reason it was decided that only the number of breeding pigs should enter the multivariable model.

Several management practices linked to herd and pen were assessed (Tables 1 and 2). The variables - region of the herd, size of the herd, source of semen, rodents control, number of pigs per pen, age of breeding sows, breeding sector room, source of feed and use of antibiotics - were selected to enter the multivariable model. Table 5 shows the final multivariable multilevel model results. The results were converted to odds ratio (OR) and the respective 95% credible intervals (OR CrI) were calculated. The posterior median was used to estimate point values of each OR, because unlike the mean, this is less affected by asymmetric distributions. Posterior distributions of all OR are highly asymmetric since they are based on the exponentiation of posteriors of the slope parameters. The convergence of MCMC calculations was considered acceptable with R-hat values of all parameters being less than 1.001. Different starting values did not affect the final results. None of the between-parameter correlations was larger than 0.5 in magnitude while the majority was less than 0.1 implying no influential confounding in any of the variables.

Table 5 Posterior results for the final multivariable categorical multilevel model for the risk factors (*Salmonella* negative as reference group)

Variable	Typhimurium or 1,4,5,12:i:-				Other serotypes			
	Coefficient	SD	OR	95% OR CrI	Coefficient	SD	OR	95% OR CrI
HERD								
Region of the herd								
Alentejo	0		1.0		0		1.0	
Centre	−1.3	1.5	0.28	0.01-4.30	1.5	0.7	**4.57**	**1.33-17.57**
Lisbon and Tagus Valley	−0.5	1.1	0.62	0.07-5.05	0.9	0.6	2.56	0.86-8.36
North	−0.1	1.7	0.88	0.03-24.31	2.6	0.8	**12.9**	**2.97-64.33**
Size of the herd: (number of breeding pigs)								
<203	0		1.0		0		1.0	
≥203	1.9	0.9	**7.04**	**1.46-60.04**	0.5	0.4	1.65	0.83-3.44
Source of semen								
Insemination centre – IC	0		1.0		0		1.0	
Own boar + IC	0.4	0.8	1.45	0.24-7.77	1.1	0.4	2.91	1.35-6.83
Boar from another herd	3.7	1.6	**41.22**	**2.46-1392.7**	1.4	0.8	4.18	0.94-19.30
Control of rodents								
No	0		1.0		0		1.0	
Yes	−2.2	1.8	0.11	0.002- 1.85	−2.0	0.7	**0.13**	**0.03-0.45**
PEN								
Number of pigs/pen								
=10	0		1.0		0		1.0	
>10	1.4	0.7	**4.06**	**1.03-19.73**	0.6	0.4	1.82	0.88-3.79
Age of the breeding sows								
Only gilts or gilts and others	0		1.0		0		1.0	
Without gilts	−1.8	0.8	**0.17**	**0.03-0.65**	0.2	0.3	1.24	0.68-2.24
Breeding sector room								
Mating	0		1.0		0		1.0	
Gestation	0.1	0.5	1.11	0.44-3.10	−0.2	0.3	0.81	0.45-1.52
Mixture of animals of different sectors	0.2	1.7	1.17	0.03-24.80	0.8	0.7	2.14	0.54-7.78
Farrowing	−1.0	0.6	0.36	0.10-1.22	−1.0	0.4	**0.38**	**0.17-0.80**
Replacement breeders	−0.9	1.1	0.40	0.04-2.72	0.1	0.7	1.15	0.29-3.88
Source of feed								
Exclusively own	0		1.0		0		1.0	
Not exclusively own	0.5	1.1	1.63	0.18-17.62	2.0	0.7	**7.29**	**2.25-29.46**
Herd random effect variance	5.8	0.66			1.4	0.24		

Legend: SD – standard deviation, OR – odds ratio, CrI – credible interval, in bold the significant OR for a 95%CrI.

Significance of the herd random effects is tested by looking at whether the variance estimates ($1/\tau_1$, $1/\tau_2$) are non-zero. Estimates of $1/\tau_1$ and $1/\tau_2$ are arbitrarily away from zero (5.8 and 1.4 respectively) and their standard errors are relatively small, indicating that both estimates are different from zero (see Table 5). The model fit was reasonably accurate with a p-value of 0.21 which means no significant differences between replicated and observed data.

It can be seen from the analysis of Table 5 that there are different risk profiles for the two *Salmonella* serotype categories when compared to category "no *Salmonella*". This is an important finding and suggests that the risk factors may be different between the categories of serotypes defined in this study. For category "Typhimurium or *S.* Typhimurium-like strains with the antigenic formula: 1,4,5,12:i:- associations with significant change in risk were: 1) *size of the herd*: herds with 203 and more breeding pigs are at higher risk of infection, 2) the source of semen: purchase of boars from other herds increase the risk of infection, 3) *number of pigs per pen*: pens with more than 10 animals per pen have increased risk of infection, and 4) the age of the sows: pens without gilts have a decreased risk of

infection. For the category "other serotypes" the significant risk associations were: 1) *region of the herd*: herds in the Centre and North Region have a higher risk of infection, 2) *the source of semen*: the use of own boar increased the herds' risk of infection, 3) *control of rodents* had a significant effect in reducing the risk at the herd level, 4) *feed source:* using feed from external sources, i.e., not exclusively from the farm increased the risk of infection, and 5) *breeding sector*: the farrowing sector had a lower risk of infection than the mating sector.

Discussion

This study investigated risk factors for *Salmonella* shedding for two different groups of serotypes using pen faecal samples from herds with breeding pigs adequately representing the Portuguese pig industry.

The outcome variable

The different serotypes of *Salmonella sp.* were divided in two groups because serotype Typhimurium is a serotype with a recognized difficult control [29] and is also the cause of many human cases of food-borne disease linked to pork meat. Serotype Typhimurium-like strains with the antigenic formula: 1,4,5,12:i:-was included in the group of serotype Typhimurium because of the genetic similarity, the similar virulence and the antimicrobial resistance characteristics existing between the two serotypes [30]. The use of composite samples increases the overall sensitivity of detection of infected pens [31] strengthening the confidence on the accuracy of our response variable. The increased sensitivity of the use of pooled faecal samples was shown by the analysis of the Baseline Survey results [32] which demonstrated that this pooled sampling process detected approximately 80% of the true *Salmonella* positive herds, and that with 10 pooled faecal samples it is possible to detect at least one positive sample in a pig herd when the animal level prevalence is at least 20%, with 95% certainty [31].

The model

It was anticipated that the hierarchical structure of the data from our sample could influence the outcome of the analysis. Therefore the statistical approach was chosen to take into consideration the multilevel structure of data from our sample where the pen faecal samples (level 1) are nested in herds (level 2). Some important remarks concerning the statistical approach deserve to be highlighted: the model implemented here showed a good fit, despite the fact there was little information to update the prior distributions. The methodology proposed could offer a general modelling approach to researchers who want to incorporate expert knowledge in the specification of the priors or for those who wish to restrict the priors accordingly to account for lack of information in the response variable which was not the case in this study. Lastly, both WinBUGS and R, are freely available software which is particularly appealing for the purpose of presenting the methodology here as a general modelling tool.

Risk factors for *Salmonella* Typhimurium and Typhimurium-like strains with the antigenic formula: 1,4,5,12:i:- infection

It can be seen from the analysis of Table 5 that there are different risk profiles for the two categories of *Salmonella*, validating our initial hypothesis that the risk factors could vary between the two categories of serotypes studied.

In category "Typhimurium or 1,4,5,12:i:-" *the size of the herd* (the number of breeding pigs being equal or greater than 203) was considered a risk factor. A similar association was found for *Salmonella* sp. in finishers [10] and also in the breeding pigs [32]. A reason for this is that in bigger herds, the risk of transmission is higher given a higher number of "infectious" and "susceptible" animals, offering increased chances of more effective contacts per unit of time. The *number of pigs per pen* was another risk factor, already reported for *Salmonella sp.* in breeding pigs by Nollet in 2005 [32]. As in the case of the size of the herd, the greater the number of pigs in the pen, the easier the transmission of infection between pigs, if there are infected pigs in that pen. Interestingly, these two factors were not found significant for "other serotypes" which suggests that "serotype Typhimurium category" could be more associated with transmission between animals than other categories. A protective association, relating to *pens without gilts* was found. A similar association was also found in the European Union Baseline survey on breeding pigs for maiden gilts [32]. One reason may be that older sows have higher immunity status to *Salmonella* Typhimurium and may be less susceptible to stress than younger sows although they could be carriers (the test used was pooled faecal culture so it could not detect carriers if they are not shedding) [33]. The last significant risk factor found in this category of the outcome was the *boar from another herd* which however, has a wide credible interval. A combination of the high odds ratio with a relatively small number of pen faecal samples in this variable category indicates that this association should be a matter of further study. Interestingly, for *rodent control*, a strong protective effect was noticed towards the Typhimurim group (noticeable by the OR = 0.11) although not statistically significant. However, it is our opinion that rodent control should not be disregarded from the list of risk factors for *S.* Typhimurim.

Risk factors for infection with other serotypes of *Salmonella*

Concerning the category "other serotypes", the *region of the herd* was found relevant: samples from herds in the North and Centre Region had higher odds of being positive than samples from herds in the Alentejo Region. Possibilities to explain this finding are that herds in the Centre and North regions are close together or share common management factors. This variable needs further studying to understand whether there are differences in management procedures that were not evaluated by this questionnaire, as this variable did not influence the results of the other variables when it entered the model. Using *semen from own boar* is a risk factor when compared to using semen from insemination centres only, where the animals are tested and if *Salmonella*-positive culled. This association has not been reported yet in the literature, probably because in the majority of the countries the semen comes from insemination centres. Pens where pigs *feed is not exclusively home produced* were at higher risk: the risk is linked to exotic serotypes such as the ones that are isolated in commercial feed; similar association was also found in other studies but for *Salmonella* sp. [15,32]. There is a protective effect for farrowing pens when compared to mating pens. This can be justified by the hormonal changes in the sow at mating which is similar to the results found in a longitudinal study for sows seven days after weaning [33] where more *Salmonella* was detected at mating than in the others sector of breeding sows. In that study, this was attributed to hormonal changes that takes place in the sows resulting in follicular growth, ovulation and oestrus behaviour, and also to rise in adrenocorticotrope hormone due to stress. So it was concluded that with stress sows are more susceptible to infection and also carrier sows are more likely to start shedding the pathogen [33]. The control of rodents was considered a protective factor for the presence of "other serotypes": the role of rodents in the transmission of this agent was also highlighted in other studies [6,8]. Since rodents could lead to the dissemination of the agent in the herd as a vector that transmits the infection between closed sectors their role must not be underestimated in a control programme. As already mentioned this variable appears as a protective factor to the group Typhimurium or 1,4,5,12:i:- although not statistically significant. This intriguing finding does not compromise the hypothesis of the importance from pig to pig transmission – direct or indirect - in the case of Typhimurium.

Application in control

The results from this work should be taken into account when implementing control and biosecurity programmes to *Salmonella* sp., since they highlight the importance to pre-define herd infection status regarding *S.* Typhimurium, and of making a risk profile based on the management practices in place before the adoption of control measures. Control measures should be adapted to suite the type of infection present bearing in mind that for serotype Typhimurium the control of animal source risk factors should be considered, whereas for the other serotypes is it the environmental source risk control that is important.

Conclusion

In Portugal, the prevalence of herds with breeding pigs that had at least one sample positive to serotype Typhimurium or *S.* Typhimurium-like strains with the antigenic formula: 1,4,5,12:i: was 13.8% and for the other serotypes 31.7%. A flexible and innovative statistical modelling approach was successfully used here. This provides a framework for similar studies of other diseases as it is straightforward to implement and can be easily generalized. The risk factors for serotype Typhimurium suggest a contagious pattern and the risk factors for other serotypes appeal to be related to environmental factors. The role of rodent control in serotype Typhimurium needs further studies. This study provided valuable information that can be incorporated in future control programmes for *Salmonella* sp. in breeding pigs in Portugal and other countries.

Additional files

Additional file 1: Model framework.

Additional file 2: WinBUGS code for the categorical multilevel model.

Competing interests

The authors do not have any competing interest.

Authors' contributions

CCG was involved in the design and performed the statistical modelling analysis and drafted the manuscript. TE performed part of the statistical analysis and was involved in the revision of the manuscript for intellectual contents. DM was involved in the revision of the manuscript for intellectual contents and statistical analysis. MVP was involved in the revision of the manuscript for intellectual contents. JNR was involved in the design of the statistical analysis, in the drafting and revision of the manuscript for intellectual content. All authors approved the final manuscript.

Acknowledgments

We would like to thank FCT for the PhD scholarship (SFRH/BD/40932/2007) and for the strategic research project Pest-OE/AGR/UIO772/2011. We would like also to thank the Portuguese official veterinary authority (DGAV) for the data.

Author details

[1]Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto (ICBAS-UP), Population Studies Department, Largo Prof. Abel Salazar, Porto 2, 4099-003, PORTUGAL. [2]Instituto de Saúde Pública da Universidade do Porto (ISPUP), Rua das Taipas, Porto 135, 4050-600, PORTUGAL. [3]CEMS, University of Exeter, Harrison Building, North Park Road, Exeter EX4 4QF, UK. [4]UTAD,

Veterinary Science Department, Apartado, Vila Real 1013, 5001-801, PORTUGAL.

Received: 16 April 2012 Accepted: 1 November 2012
Published: 21 November 2012

References

1. EFSA: **The community summary report on trends and sources of Zoonoses and zoonotic agents and food-borne outbreaks in the European union 2008.** *The EFSA Journal* 2010, **1496:**288.
2. Hald T, Vose D, Wegener HC, Koupeev T: **A Bayesian approach to quantify the contribution of animal-food sources to human salmonellosis.** *Risk Anal* 2004, **24**(1):255–269.
3. EFSA: **Analysis of the baseline survey on the prevalence of Salmonella in holdings with breeding pigs, in the EU, 2008, Part A: Salmonella prevalence estimates.** *EFSA Journal* 2009, **7**(12):1377.
4. EFSA: **Opinion of the scientific panel on biological hazards on "risk assessment and mitigation options of *Salmonella* in pig production".** *The EFSA Journal* 2006, **341:**1–131.
5. Baptista FM, Alban L, Nielsen LR, Domingos I, Pomba C, Almeida V: **Use of herd information for predicting salmonella status in Pig herds.** *Zoonoses Public Health* 2010, **57:**49–59.
6. Skov MN, Madsen JJ, Rahbek C, Lodal J, Jespersen JB, Jørgensen JC, Dietz HH, Chriél M, Baggesen DL: **Transmission of Salmonella between wildlife and meat-production animals in Denmark.** *J Appl Microbiol* 2008, **105**(5):1558–1568.
7. Letellier A, Messier S, Paré J, Ménard J, Quessy S: **Distribution of Salmonella in swine herds in Québec.** *Vet Microbiol* 1999, **67**(4):299–306.
8. Meerburg BG, Kijlstra A: **Role of rodents in transmission of Salmonella and Campylobacter.** *J Sci Food Agric* 2007, **87**(15):2774–2781.
9. Lo Fo Wong DM, Dahl J, Stege H, Van der Wolf PJ, Leontides L, Von Altrock A, Thorberg BM: **Herd-level risk factors for subclinical Salmonella infection in European finishing-pig herds.** *Prev Vet Med* 2004, **62**(4):253–266.
10. Poljak Z, Dewey CE, Friendship RM, Martin SW, Christensen J: **Multilevel analysis of risk factors for Salmonella shedding in Ontario finishing pigs.** *Epidemiol Infect* 2008, **136**(10):1388–1400.
11. Funk J, Gebreyes WA: **Risk factors associated with Salmonella prevalence on swine farms.** *Journal of Swine Health and Production* 2004, **12**(5):246–251.
12. Nollet N, Maes D, De Zutter L, Duchateau L, Houf K, Huysmans K, Imberechts H, Geers R, de Kruif A, Van Hoof J: **Risk factors for the herd-level bacteriologic prevalence of Salmonella in Belgian slaughter pigs.** *Prev Vet Med* 2004, **65**(1–2):63–75.
13. Rossel R, Rouillier J, Beloeil PA, Chauvin C, Basta F, Crabos JP, Theau-Audin S: **Salmonella en élevage de porcs du Sud-Ouest de la France: séroprévalence en fin d'engraissement et facteurs de risque associés.** *Journées de la Recherche Porcine* 2006, **38:**371–378.
14. Bahnson PB, Fedorka-Cray PJ, Ladely SR, Mateus-Pinilla NE: **Herd-level risk factors for Salmonella enterica subsp. enterica in U.S. market pigs.** *Prev Vet Med* 2006, **76**(3–4):249–262.
15. Benschop J, Stevenson MA, Dahl J, French NP: **Towards incorporating spatial risk analysis for Salmonella sero-positivity into the Danish swine surveillance programme.** *Prev Vet Med* 2008, **83**(3–4):347–359.
16. Beloeil PA, Chauvin C, Proux K, Fablet C, Madec F, Alioum A: **Risk factors for Salmonella seroconversion of fattening pigs in farrow-to-finish herds.** *Vet Res* 2007, **38**(6):835–848.
17. Funk J, Wittum TE, LeJeune JT, Rajala-Schultz PJ, Bowman A, Mack A: **Evaluation of stocking density and subtherapeutic chlortetracycline on Salmonella enterica subsp enterica shedding in growing swine.** *Vet Microbiol* 2007, **124:**202–208.
18. van der Wolf PJ, Wolbers WB, Elbers ARW, van der Heijden HMJF, Koppen JMCC, Hunneman WA, Van Schie FW, Tielen MJM: **Herd level husbandry factors associated with the serological Salmonella prevalence in finishing pig herds in The Netherlands.** *Vet Microbiol* 2001, **78**(3):205–219.
19. Beloeil PA, Fravalo P, Fablet C, Jolly JP, Eveno E, Hascoet Y, Chauvin C, Salvat G, Madec F: **Risk factors for Salmonella enterica subsp. enterica shedding by market-age pigs in French farrow-to-finish herds.** *Prev Vet Med* 2004, **63**(1–2):103–120.
20. Farzan A, Friendship RM, Dewey CE, Poppe C, Funk J: **Evaluation of the risk factors for shedding salmonella with or without antimicrobial resistance in swine using multinomial regression method.** *Zoonoses Public Health* 2010, **57:**85–93.
21. Anonymous: **Report of the task force on Zoonoses data collection on a proposal for technical specifications for a baseline survey on the prevalence of Salmonella in breeding pigs.** *The EFSA Journal* 2007, **99:**1–28.
22. Hoorfar J, Mortensen AV: **Improved culture methods for isolation of Salmonella organisms from swine feces.** *Am J Vet Res* 2000, **61**(11):1426–1429.
23. Arnold ME, Cook A, Davies R: **A modelling approach to estimate the sensitivity of pooled faecal samples for isolation of Salmonella in pigs.** *J R Soc Interface* 2005, **2**(4):365–372.
24. Gelman A, Rubin DB: **Inference from iterative simulation using multiple sequences.** *Stat Sci* 1992, **7:**457–511.
25. Kostoulas P, Leontides L, Enøe C, Billinis C, Florou M, Sofia M: **Bayesian estimation of sensitivity and specificity of serum ELISA and faecal culture for diagnosis of paratuberculosis in Greek dairy sheep and goats.** *Prev Vet Med* 2006, **76**(1–2):56–73.
26. Best NG, Cowles MK, Vines SK: *CODA Manual Version 0.30*. Cambridge, UK: MCR Biostatistics Unit; 1995.
27. Congdon PD (Ed): *Applied Bayesian hierarchical methods*. Boca Raton: Chapman and hall/ CRC; 2010.
28. Gilks WR, Richardson S, Spiegelhalter DJ: *Markov chain Monte Carlo in practice*. Boca Raton: Chapman and Hall/CRC; 1996.
29. Alban L, Olsen AM, Nielsen B, Sorensen R, Jessen B: **Qualitative and quantitative risk assessment for human salmonellosis due to multi-resistant salmonella typhimurium DT104 from consumption of Danish dry-cured pork sausages.** *Prev Vet Med* 2002, **52**(3–4):251–265.
30. (BIOHAZ) EPoBH: **Scientific opinion on monitoring and assessment of the public health risk of "Salmonella Typhimurium-like" strains.** *EFSA Journal* 2010, **8**(10):48.
31. Arnold ME, Cook AJ: **Estimation of sample sizes for pooled faecal sampling for detection of Salmonella in pigs.** *Epidemiol Infect* 2009, **137**(12):1734–1741.
32. EFSA: **Analysis of the baseline survey on the prevalence of Salmonella in holdings with breeding pigs, in the EU, 2008 Part B: factors associated with Salmonella pen positivity.** *The EFSA Journal* 2011, **9**(7):2329.
33. Nollet N, Houf K, Dewulf J, De Kruif A, De Zutter L, Maes D: **Salmonella in sows: a longitudinal study in farrow-to-finish pig herds.** *Vet Res* 2005, **36**(4):645–656.

doi:10.1186/1746-6148-8-226
Cite this article as: Correia-Gomes *et al.*: Assessing risk profiles for *Salmonella* serotypes in breeding pig operations in Portugal using a Bayesian hierarchical model. *BMC Veterinary Research* 2012 **8**:226.

Submit your next manuscript to BioMed Central and take full advantage of:

- **Convenient online submission**
- **Thorough peer review**
- **No space constraints or color figure charges**
- **Immediate publication on acceptance**
- **Inclusion in PubMed, CAS, Scopus and Google Scholar**
- **Research which is freely available for redistribution**

Submit your manuscript at
www.biomedcentral.com/submit

Additional File 1 – Model framework

Level 1- pen samples:

$$Y_{khi} = \begin{cases} 1\ (no\ Salmonella) \\ 2\ (Typhimurium\ or\ 1{,}4{,}[5]{,}12{:}i{:}-) \\ 3\ (other\ serotype) \end{cases}$$

where

$\Pr(Y_{khi}) = p_{khi}$; $k = 1, 2, 3$ for outcome variable; $h = 1, \ldots 1670$ for samples ; $i = 1, \ldots, 167$ for herds and

$\sum_{k=1}^{3} p_{khi} = 1$

is the probability of occurrence for each category of the outcome variable Y. These probabilities are themselves modelled using explanatory variables and random effects:

$$\text{logit}(p_{khi}) = \alpha_k + \beta_{jk}\text{herd variables}_{ih} + \beta_{jk}\text{pen variables}_h + \beta_{jk}\text{herd variables}_{ih}*\text{herd variables}_{ih} + \beta_{jk}\text{pen variables}_h*\text{pen variables}_h + \beta_{jk}\text{herd variables}_{ih}*\text{pen variables}_h + b2_{ik}$$

where j is the number of explanatory variables.

Note that with the use of random effects, the probabilities of Y=1, 2 or 3 are herd specific.

The probability for each category of Y is modelled using the same explanatory variables but different slope parameters (β_{jk}) to assess whether those variables affect each category in a different way. The reference category is Y=1 (no *Salmonella*) and all the results from each of the categories Y=2 and 3 are compared to the reference category.

Level 2 - herds:

$b2_{i1} = 0$

$b2_{i2} \sim N(0, 1/\tau_1)$

$b2_{i3} \sim N(0, 1/\tau_2)$ where $1/\tau_1$ and $1/\tau_2$ are the variances for category "serotype Typhimurium or serotype 1,4,5,12:i:-" and "other serotypes" respectively.

The $b2_{ik}$ are the random effects allowing for the fact that the observations are 'nested' in herds (this reduces the effective number of model parameters by 'pooling' herd information, while retaining model flexibility). Treating the herd effect as random, also allows for the fact that the number of herds here (167) is a sample of all existing herds. The prior distributions for the model parameters:

$\alpha_1 = 0$

$\alpha_k \sim N(0,100)$ where k = 2,3 for the intercepts in each category of Y_{khi}.

$\beta_{j1} = 0$ where j = 1,2,…,14 for the reference category of the explanatory variables.

$\beta_{jk} \sim N(0,100)$ where j = 1,2,…,14 and k =2,3. These are the fixed effects of the explanatory variables in the other two categories of the Y_{khi}.

$\tau_1 \sim \text{Gamma}(0.5, 0.001)$,

$\tau_2 \sim \text{Gamma}(0.5, 0.001)$ for the variance of the herd random effects.

All prior distributions were chosen to be as uninformative as possible. For parameters with infinite support, Gaussian priors with large variance are conventionally used to express lack of information [34]. For variance parameters with strictly positive support, the inverse of the variance (precision) is given an uninformative gamma distribution implying that the variance is given an inverse gamma. The inverse gamma is the conjugate prior for a Gaussian random effect therefore it is a natural choice which aids computation. A Gamma(0.5,0.001) was chosen which has mean 500 and variance of 500000, implying it is a very flat or uninformative prior distribution.

Additional File 2 – WinBUGS code

Figure1. WinBUGS code for the categorical multilevel model

Legend: H = number of cases, K = number of categories in the outcome variable = 3 (1-no *Salmonella*, 2 - serotype Typhimurium or serotype 4,5,12:i-, 3 - other serotypes), sero[h] = outcome variable, cod.herd[h] = number of the herd, α = intercept for each outcome result, β = fixed effects, b2 = random effects considering herd level, I = number of herds, Variables: rod = rodents control, sem2 = mixture of own boar semen and semen form insemination centres, sem3 = semen of boar from another herd, reg2 = Centre region, reg3 = Lisbon and Tagus Valley region, reg4 = North region, herdsize = size of the herd: number of breeding pigs (equal or more than 203), feed = source of feed in the pen, sec2 = gestation pen, sec3 = mixture of animals of different sector in the pen, sec4 = farrowing pen, sec5 = replacement breeders pen, num = more than 10 animals per pen, age = age of the breeding sows in the pen, sig1 = standard deviation of category Typhimurium or 4,5,12:i-, sig2 = standard deviation of category other serotypes

WinBUGS model file for categorical multilevel model

```
model{
  for(h in 1:H){
   for(k in 1:K){
#likelihood for categorical outcome
     p[h,k]<-expecta[h,k]/sum(expecta[h,1:K])
     expecta[h,k]<-exp(eta[h,k])
     eta[h,k]<-α[1,k]+b2[cod.herd[h],k]+β[1,k]*rod[h]+β[2,k]*sem2[h]+β[3,k]*sem3[h]+β[4,k]*reg2[h]+
          β[5,k]*reg3[h]+β[6,k]*reg4[h]+β[7,k]*herdsize[h]+β[8,k]*feed[h]+β[9,k]*sec2[h]+
          β[10,k]*sec3[h]+ β[11,k]*sec4[h]+β[12,k]*sec5[h]+β[13,k]*num[h]+β[14,k]*age[h]
     }
    sero[h]~dcat(p[h,1:K])
  }
#Priors for intercept
for (j in 1:1){
α[j,1]<-0.0
for(k in 2:K){
α[j,k]~dnorm(0,0.01)}
}
#Priors for random cohort effects
tau1~dgamma(0.5,0.001)
sig1<-1/sqrt(tau1)
tau2~dgamma(0.5,0.001)
sig2<-1/sqrt(tau2)

for (i in 1:I){
b2[i,1]<-0.0
b2[i,2]~dnorm(0,tau1)
b2[i,3]~dnorm(0,tau2)
}
#Priors for fixed effects
for(a in 1:14){
β[a,1]<-0.0
for(k in 2:K){
β[a,k]~dnorm(0,0.01)}
}
}
```

Manuscript 4

"Transmission parameters estimated for *Salmonella* Typhimurium in swine using susceptible-infectious-resistant models and a Bayesian approach"
Submitted to BMC Veterinary Research

1 # Transmission parameters estimated for *Salmonella* Typhimurium in swine
2 # using susceptible-infectious-resistant models and a Bayesian approach
3

4 **Carla Correia-Gomes[1,7*], Theodoros Economou[2], Trevor Bailey[2], Pavel Brazdil[3,4], Lis**
5 **Alban[5], João Niza-Ribeiro[1,6]**
6

7 [1]ICBAS-UP - Department of Population Studies, Instituto de Ciências Biomédicas Abel
8 Salazar – Universidade do Porto, Rua de Jorge Viterbo Ferreira, nº228, 4050-313 Porto,
9 Portugal
10 [2] CEMPS, University of Exeter, Harrison Building, North Park Road, Exeter, EX4 4QF,
11 United Kingdom
12 [3] LIAAD – INESC Porto L.A. Universidade do Porto, Rua de Ceuta, 118, 6º, 4050-190 Porto,
13 Portugal
14 [4] FEP-UP – Faculty of Economics – Universidade do Porto, Rua Dr. Roberto Frias, 4200-464
15 Porto, Portugal
16 [5]DMA - Danish Agriculture and Food Council, Axelborg, Axeltorv 3, DK-1609 Copenhagen,
17 Denmark
18 [6] ISPUP – Institute of Public Health – Universidade do Porto, Rua das Taipas, 4099-003
19 Porto, Portugal
20 [7]ERU-SRUC – Epidemiology Research Unit, Future Farming Systems, SRUC,
21 Drummondhill, Inverness, IV2 4JZ, United Kingdom
22 *Corresponding author
23

24 Email address:
25 CCG: carla.gomes@sruc.ac.uk

26 TE: T.Economou@exeter.ac.uk

27 TB: T.C.Bailey@exeter.ac.uk

28 PB: pbrazdil@inescporto.pt

29 LA: LIA@lf.dk

30 JNR: nizaribeiro@gmail.com

31

32 Abstract

33 Background

34 Transmission models can help in understanding disease dynamics and can then be used to test
35 the efficiency of control measures. The aim of this study was to formulate an appropriate
36 stochastic Susceptible-Infectious-Resistant/Carrier (SIR) model for *Salmonella* Typhimurium
37 in pigs and thus estimate the transmission parameters between states.

38 Methods

39 The transmission parameters were estimated using data from a longitudinal study of three
40 Danish farrow-to-finish pig herds known to be infected. A Bayesian model framework was
41 proposed, which comprised of Binomial components for the transition 1) from susceptible to
42 infectious and 2) infectious to carrier; and a Poisson component for carrier to infectious.
43 Cohort random effects were incorporated into these models to allow for unobserved cohort-
44 specific variables as well as unobserved sources of transmission, thus enabling a more
45 realistic estimation of the transmission parameters. In the case of the transition from
46 susceptible to infectious, a cohort time-varying random effect was used. The number of
47 infectious pigs not detected by the parallel testing was treated as unknown, and the probability
48 of non-detection was estimated using information about the sensitivity and specificity of the
49 bacteriologic and serologic tests.

50 Results

The estimate of the transmission rate 1) from susceptible to infectious was 0.33 [0.06, 1.52], 2) from infectious to carrier was 0.18 [0.14, 0.23] and 3) from carrier to infectious was 0.01 [0.0001, 0.04]. The estimate for the basic reproduction ration (R_0) was 1.91 [0.78, 5.24]. The probability of non-detection was estimated to be 0.18 [0.12, 0.25].

Conclusions

The proposed framework for stochastic SIR models was successfully implemented to estimate transmission rate parameters for *Salmonella* Typhimurium in swine field data. R_0 was 1.91, implying that there was dissemination of the infection within pigs of the same cohort. There was significant temporal-cohort variability, especially in the susceptible to infectious stage. The model adequately fitted the data, allowing for both observed and unobserved (cohort effects, test sensitivity) sources of uncertainty, indicating reliable estimates of transmission parameters.

Keywords: *Salmonella* Typhimurium; transmission parameters; Bayesian approach

Background

Salmonella Typhimurium is one of the major food-borne pathogens currently causing disease in humans [1] and it is often related with the consumption of pork products. Given its relevance to consumer food safety, *Salmonella spp.* control was considered necessary by the European food-safety policy makers under the EC Regulation 2160/2003. In the near future, it is possible that a mandatory target reduction will be put in place in the European Union, regarding the *Salmonella* prevalence for pigs.

However, in practice, the control of this agent has proved to be difficult and expensive at the farm level [2]. Consequently, the evaluation of the efficiency of control strategies relating to

75 this agent has become an important issue, as stated in recent reports [3]. Modelling the
76 dynamics of *Salmonella* Typhimurium in pigs is important in evaluating alternative control
77 strategies. The basic reproduction ratio expresses the secondary number of cases that a
78 primary case infects during the infectious period. If less than unity the disease is receding, but
79 when higher than unity the disease is spreading.
80 Susceptible – Infectious – Resistant (SIR) models are attractive tools that help in
81 understanding the disease dynamics. The SIR model formulates the changes of individuals in
82 the population between different disease states in terms of a system of ordinary differential
83 equations (ODE), known as the Kermack-McKendrick ODE model [4]. The variables in the
84 system are given by the three components: group of susceptible (S), group of infectious (I)
85 and group of carriers (R). SIR models include a mathematical specification of the movement
86 in and out of the three components. The key parameter in each of these mathematical
87 specifications is the transition rate: from S to I (β), from I to R (α) and from R to I (ν). If such
88 modelling is to be helpful in infectious disease control, it is critical to have the best possible
89 estimates of these rates (β, α and ν), as all three of them are important in modelling the spread
90 of the infection. Transmission data, generated under controlled conditions are necessary to
91 estimate the transition rates as accurately as possible.
92 In most cases *Salmonella* Typhimurium causes subclinical infection with no apparent
93 symptoms of disease in swine which makes it difficult to assess the infection status of
94 individual pigs in an infected population without testing each animal several times. One of the
95 difficulties in obtaining accurate estimates for β in *Salmonella* Typhimurium studies stems
96 from the fact that the currently available bacteriological and serological tests used to assign
97 the infection status are imperfect, introducing uncertainty when trying to classify each animal.
98 Yet another source of uncertainty comes from the fact that pigs, once infected, shed the agent
99 intermittently.

100 In the literature, it is conventional to use generalised linear models (GLMs) to describe the
101 counts of animals, e.g. from S to I using either Poisson [5-8] or Binomial models [4, 9]. In
102 fact, GLMs can be used to estimate all three transmission parameters although they lack
103 flexibility, for instance to also capture the effect of sensitivity and specificity of the diagnostic
104 test used. GLMs also lack the flexibility to allow for unobserved effects from variables not
105 recorded in the data, but which influence the outcomes. The Bayesian framework proposed in
106 this paper is flexible enough to incorporate such effects, but also quantifies the uncertainty
107 due to imperfect diagnostic tests.
108 To follow cohorts of animals in order to determine the dynamics of *S.* Typhimurium in
109 susceptible populations it is a very expensive procedure, so only few of such studies exist. In
110 this paper, we use data from a previous observational study designed and performed by
111 Kranker *et al* [10].
112 A Bayesian modelling framework was proposed and used to estimate transmission parameters
113 (transition rate from S to I, transition rate from I to R and transition rate from R to I) for
114 *Salmonella* Typhimurium in pig herds, using the longitudinal data from Kranker *et al* [10].
115 The sensitivity and specificity of the tests used to classify the animals in the Kranker study
116 were allowed for in the statistical model, which also incorporated random effects to allow for
117 cohort heterogeneity.
118

119 Methods

120 *Study herds, sampling, bacteriology and ELISA test*
121 The data used have been previously described by Kranker *et al* [10] and originate from three
122 Danish pig herds known to be infected with *Salmonella* Typhimurium. The herds had
123 moderate to high levels of *Salmonella* Typhimurium and therefore the within-herd prevalence

124 was 40% or higher based on meat-juice samples collected over three months, evaluated by use
125 of a cut-off of 20 optical density percentage (OD%). Two of the farms, with 650 and 440
126 sows, respectively, were two-site operations while the remaining farm was a three-site
127 operation with 300 sows. The three herds were self-supplying. In each herd, 10 litters were
128 randomly selected, and in each litter, the ears of six randomly selected piglets were tagged. To
129 account for variations in *Salmonella* shedding over time, litters from each herd were divided
130 into two groups of five litters that were raised at approximately one-month intervals. Thus, on
131 each farm there were two cohorts consisting of 30 pigs each, yielding a total of 180 piglets at
132 the start of the study. All ear-tagged pigs from a given cohort were supposed to be raised
133 together for the entire observation period. The animals were followed longitudinally [10] and
134 were first tested at the age of four weeks and thereafter at two to five week intervals until the
135 age of slaughter (varied between cohorts but on average around 25 weeks). The testing
136 occasions varied between cohorts (six to seven times). At each testing occasion, sera and
137 faeces from the animals were collected and tested for the presence of *Salmonella spp.* (at the
138 age of four weeks only faeces were collected, because maternal antibodies still present could
139 give a false positive result). An animal was considered serologically positive, wherever the
140 serological test revealed a result of OD% >20, and bacteriological positive if *Salmonella* was
141 isolated from the faeces. The serological test used at this cut-off value is considered to have a
142 sensitivity of 68% and to be 100% specific [11]. The bacteriological test is considered to be
143 100% specific and the sensitivity is around 30 to 55% [12]. These test characteristics were
144 incorporated in the statistical model.
145

146 *Infection status of the pigs*

147 The testing time interval was different in each cohort, specifically it varied from two to five
148 weeks. A homogenous data set was derived by inferring the infection status of each pig, every

149 two weeks. The time step of two weeks was chosen because on average it takes two weeks for
150 an animal to test positive to serology after being infected. It was therefore assumed that an
151 animal was infectious in the two weeks before being seropositive. The most likely infection
152 status of each pig was determined for each time step (two weeks) based on both the faecal
153 shedding and the serology of every sampling period. Each animal was categorized as
154 susceptible (S), infectious (I) or carrier (R). A susceptible animal was considered to be an
155 animal without the agent and susceptible to be infected. An infectious animal was considered
156 to be an infected animal shedding the agent, meaning it could infect other animals. A carrier
157 animal was considered to be an infected animal not shedding the agent and therefore not able
158 to infect other animals. In the absence of reasonable sensitivity of the bacteriological culture
159 method, serology offered an alternative and complementary way to assign the infection status
160 of a pig.
161 Pigs were attributed status S when there was no presence of bacteria in the faecal samples and
162 the OD% was below 20. Status I was assigned from the date when a pig was found
163 bacteriologic-positive until it stopped being bacteriologic-positive. Additionally, pigs were
164 assigned to status I based on seroconversion. The beginning of the infectious period was set to
165 two weeks prior to the recorded date of seroconversion [14, 15] and the duration was set to
166 four weeks, assuming that a pig would shed *Salmonella spp.* within an average of four weeks.
167 This average period was based on data regarding the shedding period duration, from
168 experimental studies [13, 14]. So for pig classification, information was used from both tests
169 in parallel. Finally, status I was followed by status R and the pigs could return to status I if
170 they were found culture positive later on during the study period. It was assumed that no pig
171 would return to the susceptible status after being infected, because of the relative short life
172 span of finisher pigs (after infection it takes around 112 days to clear the agent from the
173 organs [16], which is too long for post-weaned pigs). A particular example of how the

174 classification was performed: if a pig was shedding at a specific testing time, it was
175 considered infectious up until a negative culture, after which it was considered as carrier. On
176 the other hand if an animal was not positive to culture nor to the ELISA test, it was
177 considered susceptible. However, if in the next testing occasion it was found positive to the
178 ELISA test (in the presence of a negative culture), then it was considered infected and
179 therefore branded as infectious for at least four weeks, beginning two weeks prior the testing
180 time.
181 Given that in the beginning of the follow-up piglets could only be tested by use of
182 bacteriology (which has low sensitivity), some piglets infected by the sow could have been
183 erroneously classified as susceptible. Therefore, the analysis in each cohort started at the time
184 infected animals were first detected (by either serology or bacteriology).
185
186 *Estimation of the transmission parameters*
187 Conventionally, transmission parameters of infectious disease, including *Salmonella spp.*, in
188 swine herds [16-22] are estimated using regression models, often based on data describing the
189 prevalence of the country or region to which the particular study refers to. As suggested in
190 some studies [5, 23, 24], stochastic SIR models were first applied here in the form of
191 Generalised Linear Models (GLMs), in order to estimate the three transmission parameters.
192 However, preliminary results (not reported here) suggested the presence of overdispersion in
193 the GLMs, hinting towards unobserved sources of variation in the data such as cohort
194 heterogeneity. Here a framework for stochastic SIR models is proposed which 1) extends the
195 current GLM framework by including random effects, 2) is implemented using a Bayesian
196 approach thus allowing incorporation of prior information (such as the sensitivity of
197 *Salmonella* tests), 3) explicitly estimates the probability of not detecting infectious animals
198 due to test sensitivity and 4) incorporates all sources of uncertainty/variation thus obtaining

199 more realistic estimates of transmission parameters. As suggested by some authors [5], the
200 inclusion of random effects automatically accounts for overdispersion by inflating the
201 variance of the response variables while at the same time allowing for cohort heterogeneity.
202 Stochastic SIR models (and other variants such as SI or SIS models) are well-established in
203 animal disease literature, for instance see [20-22] for recent examples, and also the book by
204 Diekmann and Heesterbeek [4], chapter 1. The benefit in using stochastic SIR model is that
205 transmission parameters can be estimated using statistical modelling, and here the
206 conventional stochastic SIR models was extended by explicitly allowing for cohort variation
207 and unobserved temporal effects. Below the three components of the stochastic SIR model are
208 described in detail.

209 *1) Transition from susceptible to infectious*

210 It was assumed that pigs become infected by "infectious contacts" defined as: either contact
211 with other infected animals, or contact with their environment (rodents, contaminated muck or
212 feed). The rate at which a given animal has infectious contacts was assumed 1) to be constant
213 in time and 2) proportional to the density of infectious animals [20], with a constant of
214 proportionality β, i.e. the transmission rate parameter. In other words, the infectious contacts
215 per animal happen randomly in time so that their occurrence can be described by a Poisson
216 process. More precisely, the number of infectious contacts per animal, in a period Δt is
217 Poisson distributed with mean $\lambda=\beta(I/N)\Delta t$, where I is the number of infectious animals and N
218 is the total number of animals, at the beginning of Δt. As such, the probability of no
219 infectious contacts per animal in Δt is $\exp(-\beta(I/N)\Delta t)$, implying that the probability of
220 infection in Δt is $p = 1- \exp(-\beta(I/N)\Delta t)$. This in turn implies that the number of new cases C at
221 the end of Δt is Binomial with parameters S and p so that the mean of C is S*p.
222 Here, the current established methodology was extended to allow for the fact that 1) λ may
223 vary in time due to exogenous factors and 2) λ may vary across cohorts due to unobserved

224 cohort effects. So, a random (scaling) effect $\exp(r_{jt})$ was included, for the jth cohort at time t,

225 to get $\lambda_{jt}=\beta(I/N)\exp(r_{jt})\Delta t$ as the mean number of infectious contacts of a random animal, in

226 herd j at time t. Note that Δt denotes the length of a time interval whereas t refers to actual

227 time. On average, $\exp(r_{jt})$ was assumed to be equal to one, so that across all cohorts and time,

228 the average transmission rate parameter is still β. By doing this, variations due to cohort or

229 unknown temporal effects was explicitly modelled, which would otherwise contribute to the

230 uncertainty in estimating β.

231 Recall that all time intervals in the data are equal to two weeks so for clarity, $\Delta t=1$ was set so

232 that one time step Δt corresponds to two weeks. This does not qualitatively affect the

233 estimation of the transmission parameters. Because of the nature of the data, time t is now

234 defined in discrete consecutive (biweekly) time steps.

235 The model may be formulated as follows:

236 $C_{jt} \sim \text{Binomial}(S_{jt}, p_{jt})$

237 $p_{jt} = 1 - \exp\{ -\beta(I_{jt-1}/N_{jt-1})\exp(r_{1jt}) \}$

238 $\text{cloglog}(p_{jt}) = \log(\beta) + \log(I_{jt-1}) - \log(N_{jt-1}) + r_{1jt}$ (1)

239 where:

240 - C_{jt} denotes the number of new infectious animals in cohort (j) at the end of the time step (t),

241 - S_{jt-1} is the number of susceptible animals in cohort (j) at the end of the time step (t-1),

242 - p_{jt} is the probability of a susceptible animal in cohort (j) at the end of time step (t-1)

243 becoming infectious by the end of time step (t),

244 - cloglog is the complementary log-log transformation,

245 - β is the transmission rate parameter for the transition from susceptible to infectious,

246 - I_{jt-1} is the number of infectious animals in cohort (j) at the end of the time step (t-1),

247 - N_{jt-1} is the total number of animals in cohort (j) at the end of the time step (t-1), and

248 - r_{1jt} is a cohort time-dependent random effect (which is zero on average).

249 Note that, at the beginning of the study, pigs were considered to be either in the S or I status
250 depending on the test results. When there was no infectious pig present at the end of the
251 previous time step, i.e. $I_{jt-1} = 0$, the probability of becoming infectious was modelled as:
252 $cloglog(p_{jt}) = log(\beta) + r_{1jt}$. This is because even if there are no infectious pigs around, animals
253 can still be infected (e.g., contaminated environment, feed, water, etc.). In this formulation, β
254 is seen as the underlying rate of transition for a random pig in an average cohort with no
255 infectious animals, while r_{1jt} allows for unobserved cohort-time effects in the data e.g.,
256 anthropogenic influence, rodents etc. Note that homogeneous mixing of the pigs in each cohort (i.e.
257 all pigs could come into contact with each other) was assumed, due to the small size of the cohorts.
258
259 In using the number of infectious pigs I_{jt}, in each cohort at the end of time step t, it was
260 necessary to account for the sensitivity of both the serological and bacteriological test. Since
261 the specificity in both tests is considered to be 100%, the parallel specificity is 1. This implies
262 that $I_{jt} = Iobs_{jt} + Inob_{jt}$, where $Iobs_{jt}$ is the observed value and $Inob_{jt}$ is the number of
263 infectious animals not detected (false negative pigs). In other words, $Iobs_{jt}$ is a lower bound
264 on the actual I_{jt}. The unobserved variable $Inob_{jt}$ may be incorporated (and thus estimated) in
265 the stochastic model and here it was assumed that it has a Binomial distribution with
266 parameters N_{jt} and pND where pND is the probability of not detecting infectious animals.
267 This probability, pND, is of course dependent on the sensitivity probabilities of each test,
268 which were assumed to be independent. $Inob_{jt}$ was modelled as follows:
269 $I_{jt} = Iobs_{jt} + Inob_{jt}$
270 $Inob_{jt} \sim Binomial(N_{jt}, pND)$
271 $pND = (1\text{-}SenC)*(1\text{-}SenE)$ (2)
272 where:
273 - *SenC* is the sensitivity probability of microbiological culture, and

274 - *SenE* is the sensitivity probability of the ELISA test.

275 Treating $Inob_{jt}$ as an unobserved random variable allows formal quantification of the
276 uncertainty in the data due to test sensitivity and constitutes one of the novelties of the
277 proposed model. The Bayesian framework (see section 5 later on) used to estimate the
278 stochastic SIR model can easily incorporate the estimation of $Inob_{jt}$ given prior information on
279 SenC and SenE.

280

281 2) *Transition from infectious (I) to resistant (R)*

282 The rate α at which a random infectious animal, in a given cohort, becomes carrier was
283 assumed to be constant in time. As such, the length of time τ until an infectious animal
284 becomes carrier can be modelled by an exponential distribution with rate parameter α. So,
285 given that the animal is infectious at the start of time interval Δt, the probability pR of
286 becoming carrier is $pR = Pr(\tau \leq \Delta t) = 1\text{-}exp(-\alpha \Delta t)$ since τ is exponentially distributed (recall
287 that Δt=1 was set for conciseness). Like before, a random cohort effect r2j was added to allow
288 for cohort heterogeneity in the data, to obtain $pR_j = 1\text{-}exp(-\alpha exp(r2_j))$. The number of new
289 carrier animals $Rnew_{jt}$ at the end of time step t, is thus Binomial with parameters I_{jt} and pR_j.
290 Note that a single parameter α was utilised, describing the rate at which a random infectious
291 animal in an average cohort, becomes carrier. However, cohort variability (not all cohorts are
292 average) was allowed for through $r2_j$, which in turn reduces uncertainty in estimating α. The I
293 to R transition was modelled as follows:

294 $Rnew_{jt} \sim Binomial(I_{jt}, pR_j)$

295 $cloglog(pR_j) = log(\alpha) + r_{2j}$ (3)

296 3) *Transition from resistant to infectious*

297 For this compartment of the model, the rate of infectious contacts ν in a random carrier animal
298 was assumed to be constant in time where ν is the transmission rate parameter for the

299 transition from carrier to infectious. With similar arguments as in the S to I compartment, the

300 number of infectious contacts per animal in time period Δt is Poisson distributed with mean

301 $\nu\Delta t$. Since this transition was actually a rare event (only happened three times in the entire

302 study), the Poisson distribution can be used, since it approximates the Binomial when its

303 probability parameter is close to zero. So if in cohort j, there are R_{jt-1} carrier animals at the

304 end of the previous time step, the number of transitions R to I in time step t may be modelled

305 as a Poisson variable with mean $\mu_{jt} = \nu R_{jt-1} \exp(r_{3j})$ or more explicitly:

306 $Inew_{jt} \sim Poisson(\mu_{jt})$

307 $\log(\mu_{jt}) = \log(\nu) + \log(R_{jt-1}) + r_{3j}$ (4)

308 where:

309 - $Inew_{jt}$ denotes the number of new infectious animals (that result from this transition) in

310 cohort (j) at the end of the time step (t),

311 - μ_{jt} is the mean number of carrier animals that become infectious in the cohort (j) during time

312 step (t),

313 - ν is the transmission rate parameter for the transition from carrier to infectious state,

314 - R_{jt-1} is the number of carrier animals at the end of the time step (t-1) in cohort (j), and

315 - r_{3j} is a cohort random effect that allows for cohort heterogeneity.

316 Note that $R_{jt-1} = 0$ is possible, in which case $\log(R_{jt-1}) = 0$ was set. The argument for doing that

317 is that the transmission rate parameter ν may be defined as the limit of μ_{jt}/R_{jt-1} as R_{jt-1} goes to

318 zero. As such, ignoring the random effect for a moment, μ_{jt}/R_{jt-1} should tend to a constant (i.e.

319 ν) as R_{jt-1} goes to zero rather than infinity. Note that in our data, $R_{jt-1} = 0$ happened on 20% of

320 the occasions. In the hypothetical case that $R_{jt-1} = 0$ for the majority of time steps and cohorts,

321 then this component of the model (i.e. the transition R to I) becomes redundant as there will

322 ultimately be almost no information with which to estimate the transition parameter.

323 *4) Cohort random effects*

324 As indicated above, random cohort effects were incorporated into each transition step to allow
325 for 1) cohort heterogeneity/variability in the data, 2) unobserved cohort-specific factors, 3)
326 unobserved temporal effects in the S to I compartment. These effects were different for each
327 transition under the assumption that any unobserved cohort factors affect each transition in a
328 different way. For the transitions S to I and R to I, these random effects also allow for factors
329 which affect the spreading of disease which are not dependent on the animals themselves (as
330 for example contaminated environment, feed, water, etc.).
331 For the transition S to I, the cohort random effects were assumed to be time-varying and auto-
332 correlated, and were modelled as:

333
$$\begin{aligned} r_{1j,t=1} &\sim Normal(0, \sigma_1^2) \\ r_{1j,t} &\sim Normal(r_{1j,t-1}, \sigma_1^2) \end{aligned} \tag{5}$$

334 where the cohort random effect (r_{1jt}) for time step t depends on the previous cohort random
335 effect at time (t-1). With this cohort time-dependent random effect any unobserved dynamic
336 behaviour in the spreading of the infection within cohorts was captured, such as the spread of
337 the infection due to infected mice.
338 For the transition I to R and R to I, the random effects were modelled as:

339
$$r_{kj} \sim Normal(0, \sigma_k^2), k = 2,3 \tag{6}$$

340 where:
341 - subscript *j* denotes cohorts and
342 - σ_k^2 is the variance of the unobserved cohorts effects.
343 In a preliminary model building stage, a cohort time-dependent random effect, r_{2jt}, for the
344 transition I to R was considered, however the results showed no improvement to the model fit.
345 The transition R to I was rare (only happened three times in the study) so there was
346 insufficient data for using a cohort time-dependent random effect.

347

348 *5) Model implementation*

349 The overall SIR model described above was implemented in a Bayesian framework and fitted
350 using Markov chain Monte Carlo (MCMC). In this framework, parameters are treated as
351 random variables whose "prior" distribution expresses our uncertainty about their value
352 before any data is observed. After data is obtained though, prior distributions (or simply
353 priors), are combined with the data through Bayes theorem to produce the posterior
354 distributions (or simply the posteriors) of each parameter. The posteriors express the
355 uncertainty about model parameters after data is observed and all statistical inference is based
356 solely on the posteriors. MCMC is a numerical technique which produces samples of values
357 that eventually converge (after a certain "burn-in" number) to samples of values from the
358 posterior (distribution) of each parameter.

359

360 There was no historical information with which to inform the prior distributions of $\log(\beta)$,
361 $\log(\alpha)$ and $\log(\nu)$, so Normal distributions with zero mean and a variance of 100, which
362 reflected prior ignorance while avoiding the use of improper prior distributions, were used
363 [25]. For the sensitivity probabilities of both serological and bacteriological tests, a Beta
364 distribution was used as a prior. Previous information about the sensitivity of both tests [11,
365 12] was used to inform those Beta distributions: a mean of 0.49 for faecal culture and a mean
366 of 0.68 for Danish mix ELISA were assumed, so SenC~Beta(48.5, 50.5) and SenE~Beta
367 (58.5, 27.5) were specified. These priors have means 0.49 and 0.68 respectively, and
368 variances that match the range of possible values dictated by the findings of [11, 12].
369 Specificity was assumed to be 100% in both tests. The precision (i.e. the inverse of the
370 variance) of the Normal distribution for each random effect was given a Gamma (0.5, 0.005)
371 prior distribution (large mean and very large variance to indicate prior ignorance).

372 The complete SIR model was implemented in the open-source statistical software WinBUGS
373 [26]. 100,000 posterior samples were collected after a 5,000 sample burn-in to ensure
374 convergence to the posterior distribution [27]. Two MCMC runs were performed, with
375 dispersed initial values, to ensure convergence and mixing. The samples were thinned by only
376 collecting one in 10 consecutive samples to eliminate autocorrelation in posterior samples (the
377 R package "coda" [28] was used), so that in total we ended up with 20,000 samples.
378 Convergence was assessed by inspection of trace-plots but also more formally using the
379 Raftery and Lewis diagnostic, and the Gelman-Rubin R-hat diagnostic which should be
380 sufficiently close to one if convergence was achieved [29, 30]. Mixing in the chains was
381 assessed by comparing the Markov Chain (MC) error with the standard deviation, for each
382 parameter. Ideally the MC error for each parameter should be less than 5% of the standard
383 deviation [31] for good mixing.
384 Posterior predictive simulation was used for model checking as described by Gilks *et al.*[25].
385 This technique is effectively testing whether the observed data are extreme in relation to the
386 posterior predictive distribution of the observations (i.e., the fitted model). The deviance was
387 the measure adopted for comparison. The technique involves the calculation of a "p-value"
388 which should not be extreme (close to 0 or 1) for good model fit.
389
390 *6) Calculations of the basic reproduction ratio (R_0)*
391 Samples from the posterior distribution of R_0 were calculated from those of β and α by use of
392 the following formula [5]:
393 $$R_0 = \beta / \alpha \quad (7)$$
394 where β is the transition rate from S to I, and α is the transition rate from I to R.
395

Results

Transmission parameters

Results, in terms of summary statistics from the posterior samples, are shown in Table 1. Note that the posterior samples are effectively samples from the posterior distribution of each model parameter and all inference is based on those samples. A point estimate, the standard error and the 95% credible interval for a parameter, are for instance calculated as the sample mean, the sample standard deviation and the sample 2.5% and 97.5% quantiles of the posterior samples for that parameter.

The MCMC convergence was considered acceptable since the R-hat for all parameters (including random effects) was never above 1.001. The results of the model did not significantly differ when the parameters of the priors for the sensitivity tests were varied (increasing and decreasing them by 10%).

The posterior distribution for transition rate α (I to R) was symmetric, but for the transition rate β (S to I) and ν (R to I), the posterior distributions were asymmetric (Fig 1, Fig 2 and Fig 3). As such, the posterior median was chosen to best summarise the value of these parameters. The median for the transition rate β was 0.33, for α it was 0.18 while for ν it was 0.01 (Table 1). The median of the variance of cohort random effects for the transitions I to R and R to I was close to zero, which implies that there was little significant variation between cohorts for these two transitions of the model. The median of the variance of cohort-time dependent random effect for the transition S to I was 2.6 (95% credible interval [0.80; 7.59]), meaning that the cohort random effect is significant for this transition (Fig 4). The overall model fit was satisfactory with a "p-value" of 0.24 implying no significant difference between posterior predictive simulations (predictions from the model) and observed data.

420 *Basic reproductive ratio (R_0)*

421 Summary statistics of the posterior distribution of the R_0 parameter are shown in Table 1. The
422 posterior median of R_0 was 1.91, with a 95% credible interval of 0.78 to 5.24. A density
423 estimate of the posterior samples of R_0, which effectively describes the spread of *Salmonella*
424 *spp.* in these three Danish pig herds known to be infected with *Salmonella*, is shown in Fig 5.
425 For moderate to high within herd *Salmonella* prevalence, this R_0 distribution suggests that
426 *Salmonella* Typhimurim can go from fading out scenarios to epidemic ones but most of the
427 time the infection spread assumes an endemic form.

428

429 *Test sensitivity (pND)*

430 Recall that this modelling framework includes the estimation of the probability of failing to
431 detect infectious animals, pND, using both the data but also prior information about the tests
432 i.e. [11, 12]. Figure 6 shows a density estimate plot of the posterior distribution of pND.

433

434 Summary and discussion

435 In this paper field data was used from a study [10] conducted in three Danish pig herds which
436 were known to be infected with *Salmonella* Typhimurium. That study was performed,
437 amongst other things, to describe the time of onset and duration of *Salmonella* shedding and
438 the patterns of bacterial transmission between individual pigs until slaughter. It is expensive
439 to undertake such studies and this limits the number of studies available. The procedure
440 followed to select the herds, the use of two tests to assess the pig status and the follow up of
441 each cohort during the whole fattening period, indicated that this dataset is sufficiently
442 reliable to be used in estimating the transmission parameter β but also R_0 for *Salmonella* in
443 finisher pigs.

444 In comparing the parameters to ones found in different studies (whether simulation- or
445 observation-based studies) it is important to take into consideration that the time interval used
446 in this study was two weeks whereas in past simulation studies it varies from one day [16, 18]
447 to one week [19]. However, as the transmission parameters are rates, they can be easily
448 transformed to relate to different time steps. Although the herds used in the Kranker study
449 [10] had moderate to high levels of *Salmonella* Typhimurium prevalence, the median
450 estimates of the transmission parameters from this study are lower than those found in
451 previous simulation studies [16, 18]. The transition rate β from S to I is slightly higher when
452 compared to the Lurette *et al*. study [19] although the other rates (α, ν) are lower than the
453 equivalent parameters in that same study. So the use of this framework to these Danish herds
454 resulted in estimates comparable to other similar studies (note that this approach could easily
455 be used with data from other countries). Moreover, the prevalence of *Salmonella* in finishing
456 pigs in Denmark is known to be the close to the average prevalence in the EU [32].
457 To the best of our knowledge this is the first study of transmission rate parameters for
458 *Salmonella* Typhimurium in swine that estimates the parameters using field data and a
459 Bayesian probabilistic approach incorporating random effects.
460

461 *Bias of the study*

462 Correct classification of the infectious status of the individual pig is difficult for *Salmonella*
463 Typhimurium infection, because the diagnostic tests currently used are imperfect [33-38].
464 Bacteriology lacks sensitivity given intermittent shedding of *Salmonella* by infected pigs,
465 whereas using serology in individuals can be associated more with a past exposition to the
466 agent than a current exposition, so it can lack specificity for detecting animals shedding.
467 Positive serology also shows a delay between infection and expression, leading to some lack
468 of sensitivity. When analysing the data, the lack of sensitivity was accounted by: 1) starting

469 the analysis when at least one infected pig per cohort was observed and 2) by using the
470 probabilistic framework to predict the infectious animals that were not detected with these
471 tests, from appropriately informed distributions based on the sensitivity of each test.
472
473 For optimal estimation of transmission parameters, the time step between each sampling
474 should preferably be as short as the average generation interval, spanning from the time when
475 one animal becomes infectious to the time when a second animal becomes infectious because
476 of the first animal. The time steps in this data (two weeks) are not ideal – preferably days or
477 perhaps one week would be better [14]. However as previously discussed, the available data
478 did not allow for such an option and it would be very costly to obtain new data. As data from
479 a published study [10] was used, the time step was set to be an approximation of the different
480 testing intervals within and between cohorts, given the limitations offered by the original set
481 of data, and an approximation to the time of seroconversion [14, 15]. This approximation
482 could have affected the estimation of parameters due to the big time interval between testing
483 occasions. Nevertheless, comparison with the results published in other studies does not seem
484 to support this hypothesis. Concerning the cohorts, it is clear from the Kranker study [10] that
485 particular attention was paid to the selection of the herds, which were taken from a large
486 population of Danish finishing herds with a well-known status for *Salmonella*. This gave us
487 confidence regarding the generalization of our results, at least for infected herds.
488
489 *Transition parameters* and R_0 values
490 Note that the stochastic SIR model presented here is of course only a discrete-time
491 approximation to the real transmission dynamics, i.e. limited to bi-weekly intervals. In
492 particular, when the number of susceptible animals is small and the infection intensity high,
493 then the expected number of infectious animals will tend to be overestimated [5].

494 The estimate of the transition rate β (from S to I), is low compared with other infectious
495 diseases (such as swine influenza) and reflects the fact that in most of the herds, *Salmonella*
496 does not cause outbreaks but maintains a residual level of infection represented by infectious
497 and carrier animals that enable the infection to persist in the herds. The credible interval for
498 the transition parameter α (from I to R), matches the variability of shedding duration that is
499 known from experimental and field studies [14, 39]. The transition rate estimate ν (from R to
500 I) is small and possibly dependent on stress events (the authors of the Kranker *et al.* study
501 [10] describe that two cohorts in which animals began shedding in a second round had a
502 slurry overflow which can be considered a stressful event). The variance of the cohort time-
503 dependent random effect was high and a possible explanation for that is the different
504 management of cohorts which in turn induces high variability (between cohorts) in the
505 transmission data. In future studies this should be taken into consideration as a way to
506 minimize transmission of infection.
507 For spread to occur, R_0 should be above one. Looking at Fig 5 we can see that there is high
508 probability that $R_0 > 1$, 94% specifically. The median R_0 value was 1.91 indicating that
509 *Salmonella* Typhimurium was spreading in most of the cohorts. The value is not high (third
510 quartile of R_0 is less than 3) implying it would not spread rapidly through the susceptible
511 populations under management systems similar to the ones used in these herds. With lower
512 probability, R_0 is high enough to cause outbreaks, e.g. probability that $R_0 > 5$ is 2.5%.
513 The R_0 95% Credible Interval (CrI) ranges from 0.78 to 5.24. The higher values reflect that
514 animals infected with a high infectious dose have a longer shedding period [14] than the ones
515 infected with low infectious dose, and so the former can cause an outbreak. This makes sense
516 because *Salmonella* Typhimurium is an agent that primarily spreads via the faecal-oral route.

517 Few studies are available to aid in defining infectious animals, but the experimental and field
518 studies conducted by some authors [13, 14] support the duration of infectiousness used in our
519 study.
520 Regarding control strategies, the authors suggest keeping the herd in a low prevalence
521 endemic state, to prevent the development of infection and reduce the probability of long-time
522 shedders, by use of proper feeding and management practices designed to decrease the
523 environmental contamination of pens.
524 A next step in our investigation will be to include the estimated transmission parameters (β, α,
525 ν) in a stochastic simulation model developed by the authors to simulate the spreading of
526 *Salmonella* Typhimurium in swine herds and thus test the effectiveness of different control
527 strategies.

528 # Conclusions

529 A Bayesian framework was proposed, in order to estimate *Samonella* Typhimurium
530 transmission parameters, and this has been successfully implemented to data from Danish pig
531 herds. The model extends current established methodology which utilises GLMs to implement
532 stochastic SIR models. Random effects were added to 1) capture unobserved sources of
533 variability due to pigs being divided in cohorts and 2) avoid the problem of overdispersion.
534 Results in terms of posterior samples allow for direct probabilistic statements about model
535 parameters, which may be also used in other analyses such as simulation models for testing
536 management strategies.
537 The issue of underestimating infectious pigs due to testing sensitivity was addressed by
538 predicting the number of non-detected pigs, using 1) prior information about test sensitivity
539 and 2) the observed data. In doing that, the probability of non-detection was treated as an
540 unknown parameter which was estimated at the same time as the transmission parameters.

541 All model unknowns (transmission parameters, cohort random effects, non-detected pigs,
542 probability of non-detection) were estimated simultaneously, implying that all possible
543 sources of uncertainty were modelled, in turn giving more confidence about the estimates of
544 the transmission parameters.

545

546 **Competing interests**

547 The authors declare that they have no competing interests.

548

549 **Author's contributions**

550 CCG and TE were involved in the design and performed the statistical modelling analysis and
551 drafted the manuscript. TB was involved in the design of the statistical analysis and the
552 revision of the manuscript for intellectual contents. LA and PB were involved in the revision
553 of the manuscript for intellectual contents. JNR was involved in the drafting and revision of
554 the manuscript for intellectual content. All authors approved the final manuscript.

555

556 **Acknowledgements**

557 We would like to thank to Danish Agriculture & Food Council and the authors of the study
558 (Kranker et al., 2003) for allowing us to be using their data.
559 We would like to thank FCT for the PhD scholarship (SFRH/BD/40932/2007) and for the
560 pluriannual support of LIAAD-INESC Porto L.A.

561

562 # References

563 1. EFSA: **The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic
564 Agents and Food-Borne Outbreaks in the European Union 2008**. *The EFSA Journal* 2010,
565 **1496**:288.

566 2. Hurd HS, Enoe C, Sorensen L, Wachman H, Corns SM, Bryden KM, Grenier M: **Risk-based**
567 **analysis of the Danish pork Salmonella program: past and future**. *Risk Anal* 2008, **28**(2):341-
568 351.
569 3. Consortium F: **Analysis of the costs and benefits of setting a target for the reduction of**
570 ***Salmonella* in slaughter pigs for European Commision Health and Consumers Directorate-**
571 **General SANCO/2008/E2/036 Final Report**. In.; 2010: 1-198.
572 4. Diekmann O, Heesterbeek JAP (eds.): **Mathematical Epidemiology of Infectious Diseases:**
573 **Model Building, Analysis and Interpretation**. Chichester: John Wiley & Sons Ltd; 2000,
574 Chapter 2.
575 5. Nielsen LR, van den Borne B, van Schaik G: **Salmonella Dublin infection in young dairy**
576 **calves: transmission parameters estimated from field data and an SIR-model**. *Prev Vet Med*
577 2007, **79**(1):46-58.
578 6. Tsutsui T, Kobayashi S, Hayama Y, Nishiguchi A, Kameyama K, Konishi M, Murakami K:
579 **Estimation of the within-herd transmission parameter of bovine leukemia virus**. *Prev Vet*
580 *Med* 2010, **95**(1-2):158-162.
581 7. Bouma A, de Jong MCM, Kimman TG: **Transmission of pseudorabies virus within pig**
582 **populations is independent of the size of the population**. *Prev Vet Med* 1995, **23**(3-4):163-
583 172.
584 8. Yoshida N, Hara T: **Global stability of a delayed SIR epidemic model with density dependent**
585 **birth and death rates**. *Journal of Computational and Applied Mathematics* 2007, **201**(2):339-
586 347.
587 9. Thomas E, Bouma A, Klinkenberg D: **A comparison of transmission characteristics of**
588 **Salmonella enterica serovar Enteritidis between pair-housed and group-housed laying**
589 **hens**. In: *Veterinary research.* 2011.
590 10. Kranker S, Alban L, Boes J, Dahl J: **Longitudinal Study of Salmonella enterica Serotype**
591 **Typhimurium Infection in Three Danish Farrow-to-Finish Swine Herds**. *J Clin Microbiol* 2003,
592 **41**(6):2282-2288.
593 11. Harris IT: **Serologic basis for assessment of subdinical Salmonella infection in swine: Part 1**.
594 *Journal of Swine Health and Production* 2003, **11**(5):247-251.
595 12. Eriksson E, Aspan A: **Comparison of culture, ELISA and PCR techniques for salmonella**
596 **detection in faecal samples for cattle, pig and poultry**. *BMC veterinary research* 2007, **3**:21.
597 13. Nielsen B, Baggesen D, Bager F, Haugegaard J, Lind P: **The serological response to Salmonella**
598 **serovars typhimurium and infantis in experimentally infected pigs. The time course**
599 **followed with an indirect anti-LPS ELISA and bacteriological examinations**. *Veterinary*
600 *microbiology* 1995, **47**(3-4):205-218.
601 14. Fravalo P, Cariolet R, Proux K, Salvat G: **Le portage asymptomatique de *Salmonella enterica***
602 **par les porcs: résultats issues de la constituition d'un modèle en conditions**
603 **expérimentales**. In: *35èmes Journées de la Recherche Porcine: 2003; Paris, France*: ITP, INRA;
604 2003: 393-400.
605 15. Beloeil PA, Chauvin C, Proux K, Rose N, Queguiner S, Eveno E, Houdayer C, Rose V, Fravalo P,
606 Madec F: **Longitudinal serological responses to Salmonella enterica of growing pigs in a**
607 **subclinically infected herd**. *Prev Vet Med* 2003, **60**(3):207-226.
608 16. Soumpasis I, Butler F: **Development and Application of a Stochastic Epidemic Model for the**
609 **Transmission of Salmonella Typhimurium at the Farm Level of the Pork Production Chain**.
610 *Risk Anal* 2009:1521-1533.
611 17. Hill AA, Snary EL, Arnold ME, Alban L, Cook AJ: **Dynamics of Salmonella transmission on a**
612 **British pig grower-finisher farm: a stochastic model**. *Epidemiology and infection* 2008,
613 **136**(3):320-333.

614 18. van der Gaag MA, Vos F, Saatkamp HW, van Boven M, van Beek P, Huirne RBM: **A state-**
615 **transition simulation model for the spread of Salmonella in the pork supply chain**.
616 *European Journal of Operational Research* 2004, **156**(3):782-798.
617 19. Lurette A, Belloc C, Touzeau S, Hoch T, Ezanno P, Seegers H, Fourichon C: **Modelling**
618 **Salmonella spread within a farrow-to-finish pig herd**. *Veterinary research* 2008, **39**(5):49.
619 20. Velthuis AGJ, De Jong MCM, Kamp EM, Stockhofe N, Verheijden JHM: **Design and analysis of**
620 **an *Actinobacillus pleuropneumoniae* transmission experiment**. *Prev Vet Med* 2003, **60**:53-
621 58.
622 21. Broens EM, Espinosa-Gongora C, Graat EAM, Vendrig N, Van der Wolf PJ, Guardabassi L,
623 Butaye P, Nielsen JP, De Jong MCM, Van De Giessen AW: **Longitudinal study on transmission**
624 **of MRSA CC398 within pig herds**. *BMC veterinary research* 2012, **8**(58):10.
625 22. Romagosa A, Allerson M, Gramer M, Soo Joo H, Deen J, Detmer S, Torremorell M:
626 **Vaccination of influenza a virus decreases transmission rates in pigs**. *BMC veterinary*
627 *research* 2011, **42**(120):1-15.
628 23. Geenen PL, Dopfer D, Van der Meulen J, De Jong MC: **Transmission of F4+ E. coli in groups of**
629 **early weaned piglets**. *Epidemiology and infection* 2005, **133**(3):459-468.
630 24. Eble P, de Koeijer A, Bouma A, Stegeman A, Dekker A: **Quantification of within- and**
631 **between-pen transmission of Foot-and-Mouth disease virus in pigs**. *Veterinary research*
632 2006, **37**(5):647-654.
633 25. Gilks WR, Richardson S, Spiegelhalter DJ (eds.): **Markov chain Monte Carlo in practice**, First
634 edn. Boca Raton: Chapman & Hall/CRC; 1996, Chapter 2 and 11.
635 26. Lunn DJ, Thomas A, Best N, Spiegelhalter D: **WinBUGS - a Bayesian modelling framework:**
636 **concepts, structure, and extensibility**. *Statistics and Computing* 2000, **10**:325-337.
637 27. Spiegelhalter D, Thomas A, Best N, Lunn D: **WinBUGS Version 1.4.3 User Manual.** In.:
638 Medical Research Council Biostatistics Unit.; 2007.
639 28. Best NG, Cowles MK, Vines SK: **CODA Manual Version 0.30**. In. Cambridge, UK: MRC
640 Biostatistics Unit; 1995.
641 29. Gelman A, Rubin DB: **Inference from iterative simulation using multiple sequences**. *Stat Sci*
642 1992, **7**:457–511, Chapter 11.
643 30. Kostoulas P, Leontides L, Enøe C, Billinis C, Florou M, Sofia M: **Bayesian estimation of**
644 **sensitivity and specificity of serum ELISA and faecal culture for diagnosis of**
645 **paratuberculosis in Greek dairy sheep and goats**. *Preventive Veterinary Medicine* 2006,
646 **76**(1-2):56-73.
647 31. Congdon PD (ed.): **Applied Bayesian Hierarchical Methods**: Chapman and Hall/ CRC; 2010,
648 Chapter 1 and 2.
649 32. EFSA: **Report of the Task Force on Zoonoses Data Collection on the analysis of the baseline**
650 **survey on the prevalence of *Salmonella* in slaughter pigs, PartA**. *The EFSA Journal* 2008,
651 **135**:111.
652 33. van Winsen RL, van Nes A, Keuzenkamp D, Urlings HAP, Lipman LJA, Biesterveld S, Snijders
653 JMA, Verheijden JHM, van Knapen F: **Monitoring of transmission of Salmonella enterica**
654 **serovars in pigs using bacteriological and serological detection methods**. *Veterinary*
655 *microbiology* 2001, **80**(3):267-274.
656 34. Nollet N, Maes D, Duchateau L, Hautekiet V, Houf K, Van Hoof J, De Zuttera L, De Kruif A,
657 Geers R: **Discrepancies between the isolation of Salmonella from mesenteric lymph nodes**
658 **and the results of serological screening in slaughter pigs**. *Veterinary research* 2005,
659 **36**(4):545-555.
660 35. Christensen J, Baggesen DL, Soerensen V, Svensmark B: **Salmonella level of Danish swine**
661 **herds based on serological examination of meat-juice samples and Salmonella occurrence**
662 **measured by bacteriological follow-up**. *Prev Vet Med* 1999, **40**(3-4):277-292.

663 36. Farzan A, Friendship RM, Dewey CE: **Evaluation of enzyme-linked immunosorbent assay**
664 **(ELISA) tests and culture for determining Salmonella status of a pig herd**. *Epidemiology and*
665 *infection* 2007, **135**(2):238-244.
666 37. Proux K, Houdayer C, Humbert F, Cariolet R, Rose V, Eveno E, Madec F: **Development of a**
667 **complete ELISA using Salmonella lipopolysaccharides of various serogroups allowing to**
668 **detect all infected pigs**. *Veterinary research* 2000, **31**(5):481-490.
669 38. Szabo I, Scherer K, Roesler U, Appel B, Nockler K, Hensel A: **Comparative examination and**
670 **validation of ELISA test systems for Salmonella typhimurium diagnosis of slaughtering pigs**.
671 *International journal of food microbiology* 2008, **124**(1):65-69.
672 39. Nicholson FA, Groves SJ, Chambers BJ: **Pathogen survival during livestock manure storage**
673 **and following land application**. *Bioresource Technology* 2005, **96**(2):135-143.

674

675

676 Figure captions

677 Figure 1: **Posterior distribution of β**

678 **Legend:** Plot of the posterior distribution for transmission parameter β, which describes the
679 rate of spread of *Salmonella* Typhimurium from susceptible to infectious animals. Also, a
680 boxplot of the posterior samples used to produce the plot where the thick line in the box
681 reflects the median.

682 Figure 2: **Posterior distribution of α.**

683 **Legend**: Plot of the posterior distribution for transmission parameter α, which describes the
684 rate of spread of *Salmonella* Typhimurium from infectious to resistant animals. Also, a
685 boxplot of the posterior samples used to produce the plot where the thick line in the box
686 reflects the median.

687 Figure 3: **Posterior distribution of ν**

688 **Legend**: Plot of the posterior distribution for transmission parameter ν, which describes the
689 rate of spread of *Salmonella* Typhimurium from resistant to infectious animals. Also, a
690 boxplot of the posterior samples used to produce the plot where the thick line in the box
691 reflects the median.

692 Figure 4: **Posterior distribution of the β random effects for cohort two over time**

693 **Legend**: Plot of the posterior distribution of the β random effects (time and cohort) for one
694 cohort over time with the mean and 95% credible intervals.
695 Figure 5: **Posterior distribution of the basic reproduction ratio (R0).**
696 Legend: Plot of the posterior distribution for the basic reproduction ratio (R0). The vertical
697 line shows the threshold value R0=1 where dissemination of the infection occurs. Also, a
698 boxplot of the posterior samples used to produce the plot where the thick line in the box
699 reflects the median.
700 Figure 6: **Posterior distribution of the probability of non-detection of infected animals**
701 **(*pND*)**.
702 **Legend**: Plot of the probability of non-detection of infected animals (*pND*) due to the test
703 characteristics. Also, a boxplot of the posterior samples used to produce the plot where the
704 thick line in the box reflects the median.
705
706
707 # Tables

708 Table 1: Summary measures of the transmission parameters and random effects variances

709 from the *Salmonella* transmission in pigs SIR model.

Parameters	Mean	Standard deviation	Quartiles					Rhat
			2,5%	25%	50%	75%	97,5%	
β	0.44	0.49	0.06	0.20	0.33	0.52	1.52	1.0021
α	0.18	0.02	0.14	0.17	0.18	0.20	0.23	1.0009
ν	0.02	0.03	0.0001	0.006	0.01	0.02	0.04	1.0009
σ^2_1	3.00	1.80	0.80	1.76	2.60	3.77	7.59	1.0011
σ^2_2	0.02	0.04	0.002	0.005	0.01	0.02	0.096	1.0009
σ^2_3	6.64	38.82	0.003	0.06	0.08	3.85	44.44	1.0010
pND	0.18	0.03	0.12	0.16	0.18	0.20	0.25	
R_0	2.20	1.25	0.78	1.46	1.91	2.56	5.24	

710 Legend: β – transition rate from susceptible to infectious, α – transition rate from infectious to

711 carrier, ν – transition rate from carrier to infectious, σ^2_1 – variance of the random effects for the

712 transition from susceptible to infectious, σ^2_2- variance of the random effects for the transition from

713 infectious to carrier, σ^2_3 - variance of the random effects for the transition from carrier to infectious,

714 pND – probability of non-detection of infectious animals, R_0 – basic reproduction ratio.

715

716

717

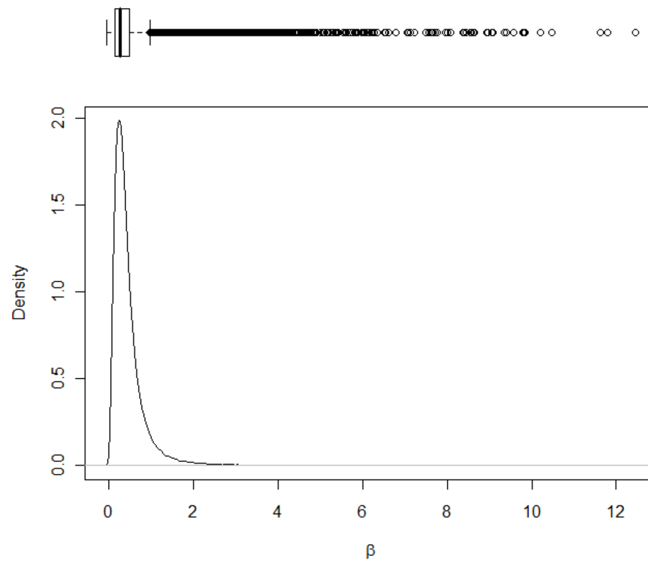
718

719

720

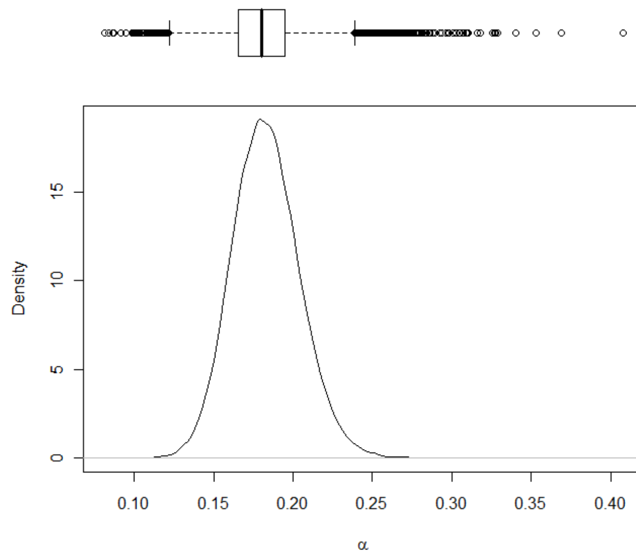
721

722 Figure 1



723

724 Figure 2

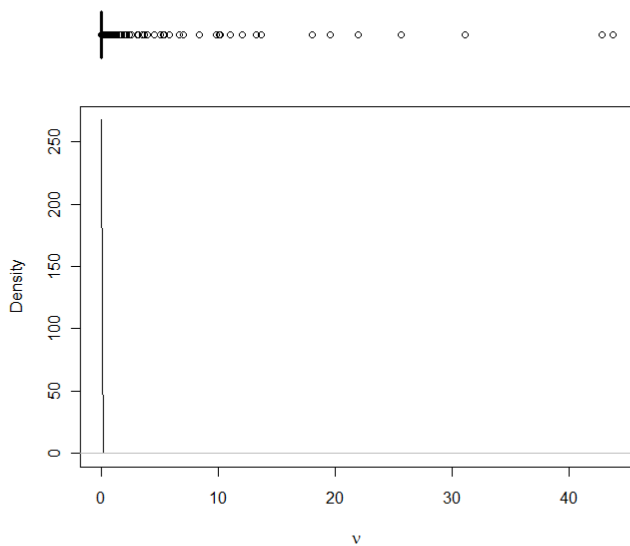


725

726

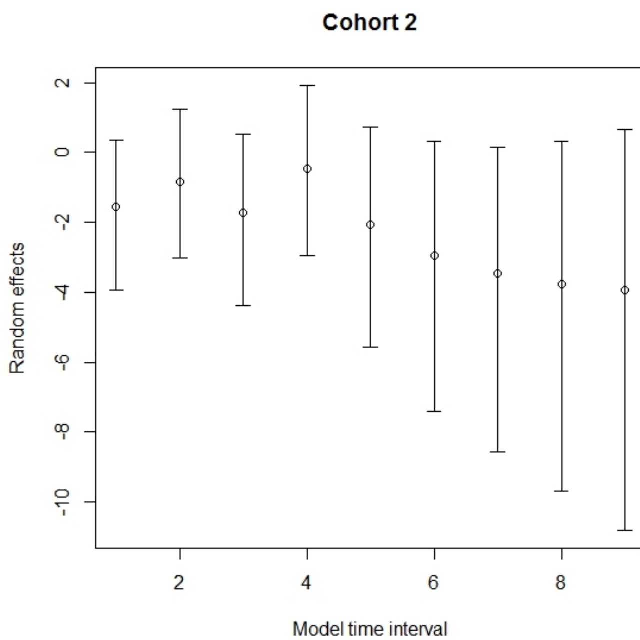
727

728 Figure 3



729

730 Figure 4

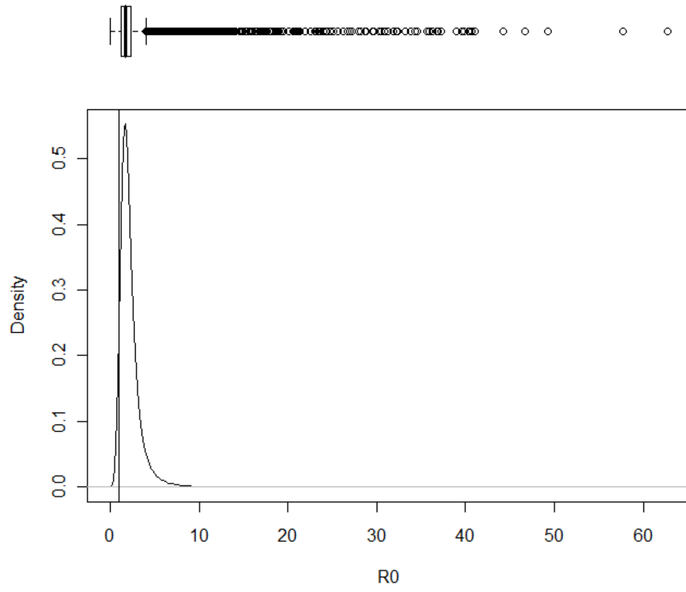


731

732

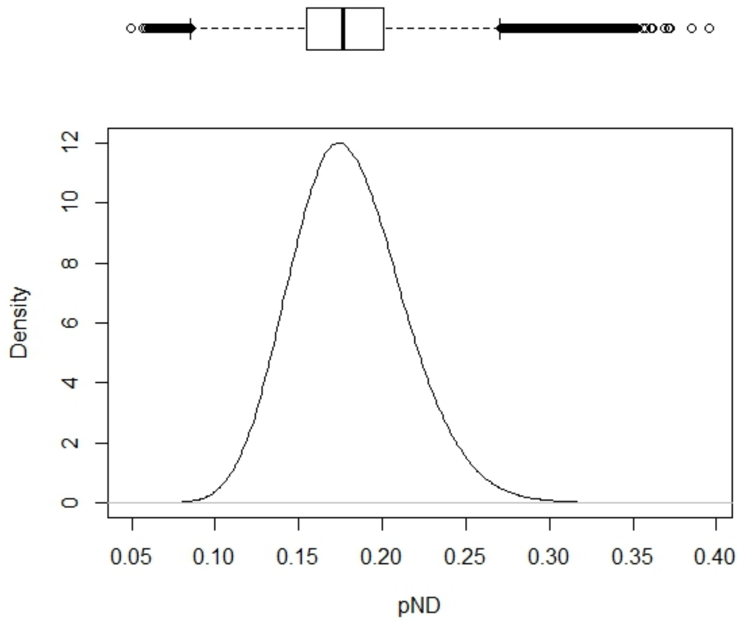
733

734 Figure 5



735

736 Figure 6



737

Manuscript 5

"Simulation model of *Salmonella* Typhimurium infection on a farrow-to-finish pig herd"

Manuscript 5 had not been yet submitted to any scientific journal due its dependency on the results from Manuscript 4.

SIMULATION MODEL FOR SALMONELLA TYPHIMURIUM INFECTION ON A FARROW-TO-FINISH PIG HERD

Carla Correia-Gomes[1,*], Theodoros Economou[2], Trevor Bailey[2], João Niza-Ribeiro[1,3]

[1]ICBAS-UP - Department of Population Studies, Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto, Rua de Jorge Viterbo Ferreira, nº228, 4050-313 Porto, PORTUGAL

[2] CEMPS, University of Exeter, Harrison Building, North Park Road, Exeter, EX4 4QF, UK

[3] ISPUP – Institute of Public Health – Universidade do Porto, Rua das Taipas, 4099-003 Porto, PORTUGAL

*Corresponding author (now at): +441463246065 (tel), +441463236579 (fax), carla.gomes@sruc.ac.uk, ERU-SRUC – Epidemiology Research Unit, Future Farming Systems, Scottish Agricultural College, Drummondhill, Inverness, IV2 4JZ, UK

Keywords: simulation model, *Salmonella* Typhimurium, farrow-to-finish herd, transmission parameters

Abstract

A stochastic model which simulates the dynamics of *Salmonella* Typhimurium in a farrow-to-finish farm in Portugal was developed and run. The model comprises two compartments with six different stages in total: three at the reproductive phase (sow-compartment) and another three for pig growth (pig-compartment). Infection dynamics of *Salmonella* is modelled for each stage with four infection transition parameters (β, α, δ, θ) and thee health status: susceptible, infectious and carrier. The parameters which influence each infection state per room were identified and discussed.

The ones that influence the infectious state most, at the end of the fattening stage, were: the transition rate from susceptible to infectious (β), and the piglets' immunity protective factor. Several control measures were suggested, so the simulation model allows estimation of cost-benefit of such control measures, if coupled with an economic model. The simulation model is flexible enough to introduce changes in the parameter values and distributions if future research and changes in the legislation so require. The model can also be adapted to different types of production (e.g. breeding, farrow-to-weaners, and finishers units) as it was built in a compartmental way.

Introduction

Salmonella spp. infection in swine in the majority of the infections does not evoke any clinical manifestation or just subclinical signs. Even so *Salmonella spp.* is one of the major causes of food-borne outbreaks in the world (the second cause in Europe)[1]. As such *Salmonella spp.* control was considered necessary by the European food-safety policy makers under the EC Regulation 2160/2003. In practice, however, the control of

this agent has proved to be difficult and expensive at farm level [2]. Consequently the evaluation of the efficiency of control strategies for this agent has become an important and stringent issue, as stated in recent reports [3].

Modelling the dynamics of *Salmonella* spp. in pigs can be useful when assessing alternative control strategies. Susceptible – Infectious – Resistant (SIR) models are attractive tools to help in assessing the disease dynamics. The SIR model describes the dynamic of different states of individuals in the population in terms of a system of ordinary differential equations. The variables in the system are given by the three compartments: group of susceptible (S), group of infectious (I) and group of resistant (R). The mathematical models provide a description of the movement in and out of the three compartments, and the transitions between compartments are governed by rates. Infection models are simple representations of the reality with the aim of simulating the dynamic of a disease so we can evaluate the disease evolution and the effect of control measures. The simulation models can be of three types: deterministic, stochastic or a mixture of the two. Deterministic models use point-values as model inputs and therefore the models outputs are also point values with some confidence interval associated with it. Stochastic models, however, incorporate uncertainty and/or natural variability into a model. Variability represents true heterogeneity in a population, e.g. the weight of a pig will vary between pigs and the fact that we cannot assigned a fixed value to the weight of a batch of pigs has nothing to do with incomplete knowledge, it is inherent to the population. On the other hand, uncertainty reflects our lack of knowledge about the exact value of a parameter. For example, the inactivation of *Salmonella* when subjected to high temperatures may be modelled by an exponential decay, dependent on time and on an inactivation parameter. This inactivation parameter is hard to measure and therefore not known exactly. In a stochastic model variability and uncertainty can be incorporated using probability distributions, instead of fixed parameter values. Incorporating distributions into the model results in a distribution for the model output; hence providing more information compared to the deterministic approach [4]. A mixture of deterministic and stochastic models is a common way of incorporating variability/uncertainly in a simulation model, and at the same time, decreasing the computation time. The uncertainty of the models needs to be appraised. A method for evaluating the uncertainty is to run alternative scenarios of the model, where the uncertain parameters were changed to a minimum and a maximum value, respectively. The resulting probability of infection is compared with the baseline results and a relative effect can be quantified. In cases where a parameter has a distribution associated with it, the alternative scenario is run with differently parameterised probability distribution [4]. In the majority of the models found in the

literature the validation of the simulations models was done comparing the results of the model (e.g. final prevalence at the end of fattening) with the observed (epidemiological) results in the populations of interest [4-7].

The management procedures (e.g. such as voluntary culling, batch production, type of pen, etc.) which occur in a farm also affects the disease dynamic and should be incorporated in the simulation models to more accurately describe what happens in the farm. *Salmonella spp.* infections in pigs are not a clinical important disease and therefore will not be expected to increase sow and pig mortality.

In the literature there are some infection models published for *Samonella spp.* in pigs which simulate the entire food chain [4, 8] or part of the food chain – mainly herd [5, 7, 9] or herd to slaughter [10, 11]. To construct such models a high number of parameters is required (e.g. such as production parameters, infection parameters, risk factors and disease prevalence, etc.). When there is not data to estimate these parameters, it is typical to call upon expert opinion to provide estimates and/or information.

The aims of this study were: a) to develop a stochastic model which incorporates a production model with an infection model (the production model simulates the management procedures of an average farrow-to-finish Portuguese pig farm, while the infection model simulates the *Salmonella* Typhimurium infection in the farm); and b) to identify the parameters which influence most the model results at different compartments and stages of life within these compartments.

Materials and Methods

Description of the simulation model – production steps

The model simulates a farrowing-to-finish herd in which batch farrowing is applied to sows, leading to batch management of pigs. This type of management is usual in countries like France and Portugal. In these herds the complete life cycle of sows is considered, from recruitment until culling/dying, and similarly for pigs, from birth till slaughter. The duration of the sow reproduction cycle depends of the weaning time of the piglets and this was fixed at 4 weeks (28 days). The pig growth period was fixed at 26 weeks (average age at slaughter in Portugal). The modelling unit was the batch (for both sows and pigs). This unit is useful because it simulates the interaction within sows and pigs which is important for infectious diseases, such as *Salmonella*, which are transmitted by close-contact between animals and by the batch environment, e.g. floors, feed, water, etc., so the exposure within batch is effectively uniform.

In the model, batches of sows are groups of sows (the same number per batch) that are mated at the same time. One week interval between two successive batch mating

was assumed. The average Portuguese farrow-to-finish herd has approximately 264 sows. Therefore using the one week batch system during the year there were 22 batches entering mating (taking into account that each sow will have 2.5 litters per year) with 12 sows per batch. The reproduction cycle is divided in three stages (mating period, gestation period and farrowing/suckling period) corresponding to the occupation of three different types of rooms. Each batch of sows was composed of gilts and sows although we did not differentiate between them in the model. Each batch of pigs was composed by the litters from the batch of sows. The pig growth is divided in three stages (sucking period, post-weaning period and fattening period) corresponding to the occupation of three different types of rooms. All animals simultaneously leave the room they occupied except for the sows which abort at gestation.

This production model describes the evolution of the number of animals within each batch and the time step is one week. The model has a stochastic element in the sense that it simulates the variability associated with biological processes such as mortality, culling, insemination failure, abortion and litter size. The model output is expressed in terms of probability distributions which in turn express: 1) the aforementioned variability, 2) the propagated uncertainty from having to estimate transmission parameters, and 3) the natural variability or randomness inherent in the behaviour of the disease (specifically, the binomial distribution was used to generate the number of animals in each production process).

The duration of the reproductive and growing stage, and therefore the duration in each room were kept fixed. The maximum capacity in each room was fixed for the maximum expected size of each batch and the pen capacity varied between batches depending on the number of animals per batch.

Reproduction cycle of the sows

The sow-compartment comprises three stages of the reproduction cycle, which take place in three different rooms:

- the mating room where the sows remain from weaning until pregnancy testing (6 weeks);
- the gestation room where the sows remain almost until the end of pregnancy (10 weeks);
- the farrowing room, in which the sows are placed 1 week before farrowing and stay until the weaning of the piglets (5 weeks).

During the reproductive cycle, the sows are subject to the following processes: mortality, artificial insemination success, abortion, culling and gilt recruitment. The probability of mortality is “applied” at each time step and is constant in time. However, it

varies between the stages of reproduction reflecting the variability that exists between the different stages. Each week the sows from a new batch enter the mating room and are inseminated. Individual pens were used at mating. At the end of the sixth week of mating, the artificial insemination success rate is used, to simulate pregnancy numbers. To represent the variability that exists between batches, the artificial insemination success rate is separately generated for each batch from a Weibull distribution. We have used field data to fit the best distribution to artificial insemination success rate using maximum likelihood. The square root of the simulated value for each batch corresponds to the probability of the artificial insemination success that is used to generate the number of sows that get pregnant and are moved to the next stage (gestation). The sows that fail to get pregnant are then either culled or moved to the following batch that will enter the mating room and be re-inseminated. The culling rate is different for mating, gestation and farrowing. In the gestation room the sows remain together in pens with a maximum of 4 sows per pen. Abortion can occur throughout the gestation period and the probability of abortion was kept constant for each week. After abortion, the sows are culled or moved to a following batch where they are going to be re-inseminated. In the farrowing room the sows are placed in individual pens (maternities). After weaning, some sows are voluntarily culled to allow renovation of the herd and the ones not culled enter in a new batch that is going to be re-inseminated in the mating room. To compensate for the mortality and culling in each batch, new gilts are introduced to ensure that the number of sows per batch is always 12.

Each batch of sows gives birth to a batch of pigs. The average litter size for each sow is drawn from a normal distribution of mean 10.45 and standard deviation of 0.87. We have used field data to fit the best distribution for litter size using maximum likelihood.

For mating the equations were:

$$Malive_t \sim Binomial(Malive_{t-1}, (1 - pmort_M))$$
$$Mpreg_{t=f} \sim Binomial(Malive_{t=f-1}, pins)$$
$$Mreturn \sim Binomial((1 - Mpreg_{t=f}), (1 - pcull_M)),\ f \text{ is the last week on mating room.}$$

For gestation the equations were:

$$Galive_t \sim Binomial(Galive_{t-1}, (1 - pmort_G))$$
$$Gpreg_t \sim Binomial(Galive_t, (1 - pabort))$$
$$Greturn_t \sim Binomial((1 - Gpreg_t), (1 - pcull_G))$$

For farrowing (sows) the equations were:

$$Falive_t \sim Binomial(Falive_{t-1}, (1 - pmort_F))$$
$$Freturn \sim Binomial(Falive_{t=i}, (1 - pcull_F)),\ i \text{ is the last week on farrowing room.}$$

Where *pmort* is the mortality probability (different for each room), *pins* is the probability of success by the artificial insemination, *pcull* is the culling probability (different for each room), *pabort* is the probability of abortion, *alive* refers to the animals that do not die, *preg* refers to the pregnant sows, *return* refers to the sows that enter a new batch for mating (due to failure to get pregnant, abortion or after farrowing).

Pig Growth

The pig-compartment comprises three different stages, which take place in three different rooms:

- farrowing/maternity room (where they stay for 4 weeks until weaning),
- post-weaning room (where they stay 8 weeks), and
- fattening room (where they stay 14 weeks).

The mortality probability is different between rooms. The number of pigs that had died in each time step is simulated using a binomial distribution.

The maximum number of pigs per pen varies between rooms. In the farrowing room the number of piglets per pen is made similar taking in consideration the litters' size to simulate the mixing of piglets that occurs in the farms with the aim of improving the quality of the batch (to develop the milk production of gilts and also to allow piglets to have access to functional teats). In the post-weaning room the maximum number of pigs per pen was 20 (with a maximum number of 6 post-weaning pens per batch) and for fattening pens this value was reduced to 15 (with a maximum number of 12 fattening pens per batch). This means that the pigs were allocated to the pens in a way that does not exceed that maximum number. These numbers were used taking into consideration the average Portuguese pen size per room, taken from an unpublished survey results [12].

For maternity (piglets) the equations were:

$$Npiglets_j \sim Normal(10.45, 0.87),\ j = \text{ number of sows per batch}$$
$$Pigalive_t \sim Binomial(Pigalive_{t-1}, (1 - pmort_{mat}))$$

For post-weaning (PW) and fattening (FA) the equations were:

$$PWalive_t \sim Binomial(PWalive_{t-1}, (1 - pmort_{PW}))$$

$$FAalive_t \sim Binomial(FAalive_{t-1}, (1 - pmort_{FA}))$$

Where *Npiglets* refers to the born alive piglets per sow (this is drawn for each sow in each batch), *pmort* is the mortality probability (different for each room), and *alive* is the number of animals alive in each room at each time step.

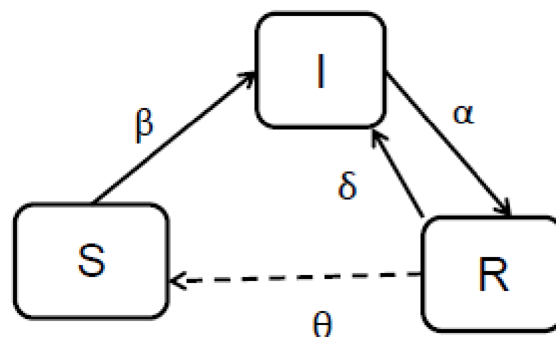
The values and sources of the production parameters are shown in Table 1.

Infection model specification

The infection model was based on a Susceptible-Infectious-Resistant/Carrier model for *Salmonella* Typhimurium. Direct transmission between the pigs in a batch was assumed but also indirect transmission via contaminated floor, rodents, etc.

The transition steps considered between the states are shown in Figure 1. For the sows, as they have a longer life span, it was assumed that they could experience all the states and transitions shown in Figure 1. For pigs, due to their short life span, it was assumed that they could not experience the transition from carrier to susceptible. The mathematical model for these transitions and the transmission parameters used in this simulation model were described and estimated in in Correia-Gomes *et al* (unpublished paper). The estimates were obtained using field data from a longitudinal study [13] which followed infected cohorts of pigs infected with *S.* Typhimurium. Although the time step in the field data was two weeks in the present model the time steps were adjusted to one week.

Figure 1: SIR model and its transition between states



Legend: S – susceptible, I – Infectious, R – resistant/carrier, β – transmission parameter for the transition between S to I, α – transmission parameter for the transition between I to R, δ – transmission parameter for the transition between R to I, θ – transmission parameter for the transition between R to S.

The binomial distribution was used to simulate the transition between susceptible and infectious state and from infectious to carrier state. For the transition between carrier state and infectious; and carrier state and susceptible, Poisson distributions were used. The transition between susceptible and infectious varied with time by parameterising it using a time-dependent cohort random effect. This random effect was used to capture the temporal structure of the spreading of infection within cohorts where the velocity of infection is dependent on the number of susceptible and infectious animals in the previous time step.

The equations used in the infection model were the following:

$$Inf_{j,t} \sim Binomial(S_{j,t-1}, p_{j,t})$$
$$\text{cloglog}(p_{j,t}) = \log(\beta) + \log(I_{j,t-1}) - \log(N_{j,t-1}) + r_{1j,t}$$
$$Rnew_{j,t} \sim Binomial(I_{j,t-1}, pr_{j,t})$$
$$\text{cloglog}(pr_{j,t}) = \log(\alpha) + r_{2j}$$
$$Inew_{j,t} \sim Poisson(\pi_{1j,t})$$
$$\log(\pi_{1j,t}) = \log(\nu) + \log(R_{j,t-1}) + r_{3j}$$
$$Snew_{j,t} \sim Poisson(\pi_{2j,t})$$
$$\log(\pi_{2j,t}) = \log(\theta) + \log(R_{j,t-1})$$
$$r_{kj} \sim Normal(0, \sigma_k^2), k = 2,3$$
$$r_{1j,t=1} \sim Normal(0, \sigma_1^2)$$
$$r_{1j,t} \sim Normal(r_{j,t-1}, \sigma_1^2)$$

Where *Inf* is the number of susceptible animals that became infectious, *S* is the number of susceptible animals at the beginning of each time interval, *p* is the probability of transition between susceptible to infectious, *β* is the transmission parameter between susceptible and infectious, *I* is the number of infectious animals at the beginning of the time interval, *N* is the total number of animals at the beginning of the time interval, r_{jt} is the cohort (j)-time (t) dependent random effects, *Rnew* is the number of animals that became carriers in each time step, *pr* is the probability for the transition between infectious and carriers, *α* is the transmission parameter from infectious to carriers, *Inew* is the number of carriers that became infectious in each time step, π_1 is the average number of carriers that became infectious, *v* is the transmission parameter between carrier to infectious, *R* is the number of carrier animals at the beginning of each time interval, *Snew* is the number of carrier animals that became susceptible (this step in the model only happens for sows), π_2 is the average number of carriers that became susceptible, *θ* is the transmission parameter between carrier to susceptible, and σ^2_k is the variance of the random effects.

So for each time step and for each pen, the number of sows in each state would be:

$$S_{jt} = S_{j,t-1} - Inf_{jt} + Snew_{jt}$$
$$I_{jt} = I_{j,t-1} + Inf_{j,t} - Rnew_{j,t} + Inew_{j,t}$$
$$R_{j,t} = R_{j,t-1} + Rnew_{j,t} - Inew_{j,t} - Snew_{j,t}$$

While for the pigs it would be:

$$S_{jt} = S_{j,t-1} - Inf_{jt}$$
$$I_{jt} = I_{j,t-1} + Inf_{j,t} - Rnew_{j,t} + Inew_{j,t}$$
$$R_{j,t} = R_{j,t-1} + Rnew_{j,t} - Inew_{j,t}$$

At the maternity stage since the litter is in contact with the sow (mother), the sow health state was allowed to affect the littler disease dynamics, however a protective factor (*pf*) for decreasing the transition rate was taken into consideration due to the sow's milk protective antibodies, as suggested by other studies [5, 9]. This protective factor was included in the model while the piglets were at the maternity stage for each batch, changing the equation for the transition from susceptible to infectious for the following:

$$Inf_{j,t} \sim Binomial(S_{j,t-1}, p_{j,t})$$
$$\text{cloglog}(p_{j,t}) = \log(\beta) + \log(pf) + \log(I_{j,t-1}) - \log(N_{j,t-1}) + r_{1j,t}$$

The study of Beloeil *et al* (2003) [14] estimated the complete loss of maternal immunity between 61 and less than 80 days. We have assumed 70 days to calculate the protective factor.

The transition rate from carrier to susceptible was calculated, taking into consideration that pigs need around 68 days to clear S. Typhimurium from their organs after being infected ([9, 15] and another 42 days to lose the protective immunity against *Salmonella* [9, 15]: a total of 110 days – 15.7 weeks - to return to susceptible state again. This value was used to calculate the transmission parameter between carrier and susceptible (1/15.7).

The production and infection parameters used in the model are shown in Table 1.

Model settings and analysis of the results

The model was built and implemented in R (CRAN project, www. R-project.org). To ensure convergence of the final results (i.e. reduce sampling uncertainty) a long run (500,000 iterations) was conducted. Before running the model it was necessary to allocate an initial state to the sows/gilts, at mating in the first batch. The allocation was 50% of susceptible, 25% of infectious and 25% of carriers.

Table 1: Production and transmission parameters and their values

Production Parameter		Random/fixed	Value	Reference
Average number of sows per herd		Fixed	264	a, b
Median number of pig per pen	Post-weaning	Fixed	25	a
	Fattening	Fixed	17	a
Duration (weeks)	Mating	Fixed	6	c
	Gestation	Fixed	10	c
	Farrowing - sows	Fixed	5	c
	Maternity - piglets	Fixed	4	b
	Post-weaning	Fixed	8	c
	Fattening	Fixed	14	c
Mortality probability (per week)	Mating	Fixed	0.000833	c
	Gestation	Fixed	0.00357	c
	Farrowing – sows	Fixed	0.001786	c
	Farrowing – piglets	Fixed	0.0275	b
	Post-Weaning	Fixed	0.00375	a
	Fattening	Fixed	0.00357	a
Artificial insemination success probability – applied in the end of mating (pins)		Squared root of a Weibull distribution	10.31 (mean), 0.77 (sd)	b
Abortion probability (per week)		Fixed	0.0025	c
Culling probability	After failing insemination	Fixed	0.017 at end of mating	c
	After abortion	Fixed	0.017/week	c
	Voluntary culling	Fixed	0.333 at end of farrowing	c
Litter size		Normal distribution (the final value was rounded)	10.45 (mean), 0.87 (sd)	b
Transmission parameter or transition rate from susceptible to infectious (β)		Random (posterior distribution)	0.34/week [0.17-0.66]	d
Transmission parameter or transition rate from infectious to carrier (α)		Random (posterior distribution)	0.27/week [0.24 – 0.30]	d
Transmission parameter or transition rate from carrier to infectious (δ)		Random (posterior distribution)	0.09/week [0.008 – 0.21]	d
Transmission parameter or transition rate from carrier to susceptible (θ)		Fixed	0.06/week	[9]
Cohort time-dependent random effect (σ^2_β)		Normal distribution	0 (mean), 1.29 (sd)	d
Piglets' protective factor due to sows passive immunity (pf)		Fixed (1/70 days)	0.1/week	[14]

Legend: sd – standard deviation

[a] Baptista *et al*, unpublished results of a survey to 109 herds in Portugal in 2009 [12]

[b] Production data of 200 Portuguese herds, collected by a software company from 2004 to 2006

[c] Expert opinion

[d] Correia-Gomes et al, unpublished (Manuscript 4)

For each model run at the end of each room the following results were saved to be analysed: the proportion of sows alive in each room, the proportion of sows pregnant at the end of mating and gestation rooms, and the proportion of sows/pigs in the different infection states.

The use of proportions was adopted instead of the number of animals (counts) because the total number of animals per room varied as function of the mortality and litter size (for pigs) making comparison between runs less straightforward. The proportions on the other hand can be directly compared.

The distributions of the results were tabled for the sows and plotted for the pigs. In the plots we have used the median as the central tendency measure because, unlike the mean, it is less affected by extreme values.

The validity of the model was assessed by comparing the predicted results from the model with observed (epidemiological) results in the population of interest: the Portuguese pig population. The results from the EU Baseline Survey on *Salmonella* Prevalence in Slaughter Pigs [16] and the EU Baseline Survey on the Prevalence of *Salmonella* in Breeding Pigs [17] were used as observed epidemiological results. The comparison was done by relating the magnitude of the predicted and observed values, and qualitatively assessing the degree of agreement/disagreement, as suggested by other authors [4, 9].

Sensitivity analysis

To perform the sensitivity analysis of the model, all the production parameters and infection parameters were perturbed, i.e. increased and decreased by 50%, and the results were compared with results from the unperturbed parameters. For the piglets' protective factor (pf) we ran several extra simulations (an increase of 250%, 500%, 750% and 1000% of the value, corresponding to the values of 0.25, 0.50, 0.75 and 1, respectively) due to the original value of the parameter being low. The lower the pf value is, the higher the protection against infection (e.g. a pf value of 0.25 means that the transmission parameter in piglets from susceptible to infectious will be reduced to 25% of it value, and in this way translating into a protective effect of 75%). For the infection state of replacement gilts, eleven combinations were tried (see Table 2) as it was not possible to execute all the possible combinations (100^3) due to limitation of time. These combinations allowed to test the effect of high and low proportions for each infection state, considered more plausible by the authors.

For the transmission parameters from S to I (β), from I to R (α), from R to I (δ) and from R to S (θ) extra simulations were run to test the simultaneous effects of increasing or decreasing each parameter in different rooms. When the perturbation is applied to all the sows' rooms, we used the suffix "sows" (i.e. β sow, α sow, δ sow and θ sow). When the perturbation occurs in the maternity for sows and piglets at the same time (only for β parameter), we used the suffix "maternity" (β maternity). When the perturbation is applied to all the pigs' rooms, we used "pigs" as a suffix (i.e. β pigs, α pigs, and δ pigs).

Table 2: Combinations for the infection state of the replacement gilts

Combination number	Proportion of each infection state		
	Susceptible	Infectious	Carriers
1	1	0	0
2	0.9	0.05	0.05
3	0.8	0.2	0
4	0.7	0.1	0.2
5	0.5	0.5	0
6	0.5	0.25	0.25
7	0.3	0.25	0.45
8	0.1	0.6	0.3
9	0	0.8	0.2
10	0.2	0.1	0.7
11	0	0	1

The perturbed and original parameters were compared by looking at the percentage change in their means – relative effect (RE):

$$RE = \frac{Mean.prop_{perturbed} - Mean.prop_{original}}{Mean.prop_{original}}$$

If RE is positive/negative the change in the parameter has increased/decreased the results (i.e. the means of the proportions). However if RE is equal to zero, the parameter change has no effect. The greater the magnitude of RE is, the greater the influence of the particular parameter.

To test if these REs were statistically significant it was used the fact that the results are in the form of samples from the distributions of the output, e.g. samples from the distribution of the proportion of sows pregnant at the end of mating. This is a direct consequence of the fact that the models used to estimate the transition parameters were Bayesian. Samples from the posterior distributions of the estimated transmission parameters from the Bayesian model were used in the simulated model to propagate the estimation uncertainty to the output from the simulated model, rather than ignoring it. Ultimately, it can be considered the output from the simulation model as samples from posterior distributions, e.g. 500,000 samples from the posterior distribution of the proportion of sows pregnant at the end of mating. It was the means of these distributions that were compared.

Therefore for each output of interest (e.g. proportion of sows pregnant at the end of mating) it was simulated a random sample from their posterior distribution. For instance, sample 1000 values from the posterior distribution of the proportion of sows pregnant at the end of mating, which means to randomly sampling 1000 values with replacement from the 500,000 samples of the output. If this procedure is done many

times, e.g. 10,000 times, and calculated the mean each time, the final result is a sample of 10,000 values for the distribution of the mean. As the interest was to compare means from the original and perturbed parameter output, the differences in the mean samples was taken to obtain a sample from the distribution of the difference. e.g. suppose μ_{1i} and μ_{2i} for i=1,…,10,000 were samples from two means distributions, then $D_i = \mu_{1i} - \mu_{2i}$ was a sample from the distribution of their difference and it was tested whether zero was a likely value from this distribution. More formally, if zero was not included in the 95% credible interval of D_I, it can be argued that the value of the two means was significantly different.

The EU legislation (Council Directive 2008/120/EC of 18 December 2008 laying down minimum standards for the protection of pigs) banned the use of sow stalls starting in January 2013, which means that the individual pens have to be adapted (the pen size has to be increased). The model was changed to ensure that this welfare legislation could be met if sows were housed in groups at mating. We opted to change the pen used in the mating rooms the same as in gestation rooms (i.e. four pens per batch). This change was also included in the sensitivity analysis.

Sensitivity analysis was also used as a way of evaluating the uncertainty linked with some parameters, as for instance the piglet's protective factor and the infection state of replacement gilts.

Considering the results statistical significant, for each outcome the parameters which influenced it more than 5%, were displayed in tables and plotted in a modified spider plot, where the percentage of change in the parameter was the x axis and the percentage of change in the outcome was the y axis.

When a variation from -50% to +50% is applied to the model the outcome varies changing positively or negatively as the parameter increases. The range of the change in the outcome due to the parameter change (from -50% to 50%) was tabled for the transmission parameters and piglets' protective factor in two separated tables, one for sows one for pigs. The criterion followed for the inclusion of each parameter in the table was to have at least a 5% variation either positive or negative. In the table a positive number means positive effect on the outcome when a variation from -50% to +50% is applied to the model (the outcome increases with the increase of the parameter); and a negative number means a negative effect on the outcome when a variation increment from -50% to +50% is applied to the model (the outcome decreases with the increase of the parameter).

Results

Prevalence results

The results of the model for the sow-compartment are shown in Table 3, showing that the majority of the sows were in the infectious and carrier states at farrowing which can be a risk for piglets in maternity.

The results for the pig-compartment are shown in Figure 2. There was a clear increase on the prevalence of infectious and carrier pigs along time, while the number of susceptible pigs went down (Figure 2).

The predicted prevalence results of the model for the infectious animals in the sow-compartment (56.9% [16.7% - 100%] of sows infectious at the end of farrowing) was higher than the one in the pig-compartment (30.9%% [0.0% - 80%] of pigs infectious at the end of fattening). The same trend was observed in the Baseline Studies [16, 17] where the prevalence of *Salmonella* Typhimurium was higher for breeding animals (13.4% [8.8% – 20.3%] of holdings positive) than for pigs at slaughter (8.4% [6.1% - 11.5%] pigs positive). The same trend was noticed for *Salmonella* spp. in those studies: 43.3% [35.6% – 52%] of positive holdings with breeding animals while 23.4% [19.4% - 28%] of positive slaughtered pigs.

Table 3: Results for the infection state in each room for the sows

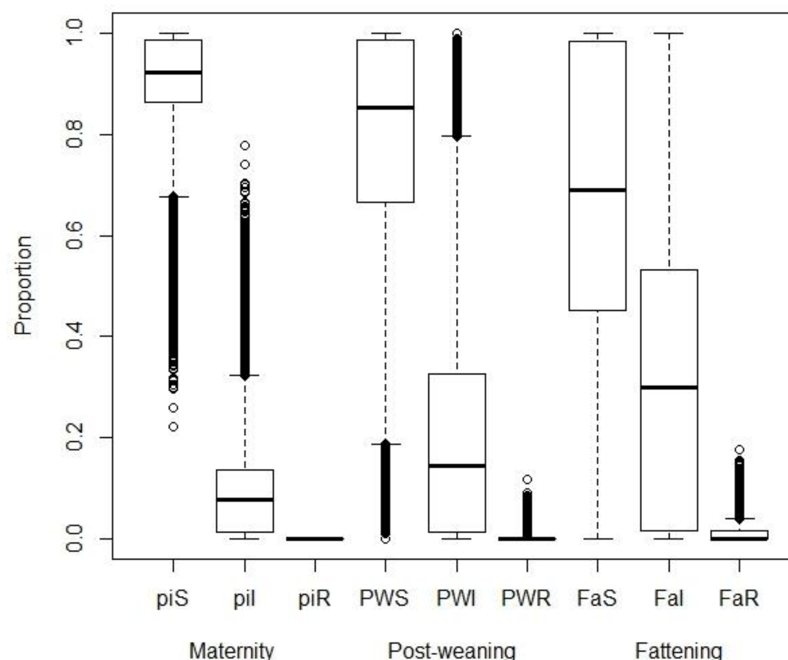
Sow production stage	Infection State	Results in proportions						
		Min	1st Q	Median	Mean	3rd Q	Max	SD
Mating	Susceptible	0.0	0.09	0.18	0.19	0.27	1.0	0.13
	Infectious	0.0	0.44	0.56	0.55	0.67	1.0	0.16
	Carriers	0.0	0.17	0.25	0.26	0.36	1.0	0.14
Gestation	Susceptible	0.0	0.10	0.20	0.23	0.33	1.0	0.16
	Infectious	0.0	0.38	0.50	0.51	0.64	1.0	0.19
	Carriers	0.0	0.17	0.25	0.26	0.36	1.0	0.14
Farrowing	Susceptible	0.0	0.0	0.14	0.16	0.25	1.0	0.16
	Infectious	0.0	0.43	0.57	0.57	0.71	1.0	0.21
	Carriers	0.0	0.14	0.25	0.27	0.40	1.0	0.19

Legend: Min – minimum, 1st Q – first quartile, 3rd Q – third quartile, Max – maximum, SD – standard deviation

Sensitivity analysis results

The magnitude of the impact of the variation of individual infection parameters in the infection states of the sows and pigs were estimated and are shown in Table 4 and 5, respectively.

Figure 2 – Dynamic of the infection states in the pig-compartment



Legend: piS – susceptible piglets at maternity, piI – infectious piglets at maternity, piR – carrier piglets at maternity, PWS – susceptible pigs at post-weaning, PWI – infectious pigs at post-weaning, PWR – carrier pigs at post-weaning, FaS – susceptible pigs at fattening, FaI – infectious pigs at fattening, FaR – carrier pigs at fattening.

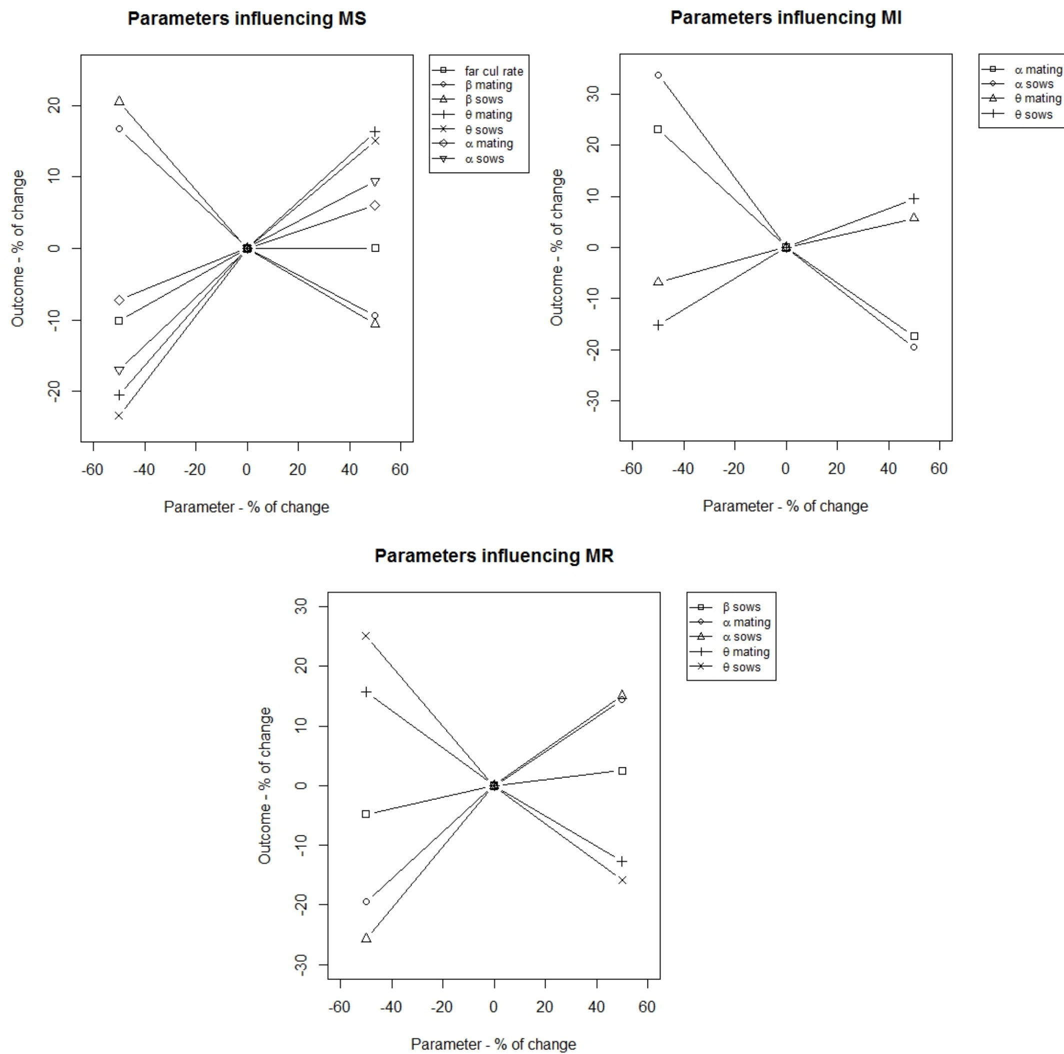
Table 4: Magnitude of the impact of the variation of individual infection parameters in the sows' infection states (only the ones ≥ 5% are shown).

Changed parameter		Results by room and infection state								
		Mating			Gestation			Farrowing		
		S	I	R	S	I	R	S	I	R
Mating	β	-28.1%	7.1%	-	-8.1%	-	-	-	-	-
	α	11.2%	-40.6%	23.9%	24.5%	-26.7%	-	13.7%	-	-
	δ	-	-	-	-	-	-	-	-	-
	θ	36.8%	12.5%	-28.4%	-	11.1%	-6.5%	-	-	-
Gestation	β	-	-	-	-7.3%	5%	-	-	-	-
	α	6.1%	-	-	25.4%	-75.6%	30.6%	22.8%	-19.9%	8.2%
	δ	-	-	-	-	10.4%	-	-	-	-
	θ	-	5.9%	-5.1%	44.7%	14.3%	-44.1%	6.6%	18.1%	-23.2%
Farrowing	β	-	-	-	-	-	-	-25.8%	9%	-
	α	5.6%	-6.8%	-	-	-	-	7.6%	-30.9%	25.5%
	δ	-	-	-	-	-	-	-	-	-
	θ	-	5.2%	-5.8%	-	-	-	31.1%	10.1%	-23.7%
Sows	β	-31.1%	6.7%	7.3%	-16.5%	8%	7.6%	-43.9%	7.4%	8.6%
	α	26.4%	-53.4%	40.8%	56.1%	-113.2%	28.7%	46.1%	-58.9%	33.9%
	δ	-	5.4%	-	-7.2%	13.9%	-	-	6.9%	-
	θ	38.4%	24.7%	-41%	43.3%	29.8%	-53.3%	45.8%	33.5%	-52.3%
Maternity	β	-	-	-	-	-	-	-23.4%	8.7%	-

Legend: β – transition rate parameter from susceptible to infectious, α – transition rate parameter from infectious to carrier, δ – transition rate parameter from carrier to infectious, θ – transition rate parameter from carrier to susceptible, S – susceptible, I – infectious, R – carrier. Interpretation: positive number - the results increased with the increase of the parameter value, negative number – the results decreased with the increase of the parameter value.

The sensitivity analysis plots (Figure 3 to 9) show which parameters are most influential on the outcomes. Modified spider plots were produced for each outcome and where each figure corresponds to a room. The influencing parameters presented in the plots were all statistically different from the original simulation.

Figure 3 – Modified spider plots for the sows susceptible (MS), infectious (MI) and carriers (MR) at mating.

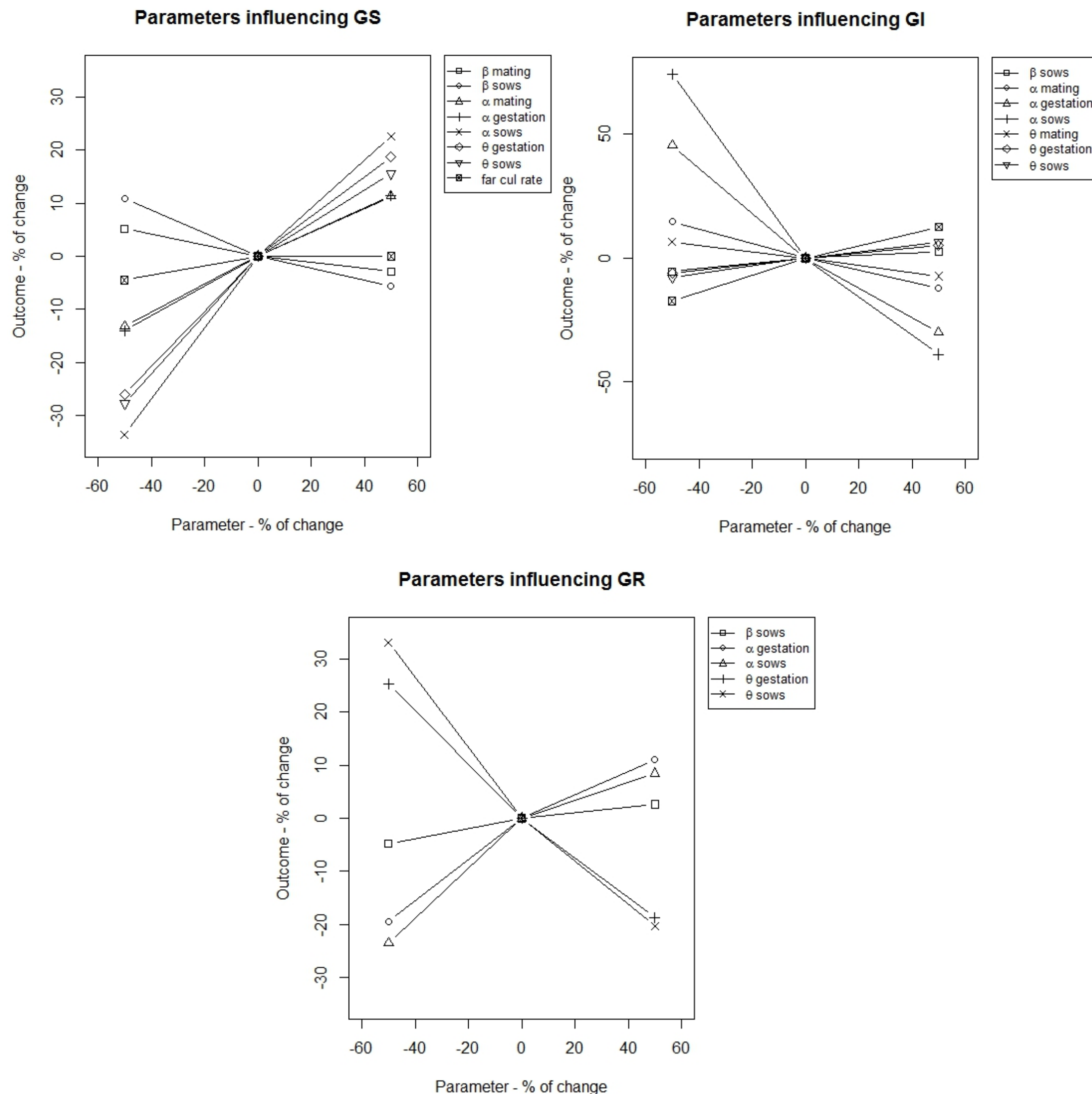


Legend: far cul rate – culling rate at farrowing, β – transition rate parameter between susceptible and infectious, α – transition rate parameter between infectious and carriers, θ – transition rate parameter between carriers to susceptible, mating – mating room, sows – change in the parameters was done at mating, gestation and farrowing.

The proportion of susceptible sows at mating was mostly influenced by the changes in transmission parameters from S to I (β) and from R to S (θ) at mating and in the sow-compartment. On the other hand, the proportion of infectious sows at mating was mostly influenced by the transmission parameter from I to R (α) at mating and in the

sow-compartment. The proportion of carriers at mating was influenced by the transmission parameter from I to R (α) and from R to S (θ) in the sow-compartment (Figure 3 and Table 4).

Figure 4 – Modified spider plots for the sows susceptible (GS), infectious (GI) and carriers (GR) at gestation.

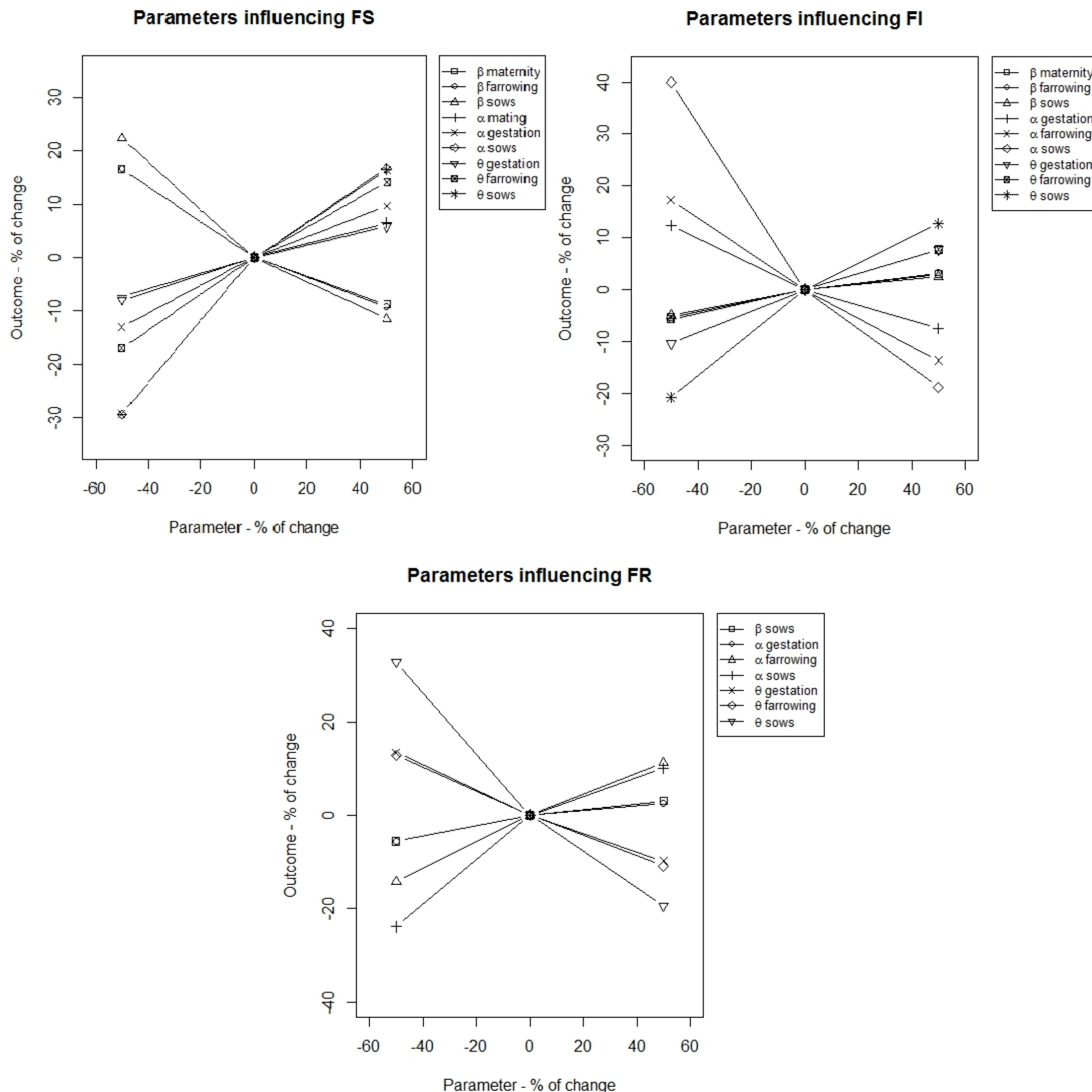


Legend: far cul rate – culling rate at farrowing, β – transition rate parameter between susceptible and infectious, α – transition rate parameter between infectious and carriers, θ – transition rate parameter between carriers to susceptible, mating – mating room, gestation – gestation room, sows – change in the parameters was done at mating, gestation and farrowing.

The proportion of susceptible sows at gestation was mostly influenced by the transition rate from R to S (θ) at gestation and in the sow-compartment; and the transition rate from I to R (α) at sow-compartment. On the other hand, the proportion of infectious sows at gestation was mostly influenced by the transition rate from I to R (α) at gestation and in the sow-compartment. The proportion of carriers at mating was

influenced by the transition rate from R to S (θ) at gestation and in the sow-compartment (Figure 4 and Table 4).

Figure 5 – Modified spider plots for the sows susceptible (FS), infectious (FI) and carriers (FR) at maternity.



Legend: β – transition rate parameter between susceptible and infectious, α – transition rate parameter between infectious and carriers, θ – transition rate parameter between carriers to susceptible, mating – mating room, gestation – gestation room, farrowing – farrowing room, maternity – change in the parameters for sows and piglets at the maternity, sows – change in the parameters was done at mating, gestation and farrowing for sows.

The β farrowing and β maternity were equal in terms of effect for the farrowing outcomes, which was expected as the piglets do not influence the infection state of sows. The θ gestation and α farrowing had a similar effect on the proportion of sows susceptible at maternity. The α sows and the θ sows exhibited similar behaviour (Figure 5 – Parameters influencing FS). This means that for the proportion of

susceptible sows at the end of maternity the change in the transmission parameter from R to S (θ) at gestation has the same influence as the change at farrowing of the transmission parameter from I to R (α). A similar effect was apparent for the same two parameters (θ and α) when we consider changes in the sow-compartment. When the transition rate from S to I in the sow-compartment increased, the proportion of sows susceptible at maternity experienced a reduction. On the other hand, an increase of the transition rate from I to R and the transition rate from R to S, in the sow-compartment, increased the proportion of sows susceptible at maternity.

The proportion of infectious sows at maternity was influenced by the α parameter (gestation, farrowing and sows), the δ sows and the θ gestation. All the β parameters (β farrowing, β maternity and β sows) and θ farrowing affected the outcome in the same way (Figure 5 – Parameters influencing FI).

Both β maternity and β sows include the same change as in the β farrowing. Therefore, as the results were the same between the three (β farrowing, β maternity and β sows), we can conclude that the change in transmission parameter from S to I (β) at farrowing was the one that influenced the proportion of infectious sows at farrowing.

In general, when the transition rate from S to I and from R to S increased, the proportion of infectious sows at maternity also increased. An increase of the transition rate parameter from I to R, reduced the proportion of infectious sows at maternity.

The proportion of carrier sows at maternity was influenced by the θ sows and α sows. The θ parameter at gestation and farrowing affected the outcome in the same way. The same happened to β sows and α gestation (Figure 5 - Parameters influencing FR).

The increase in the transmission parameter from R to S at sow-compartment reduced the proportion of carrier sows at maternity. The increase at farrowing and in the sow-compartment of the transmission parameter from I to R resulted in an increase in the proportion of carrier sows at maternity.

The transition rate from R to S at gestation and farrowing influenced the proportion of carrier sows at maternity in the same way. Their 50% reduction increased the proportion of carrier sows at maternity. The same happened to the change in the sow-compartment of the transmission parameter from S to I and the change at gestation of the transmission parameter from I to R, but in this case their increase, increased the proportion of carrier sows at maternity.

Table 5: Magnitude of the impact of the variation of individual infection parameters in the pigs' infection states (only the ones ≥ 5% are shown).

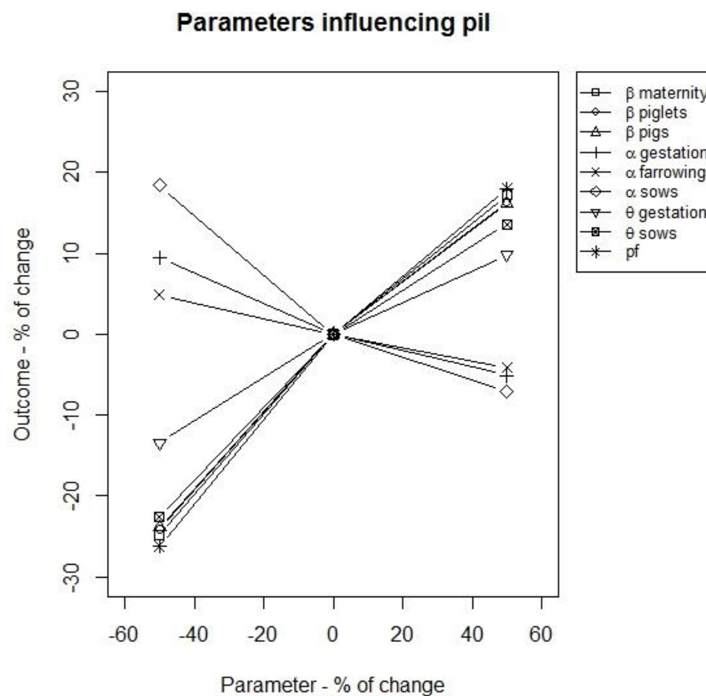
Changed parameter		Results – infection state							
		Piglets		Post-weaning			Fattening		
		S	I	S	I	R	S	I	R
Gestation	β	-	-	-	-	-	-	-	-
	α	-	-14.6%	-	-10.4%	-13.9%	6%	-8.7%	-11.4%
	δ	-	-	-	-	-	-	-	-
	θ	-	23.2%	-6.1%	17.8%	23.7%	-6%	14.8%	19.7%
Farrowin g	β	-	-	-	-	-	-	-	-
	α	-	-9%	-	-7%	-8.9%	-	-6%	-7.5%
	δ	-	-	-	-	-	-	-	-
	θ	-	7.9%	-	-	8%	-	5%	5.4%
Sows	β	-	-	-	-	-	-	-	-
	α	-	-25.5%	6.2%	-17.9%	-23.6%	10.6%	-15.2%	-19.9%
	δ	-	-	-	-	-	-	-	-
	θ	-	46.2%	-9.8%	22.5%	37.2%	-10.6%	23.3%	30.4%
Maternity	β	-5%	42%	-10.7%	31%	41.8%	-17.8%	25.5%	34.3%
Piglets	β	-5%	40.5%	-10.3%	29.4%	40.5%	-16.8%	24%	33%
Post-weaning	β	-	-	-	14.6%	-	-5.8%	8.5%	10%
	α	-	-	-	-	95%	-	-	27.2%
	δ	-	-	-	-	-	-	-	-
Fattening	β	-	-	-	-	-	-	6.3%	-
	α	-	-	-	-	-	-	-5.6%	54.4%
	δ	-	-	-	-	-	-	-	-
Pigs	β	-	40.3%	-14.6%	42.9%	39.6%	-25.9%	37.8%	42.1%
	α	-	-	-	-	95.4%	-	-8.8%	92.6%
	δ	-	-	-	-	-	-	-	-
Pf			44.2%	-8.4%	34.3%	44.2%	-15%	29.5%	37.7%

Legend: β – transition rate parameter from susceptible to infectious, α – transition rate parameter from infectious to carrier, δ – transition rate parameter from carrier to infectious, θ – transition rate parameter from carrier to susceptible, S – susceptible, I – infectious, R – carrier. Interpretation: positive number - the results increased with the increase of the parameter value, negative number – the results decreased with the increase of the parameter value.

The proportion of susceptible pigs at the maternity (piS) was only influenced in more than 5% by the piglets protective factor (pf) in extreme values, therefore a plot for this outcome was not shown. The influence of the pf parameter in this outcome is shown in Figure 9.

The proportion of infectious piglets at maternity was influenced by the piglets' protection factor (pf), the θ gestation and θ sows, the β maternity, β piglets and β pigs, and the α gestation, α farrowing and α sows. The increase of the pf value, the β parameters and the θ parameters, increased the proportion of infectious piglets. While the increase of the α parameters decreased the proportion of infectious piglets. The pf and β had similar results (Figure 6 and Table 5).

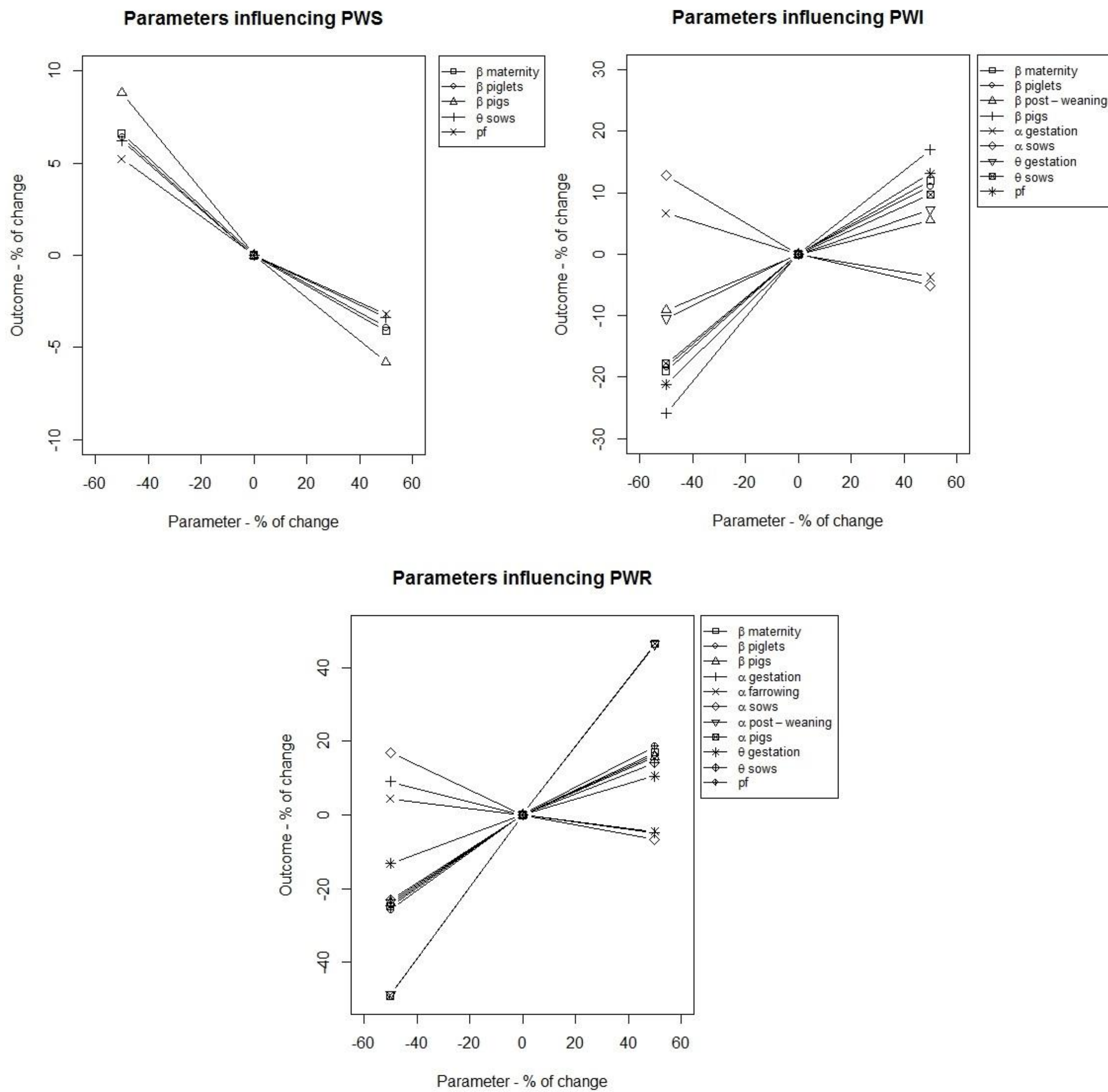
Figure 6 – Modified spider plots for the infectious piglets (piI) at maternity.



Legend: β – transition rate parameter between susceptible and infectious, α – transition rate parameter between infectious and carriers, θ – transition rate parameter between carriers to susceptible, gestation – gestation room, farrowing – farrowing room, piglets – piglets on maternity, maternity – change in the parameters for sows and piglets at the maternity, sows – change in the parameters was done at mating, gestation and farrowing for sows, pigs – change in the parameters for pigs at maternity, post-weaning and fattening, pf – piglets protective factor.

The infection state of pigs at post-weaning was influenced by the β maternity, β piglets, β post-weaning and β pigs; the α gestation, α farrowing, α sows, α post-weaning and α pigs; the θ gestation and θ sows; and the pf parameter (Figure 7 and Table 5). The increase in the mentioned βs increased the number of infectious and carrier pigs at post-weaning, while it decreased the number of susceptible at post-weaning. The increase in the transition rate from I to R (α) in the sow-compartment decreased the proportion of carriers at post-weaning. However if the increase from I to R occurred in the pig-compartment there was an increase in the proportion of carriers at post-weaning. The increase in the transition rate from R to S (θ) in the sow-compartment and pf parameter, decreased the proportion of susceptible at post-weaning while it increased the proportion of infectious and carriers at post-weaning.

Figure 7 – Modified spider plots for the susceptible (PWS), infectious (PWI) and carrier (PWR) pigs at post-weaning.



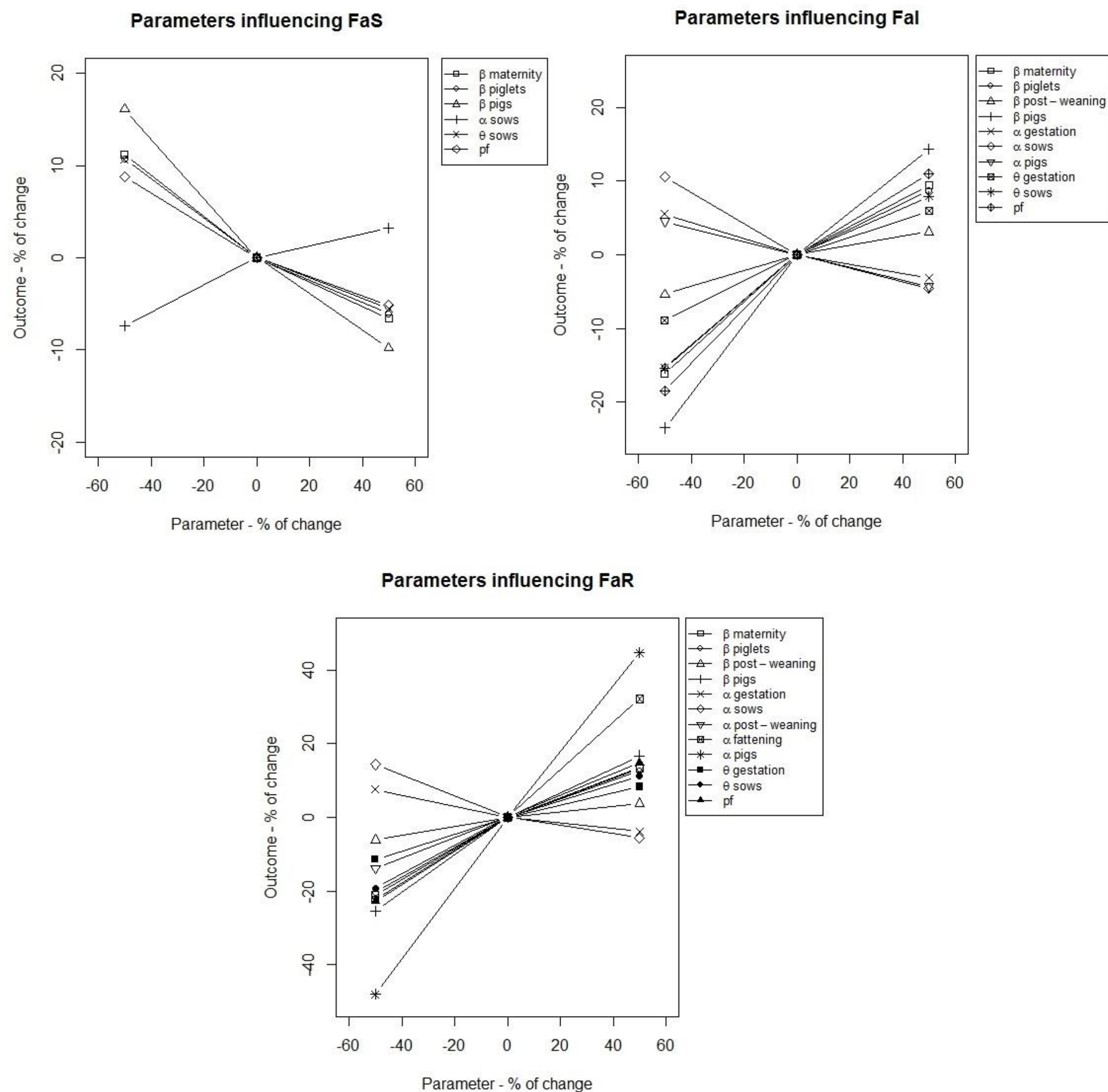
Legend: β – transition rate parameter between susceptible and infectious, α – transition rate parameter between infectious and carriers, θ – transition rate parameter between carriers to susceptible, gestation – gestation room, farrowing – farrowing room, piglets – piglets on maternity, post-weaning – post-weaning room, maternity – change in the parameters for sows and piglets at the maternity, sows – change in the parameters was done at mating, gestation and farrowing for sows, pigs – change in the parameters for pigs at maternity, post-weaning and fattening, pf – piglets protective factor.

The transition rate from S to I at post-weaning and the transition rate from R to S at gestation had the same results in terms of changes to the proportion of infectious pigs at post-weaning. Additionally, the pf parameter, the transition rate from S to I at maternity (sows and piglets) and piglets (only piglets), and the transition rate from R to S at the sow-compartment influenced in the same way the proportion of infectious and carrier pigs at post-weaning. These similar results for the β parameters (β maternity, β piglets and β pigs) suggest that the change in the transition rate from S to I in the

piglets at maternity was the major responsible for all the changes in the proportion of infectious and carriers at post-weaning due to the β parameters.

For carrier pigs, the post-weaning and pig-compartment changes of the transition rate from I to R showed the same results, suggesting the change is due to the post-weaning only.

Figure 8 – Modified spider plots for the pigs susceptible (FaS), infectious (FaI) and carriers (FaR) at fattening.



Legend: β – transition rate parameter between susceptible and infectious, α – transition rate parameter between infectious and carriers, θ – transition rate parameter between carriers to susceptible, gestation – gestation room, piglets – piglets on maternity, post-weaning – post-weaning room, fattening – fattening room, maternity – change in the parameters for sows and piglets at the maternity, sows – change in the parameters was done at mating, gestation and farrowing for sows, pigs – change in the parameters for pigs at maternity, post-weaning and fattening, pf – piglets protective factor.

The infection state of pigs at fattening (Figure 8 and Table 5) was influenced by the transition rate from S to I at maternity and the pig-compartment (mainly piglets at

maternity and pigs at post-weaning). The increase of the β parameters caused a decrease in the proportion of susceptible and an increase in the proportion of infectious and carriers.

The transition rate from I to R (α) also influenced the infection status of the pigs at fattening. An increase on this parameter at the pig-compartment caused an increase of carrier pigs and a decrease in infectious pigs. On the other hand, an increase of α parameters at the sow-compartment caused a decrease in the proportion of carriers associated also with a decrease in the proportion of infectious pigs.

The transition rate from R to S (θ) in the sow-compartment caused a decrease in the proportion of carrier pigs at fattening while increased the proportion of infectious at fattening.

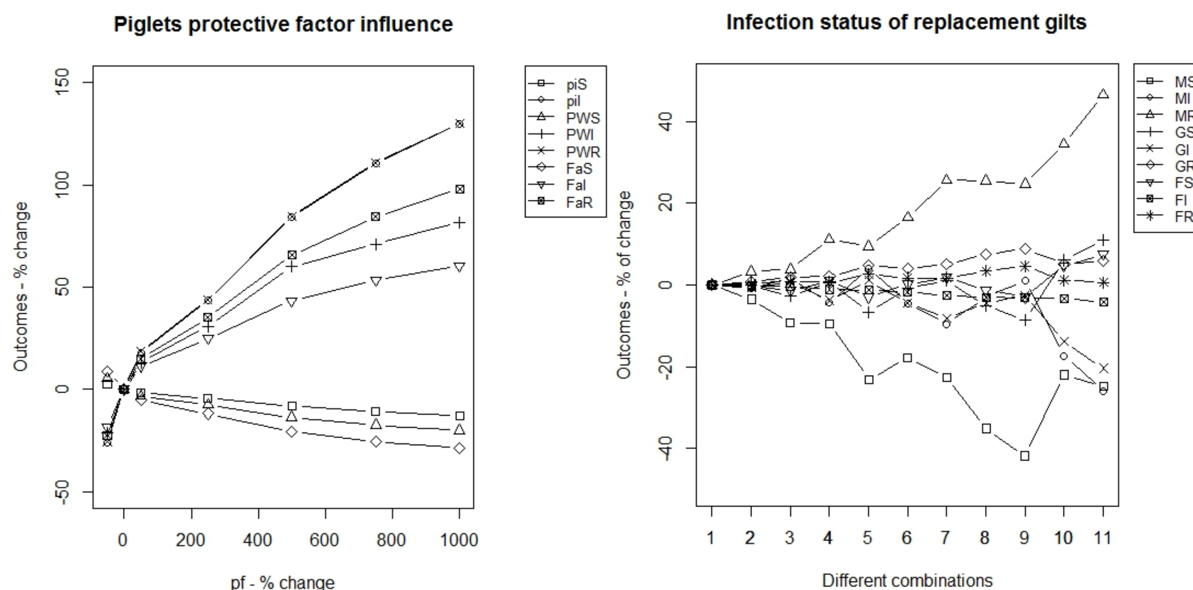
The piglets' protective factor also influenced the infection state of the pigs at fattening.

The transmission parameter from R to S at the sow-compartment produced the same change in the infectious pigs at fattening as the transmission parameter between S to I at maternity. For carrier pigs, changes of the transition rate from S to I at maternity, piglets and the pig-compartment, the pf parameter and the transmission parameter from R to S at the sow-compartment, caused similar changes in the proportion of carriers.

The piglets' protective factor influence was assessed not only by increasing and reducing the parameter in 50%, but also using higher percentages. The pf value in the model was assumed to be 0.1 (90% of protection). As the value was quite low, the values of 0.25 (75% of protection), 0.50 (50% of protection), 0.75 (25% of protection) and 1.0 (no protection) were used to evaluate its influence in the pig-compartment. The results (Figure 9 – left panel) show that the pf parameter, when assumed extreme values, influenced the pigs' infection state by more than 50% for the infectious and carrier pigs at all the pig's rooms.

The infection state of the replacement gilts only influenced, by more than 5%, the sow-compartment, therefore only the sows' outcomes were used in the plot (Figure 9 – right panel). The results show that perturbations in this parameter have not resulted in major changes in the infection state of sows at farrowing, being more influential for the infection state of sows at mating. All the combinations produced an increase in the proportion of carrier sows at mating while decreasing the proportion of susceptible sows at mating, when compared to the combination where all the gilts were susceptible (combination number 1).

Figure 9 – Modified spider plots for the influence of the piglets' protective factor (pf) and the infection state of replacement gilts (see Table 2 for the combinations values).



Legend: MS – sows susceptible at mating, MI – sows infectious at mating, MR – sows carriers at mating, GS - sows susceptible at gestation, GI – sows infectious at gestation, GR – sows carriers at gestation, FS - sows susceptible at farrowing, FI – sows infectious at farrowing, FR – sows carriers at farrowing, piS – piglets susceptible at maternity, piI – piglets infectious at maternity, PWS – pigs susceptible at post-weaning, PWI – pigs infectious at post-weaning, PWR – pigs carriers at post-weaning, FaS – pigs susceptible at fattening, FaI – pigs infectious at fattening, FaR – pigs carriers at fattening, pf – piglets protective factor.

The change to housing grouped sows at the mating room (in the same way as the gestation room) did not cause any major changes to the results. The highest percentage of change was for the proportion of susceptible sows at gestation room that increased by 3%. The pigs' infection states were influenced by less than 1%.

Discussion

Validation of the model

The predicted prevalence results of the model for the infectious animals in the sow-compartment (56.9% [16.7% - 100%] of sows infectious at the end of farrowing) compared to the pig's results (30.9% [0.0% - 80%] of pigs infectious at the end of fattening) show a decreasing trend from the sow-compartment to the pig-compartment. The same decreasing trend was observed in the Baseline Studies [16, 17] for *Salmonella* Typhimurium, where the prevalence was higher for breeding animals (13.4% [8.8% – 20.3%] of holdings positive) than for pigs at slaughter (8.4% [6.1% - 11.5%] pigs positive); and for *Salmonella* spp., where the prevalence was also higher

for breeding animals (43.3% [35.6% – 52%] of holdings positive) than for pigs at slaughter (23.4% [19.4% - 28%] pigs positive).

The prevalence figure for the breeding animals in the Baseline Study was at holding level which does not allow such a straightforward comparison with the pig at slaughter results. However the used of pooled pen samples allowed to detect, with 95% certainty, at least one positive sample in a pig herd when the animal level prevalence was at least 20% [18], and the within-holdings pen positive results in Portugal was around 20% to 80% [19], which allow us to assume that the prevalence of sows infected was around 20% (detection threshold). This value is higher than the prevalence of *S.* Typhimurium in slaughter pigs.

Note that the observed (epidemiological) data to which the estimated parameters are being compared includes uncertainty due to sampling error (statistical uncertainty), and imperfect test sensitivity and/or specificity. In addition, the samples whereupon the epidemiological data (representative sample of pigs at slaughter and of holdings with breeding pigs) are based do not match the units in which we worked within the model (sows and pigs from infected farms). Therefore, the comparison of predicted and observed values was done on a qualitative basis with focus on whether the model was predicting the same trends that were indicated by the observed data. Even so the proportion of infectious sows at maternity can be considered relatively high while the proportion of carrier sows seems low when compared to other simulation studies [5]. Reinforced by the evidence that are higher odds of sows at mating being shedding *Salmonella* when compared to maternity [20], even if this happen to other serotypes non Typhimurium like leads us to suggest that some assumptions made in the transmission parameters, which where estimated using data from growing pigs, are not suitable to be used in sows. Therefore care should be taken in the interpretation of the proportion of the infectious states at the sow compartment.

Sensitivity analysis of the model

The sensitivity analysis shows the influence on the results of the model due to changes in the parameters, and can also give us valuable information about the uncertainty of the assumptions made.

The sensitivity analysis results section shows for each outcome (infection state per room) which parameters influenced them in more than 5% (differences of less than 5% were not considered for discussion even if statistical significant).

The parameters which influenced most (higher than 30%) the sow-compartment were (Table 4): the transition rate from infectious to carrier (α), from carrier to susceptible (θ), and from susceptible to infectious (β). The β increase, reduced the proportion of

susceptible animals; the α increase, increased the proportion of infectious and carrier animals; and the θ increase, reduced the proportion of carrier while increased the proportion of susceptible and infectious. Therefore all the factors which influence the time as carrier (reduction of θ, increase of α) will help the reduction of infectious sows in infected farms.

The parameters which influenced most (higher than 30%) the pig-compartment were (Table 5): the transition rate from infectious to carrier (α) in pigs and sows, from carrier to susceptible (θ) in sows, and from susceptible to infectious (β) in pigs; and the piglets' protective factor (pf). The transition from carrier to infectious (δ), whether in pigs or sows, did not influence in a major extent the results. The β increase in pigs, reduced the proportion of susceptible animals while increased the proportion of infectious and carrier animals; the α increase in pigs, increased the proportion of carrier animals; the α increase in sows, reduced the proportion of infectious and carrier pigs while increased the proportion of susceptible; the θ increase in sows, reduced the proportion of susceptible pigs while increased the proportion of infectious and carrier pigs; and the pf parameter increase, increased the proportion of infectious and carrier pigs. The aim in the pig-compartment should be to reduce the number of infectious and carrier animals and this can be achieved by applying control measures which influence the infection of pigs (reduction of β) and piglets (reduction of pf), and increase the time as carrier for sows (reduction of θ and increase of α).

The outcomes which are of great importance in terms of public health are the infection state for sows at maternity (which contributes to the piglets' infection state) and pigs at fattening (which are going to be slaughtered and will contribute to human infections due to the consumption of pork meat). Therefore the parameters which influence them were analysed in more detail.

Parameters that influence the infection of sows at maternity

The infection state of sows at maternity room was influenced by several parameters. The proportion of susceptible sows increased 46.1% and 45.8% with the increase of the transition rate from I to R and from R to S when applied to the sow-compartment, respectively (Table 4). The parameter which influenced by a highest percentage the infectious sows at maternity was the transition rate from I to R when applied to the sow-compartment. The decrease of 50% in this parameter increased the proportion of sows infectious around 40%, but a 50% parameter increase only reduced the proportion of sows infectious around 20% (Figure 5). The same amount of reduction was achieved when reducing by 50% the transmission parameter from R to S in the sow-compartment. This makes sense, as increasing the R to S transition rate, increases the

number of susceptible sows that can be subjected to infection again. Regarding the carrier sows at maternity, increasing the R to S transition rate in the sow-compartment decreased the amount of carriers, while increased I to R transition rate, again in the sow-compartment, increased the amount of carriers. As only the infectious sows can infect the piglets it is desirable to reduce the proportion of infectious sows at farrowing. The results show that this can be done by promoting the increase of the transition rate from infectious to carrier. Although the majority of the control measures described in the literature are for pigs and not sows, we can assume that they will also applied to sows. Therefore, increasing the transition from I to R, can be done by promoting the use of organic acids in feed/water or fermented by products [21], which decreases the shedding duration. The super-shedders and long-shedders should also be reduced as they reduce the transition rate from I to R. Increasing the immune status of the farm to other disease known to interfere with immunity (e.g. PRRS, parasitosis, etc.) [22] will help decreasing the number of super-shedders or long-time shedders. The increase of susceptible sows at maternity can be achieved by the reduction of the transition rate from S to I. Effective cleaning and disinfection procedures between batches [21, 23], change in feed strategy from pelleted feed to non-pelleted feed, fermented wet feed or partially non-heat-treated feed could help lower the exposure to *Salmonella* and increase the resistance to infection [21, 24]. The reduction of the number of animals per pen can also decrease the transmission of the infection [20]. The combination of several measures will be ideal for achieving the reduction wanted [21]. The use of "*Salmonella*-free" replacement stock has been advocated [21], but the results of the model show that, at least in infected farms, the relative effect of this measure is low to achieve a reduction in infectious sows at farrowing/maternity room. In farms infected with a low prevalence, the use of "*Salmonella*-free" replacement stock is an important control measure, especially if the farm is implementing measures of selective culling of infectious sows.

The use of vaccines in sows can be advocated if vaccination will be able to reduce the rate of infection (providing persistent immunity during the life-span of sows), or, at least, to reduce the shedding duration, and in this way increase the time as carrier in sows (especially at maternity).

Parameters that influence the infection of pigs at fattening

The parameters which influenced most the infection state of pigs at fattening were: the transition rate from S to I (β) and the transition rate from I to R (α), when changes applied to the pig-compartment; the transition rate from R to S (θ) when changes applied to the sow-compartment, and the piglets' protective factor (pf). The parameter

which influenced most the infectious pigs at fattening was the transition rate from S to I (β) when changed in the pig-compartment, where a reduction in 50% caused a 20% reduction of infectious pigs. A reduction by 50% of pf value caused also the same 20% of reduction of infection pigs. The increase by 50% of the transition rate from I to R (α) in all the sow and pig-compartment caused also a reduction in the infectious animals, although by less than 10%. The reduction by 50% in the sow-compartments of the transition rate from R to S, reduced by 15% the infectious pigs. This reduction was probably due to the reduction in infectious sows at maternity, which we have discussed previously. The parameter which influenced most the proportion of carrier pigs at fattening was the transition rate from I to R (α) in the pig-compartment, where an increase of 50% caused an increase of about 45% of carrier pigs. The increase of the transition rate from R to S in the sow-compartment, the pf increase and the increase in the transition rate from S to I in the pig-compartment also caused an increase in the number of carrier pigs. At the end of fattening the pigs are sent to the abattoir for slaughter. The transport to the abattoir and the lairage waiting time, are stressful events which potentially increase the transition rate from S to I and from R to I in infected pigs. Therefore the aim should be to reduce the proportion of infectious and carrier animals. This can be achieved by decreasing at the pig-compartment the transmission parameters from S to I, decreasing the piglets' protective factor value and the transmission parameter from R to S at the sow-compartment. To reduce the transition rate parameter from S to I, several measures can be put in place like adding organic acids to the feed or water [21], change the type of feed to a wet feed or non-pelleted feed [25-27], cleaning frequently the pen floor [22, 28-30], minimizing the mixture of litters at post-weaning and fattening [31, 32], and reducing stock density per pen [20, 29]. Control of rodents and other vectors is advocated [21], although it was not considered a significant risk factor for *S.* Typhimurium in one study [20]. The reduction of the pf value (which means increasing the piglets' protective factor) can be achieved by allowing the correct consumption of colostrum by the piglets [33] and by the reduction of all the stressful events [32] or concomitant diseases [34], which decrease the piglets' immunity.

The use of vaccines which enable to reduce the infection of pigs during their life-span will have a positive effect in the reduction of the infectious and carrier pigs at the end of fattening. The vaccine effect would be similar to have a pf effect upon the post-weaning and fattening stage.

The results of the sensitivity analysis have also shown that the parameters which depended on expert opinion have not caused a major change in the results of the

simulation model. The most influencing parameters were the transition rates and the piglets' protective factor. The transmission parameters were based in a field study which followed cohorts of pigs on infected farms [13]. The estimation of the values was performed in Correia-Gomes *et al* (unpublished). The details of the estimation are described there. These values were estimated for *Salmonella* Typhimurium infection in growing cohorts in Denmark. The cohorts followed belong to farrow-to-finish farms which were self-supplying and applied batch management [13]. We have no reason to assume that the same transition rates are not applicable to Portuguese infected cohorts. As there was not any study which estimates the transmission parameters for sows, or data available for estimating these parameters; we have assumed that the transmission parameters estimated for the pigs could be applied also to sows. This assumption, as discussed previously, may prove to be wrong and further research is needed to update these values. The value of the transition rate from R to S and the piglets' protective factor were based on existing literature. The piglets' protective factor was not specific for *Salmonella* Typhimurium. The sensitivity analysis shows that this parameter influences in a great extension the results (Table 5 and Figure 9), reflecting the uncertainty around this parameter. More research in the transition rate parameters for sows and pigs, and the piglets' protective factor would be needed to improve the simulation model accuracy.

Contribution of this model to Portuguese pig production

In Portugal 13.4% of the production holdings with breeding animals (the majority farrow-to-finish farms) were considered to be positive to *Salmonella* Typhimurium [15]. If we consider also the strains similar to *Salmonella* Typhimurium and therefore with basis to assume similar behaviour, the value increases to 25.6% [20]. The infected holdings in Portugal have a higher number of animals per farm than the negative ones [20], and it can be assumed that they will contribute with a higher number of fattening pigs to slaughter and therefore with pork for human consumption. The simulation model can, then, be used to test control measures in terms of their cost-benefit, and the reduction of the prevalence in these farms will have an impact on human burden.

Conclusion

A stochastic model which simulates the dynamics of *Salmonella* Typhimurium in a farrow-to-finish farm was constructed. The parameters which influence each infection state per room were identified and the possible control measures for the fattening room were discussed. The simulation model potentially allows estimation of cost-benefit

control measures if coupled to an economic model. The simulation model is flexible enough to introduce changes in the parameter distributions or values if future research and legislation so require. At the same time the model can be adapted to different types of production (e.g. breeding units, finisher units) as it was built in a compartmental way.

Acknowledgements

We would like to thank to the authors of the Portuguese survey (Baptista et al, 2010) for given us access to the production parameters results, to IBERSAN for given us access their production data and to Dr Carmo Reis for is valuable expert opinion.

References

[1] EFSA, ECDC, The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010, EFSA Journal. (2012) 10:2597.
[2] Hurd H.S., Enoe C., Sorensen L., Wachman H., Corns S.M., Bryden K.M., Grenier M., Risk-based analysis of the Danish pork Salmonella program: past and future, Risk Anal. (2008) 28:341-351.
[3] Consortium F., Analysis of the costs and benefits of setting a target for the reduction of *Salmonella* in slaughter pigs for European Commision Health and Consumers Directorate-General SANCO/2008/E2/036 Final Report, 2010, pp. 1-198.
[4] VLA-DTU-RIVM, EFSA Quantitative Microbiological Risk Assessment on Salmonella in Slaughter and Breeder Pigs: Final Report. Question No EFSA-Q-2007-00245. Grant number CFP/EFSA/BIOHAZ/2007/01, Veterinary Laboratories Agency., 2010, pp. 437.
[5] Lurette A., Belloc C., Touzeau S., Hoch T., Ezanno P., Seegers H., Fourichon C., Modelling Salmonella spread within a farrow-to-finish pig herd, Veterinary research. (2008) 39:49.
[6] (BIOHAZ) E.P.o.B.H., Scientific Opinion on monitoring and assessment of the public health risk of "Salmonella Typhimurium-like" strains, EFSA Journal. (2010) 8:48.
[7] Hill A.A., Snary E.L., Arnold M.E., Alban L., Cook A.J., Dynamics of Salmonella transmission on a British pig grower-finisher farm: a stochastic model, Epidemiology and infection. (2008) 136:320-333.
[8] McNamara P.E., Miller G.Y., Liu X., Barber D.A., A farm-to-fork stochastic simulation model of pork-borne salmonellosis in humans: Lessons for risk ranking, Agribusiness. (2007) 23:157-172.
[9] Soumpasis I., Butler F., Development and Application of a Stochastic Epidemic Model for the Transmission of Salmonella Typhimurium at the Farm Level of the Pork Production Chain, Risk Anal. (2009):1521-1533.
[10] Krieter J., Evaluation of Salmonella surveillance in pigs using a stochastic simulation model, Arch. Tierz.-Arch. Anim. Breed. (2004) 47:337-349.

[11] van der Gaag M.A., Vos F., Saatkamp H.W., van Boven M., van Beek P., Huirne R.B.M., A state-transition simulation model for the spread of Salmonella in the pork supply chain, European Journal of Operational Research. (2004) 156:782-798.
[12] Baptista F.M., Alban L., Nielsen L.R., Domingos I., Pomba C., Almeida V., Use of Herd Information for Predicting Salmonella Status in Pig Herds, Zoonoses and public health. (2010) 57:49-59.
[13] Kranker S., Alban L., Boes J., Dahl J., Longitudinal Study of Salmonella enterica Serotype Typhimurium Infection in Three Danish Farrow-to-Finish Swine Herds, J. Clin. Microbiol. (2003) 41:2282-2288.
[14] Beloeil P.A., Chauvin C., Proux K., Rose N., Queguiner S., Eveno E., Houdayer C., Rose V., Fravalo P., Madec F., Longitudinal serological responses to Salmonella enterica of growing pigs in a subclinically infected herd, Prev Vet Med. (2003) 60:207-226.
[15] Nielsen B., Baggesen D., Bager F., Haugegaard J., Lind P., The serological response to Salmonella serovars typhimurium and infantis in experimentally infected pigs. The time course followed with an indirect anti-LPS ELISA and bacteriological examinations, Veterinary microbiology. (1995) 47:205-218.
[16] EFSA, Report of the Task Force on Zoonoses Data Collection on the analysis of the baseline survey on the prevalence of *Salmonella* in slaughter pigs, PartA, The EFSA Journal. (2008) 135:111.
[17] EFSA, Analysis of the baseline survey on the prevalence of Salmonella in holdings with breeding pigs, in the EU, 2008, Part A: Salmonella prevalence estimates, EFSA Journal. (2009) 7:1377.
[18] Arnold M.E., Cook A.J., Estimation of sample sizes for pooled faecal sampling for detection of Salmonella in pigs, Epidemiology and infection. (2009):1734-1741.
[19] EFSA, Analysis of the baseline survey of Salmonella in holdings with

breeding pigs, in the EU, 2008; Part B: Analysis of factors potentially associated with Salmonella pen positivity., EFSA Journal. (2011) 9:2329.
[20] Correia-Gomes C., Economou T., Mendonça D., Vieira-Pinto M., Niza-Ribeiro J., Assessing risk profiles for Salmonella serotypes in breeding pig operations in Portugal using a Bayesian hierarchical model, BMC veterinary research. (2012) 8:226.
[21] Lo Fo Wong D.M.A., Hald T., van der Wolf P.J., Swanenburg M., Epidemiology and control measures for Salmonella in pigs and pork, Livestock Production Science. (2002) 76:215-222.
[22] Beloeil P.A., Fravalo P., Fablet C., Jolly J.P., Eveno E., Hascoet Y., Chauvin C., Salvat G., Madec F., Risk factors for Salmonella enterica subsp. enterica shedding by market-age pigs in French farrow-to-finish herds, Prev Vet Med. (2004) 63:103-120.
[23] Funk J., Gebreyes W.A., Risk factors associated with Salmonella prevalence on swine farms, Journal of Swine Health and Production. (2004) 12:246-251.
[24] Berends B.R., Urlings H.A., Snijders J.M., Van Knapen F., Identification and quantification of risk factors in animal management and transport regarding Salmonella spp. in pigs, International journal of food microbiology. (1996) 30:37-53.
[25] Bahnson P.B., Fedorka-Cray P.J., Ladely S.R., Mateus-Pinilla N.E., Herd-level risk factors for Salmonella enterica subsp. enterica in U.S. market pigs, Prev Vet Med. (2006) 76:249-262.
[26] Farzan A., Friendship R.M., Dewey C.E., Warriner K., Poppe C., Klotins K., Prevalence of Salmonella spp. on Canadian pig farms using liquid or dry-feeding, Preventive Veterinary Medicine. (2006) 73:241-254.
[27] Leontides L.S., Grafanakis E., Genigeorgis C., Factors associated with the serological prevalence of Salmonella enterica in Greek finishing swine herds, Epidemiology and infection. (2003) 131:599-606.

[28] Beloeil P.A., Chauvin C., Proux K., Fablet C., Madec F., Alioum A., Risk factors for Salmonella seroconversion of fattening pigs in farrow-to-finish herds, Veterinary research. (2007) 38:835-848.
[29] Funk J.A., Davies P.R., Gebreyes W., Risk factors associated with Salmonella enterica prevalence in three-site swine production systems in North Carolina, USA, Berliner und Munchener tierarztliche Wochenschrift. (2001) 114:335-338.
[30] Lo Fo Wong D.M., Dahl J., Wingstrand A., van der Wolf P.J., von Altrock A., Thorberg B.M., A European longitudinal study in Salmonella seronegative- and seropositive-classified finishing pig herds, Epidemiology and infection. (2004) 132:903-914.
[31] Rossel R., Rouillier J., Beloeil P.A., Chauvin C., Basta F., Crabos J.P., Theau-Audin S., Salmonella en élevage de porcs du Sud-Ouest de la France: séroprévalence en fin d'engraissement et facteurs de risque associés., Journées de la Recherche Porcine. (2006) 38:371-378.
[32] Callaway T.R., Morrow J.L., Edrington T.S., Genovese K.J., Dowd S., Carroll J., Dailey J.W., Harvey R.B., Poole T.L., Anderson R.C., Nisbet D.J., Social stress increases fecal shedding of Salmonella Typhimurium by early weaned piglets, Current Issues in Intestinal Microbiology. (2006) 7:65-71.
[33] Lo Fo Wong D.M., Dahl J., Stege H., van der Wolf P.J., Leontides L., von Altrock A., Thorberg B.M., Herd-level risk factors for subclinical Salmonella infection in European finishing-pig herds, Prev Vet Med. (2004) 62:253-266.
[34] Møller K., Jensen T.K., E. Jorsal S., Leser T.D., Carstensen B., Detection of Lawsonia intracellularis, Serpulina hyodysenteriae, weakly beta-haemolytic intestinal spirochaetes, Salmonella enterica, and haemolytic Escherichia coli from swine herds with and without diarrhoea among growing pigs, Veterinary microbiology. (1998) 62:59-72.

Chapter 4 – Discussion and Conclusions

The aim of the PhD was to improve the epidemiologic knowledge of *Salmonella* spp. disease dynamics in pig farms in Portugal, and therefore to contribute to a better use of the available cost-effective control measures at farm level, taking into consideration the prevalence of the agent, the risk factors associated with *Salmonella* spp. and the Portuguese production system. To achieve this, the work was divided into two objectives: Objective 1 - the improvement of the knowledge of the risk factors for *Salmonella* spp. in Portugal at farm and abattoir; and Objective 2 - the development of a simulation model that describes *Salmonella* Typhimurium dynamic in a pig farm.
For fulfilling these Objectives several datasets made available to the authors were used. For Objective 1, the datasets of the Baselines studies on *Salmonella* prevalence at slaughter and at farms with breeding pigs in Portugal were made available by the Portuguese Veterinary Authorities.

Risk Factors in Portugal

The dataset of the Baseline Survey on *Salmonella* Prevalence in Slaughter Pigs in Portugal allowed testing for risk factors at abattoir level. In this survey some information was collected about the abattoir characteristics (e.g. slaughter volume, region), and no information about the pig's farm of origin. Even so it was possible to assess some possible risk factors (Manuscript 1). The abattoir region and the sample collection time were considered as significant risk factors. The abattoir region could be associated with different abattoir management practices or with the herds that supply the abattoirs but this hypothesis could not be tested. The dataset was insufficient to give a response to such questions and more work should be done in the future to evaluate the risk factors at abattoir level. In the categorical model, a variable found to be significant was the sample collection hour (afternoon at lower risk when compared to morning) for the category of serotype Typhimurium or serotype 1,4,[5],12:i:-. A biological explanation for this association could be that the animals slaughtered in the afternoon have spent less time in the lairage because they enter the slaughterhouse early morning to be culled in the same day. Because the transmission of this type of *Salmonella* is strongly associated with transmission between live animals, the reduction at lairage, of the contact between pigs from different sources could play an important role in explaining this finding.
In the majority of the positive lymph node samples the serotype Typhimurium was isolated, followed by serotype Rissen, Derby and *S.* Typhimurium-like strains.
Likewise in the data of the prevalence of *Salmonella* at farms with breeding pigs (Baseline Survey on the Prevalence of *Salmonella* in Breeding Pigs in Portugal), the serotype Typhimurium was also the predominant serotype, followed by serotype

Rissen, London and Derby. This shows that the majority of the serotypes present at herd level are also present at abattoir level, suggesting that the farm of origin is a source of contamination.

In the analyses of the breeding pig's dataset, several management factors linked to herd and pen were evaluated. The *Salmonella* spp. risk factors found were: region of the herd, size of the herd (in terms of sows), management of breeding boars, source of semen, rodents control, number of animals per pen, breeding sector, and source of feed. Some of these risk factors had already been previously identified in other studies (e.g. size of the herd, rodents control, number of animals per pen, source of feed)[71, 75, 168]. However it was the first time that the management of breeding boars and source of semen were identified as risk factors for *Salmonella*. This can be explained by the characteristics of Portuguese swine production, where a substantial proportion of the semen used does not come from insemination centres. Even so the identification of these risk factors contributes to the improvement of knowledge, and should be used in the elaboration of control programmes adapted to the Portuguese situation.

The data from the Baseline Survey also shows that in Portugal 9.1% of the breeding holdings were positive to *Salmonella* Typhimurium and 33.3% were positive to other serotypes than Typhimurium and Derby. In the work reported in Manuscript 3 we answered the question if the risk factors for infection were the same for all serotypes of *Salmonella*. As the number of samples per serotype was low we could not analyse all of them separately. Therefore the different serotypes of *Salmonella* spp. were divided in two groups: serotype Typhimurium and *S.* Typhimurium-like strains with the antigenic formula: 1,4,[5],12:i:-., and other serotypes. These groups were formed because serotype Typhimurium is recognized as a serotype difficult to control [169] and is also the cause of many human cases of food-borne disease linked to pork meat. Serotype Typhimurium-like strains with the antigenic formula: 1,4,[5],12:i:-. were included in the group of serotype Typhimurium because of the genetic similarity, the similar virulence and the antimicrobial resistance characteristics existing between the two serotypes [170]. After the serotypes were grouped and analysed using a categorical model, it was found that the risk factors were different between the two groups of serotypes. The group "Typhimurium" was associated with the stock density (number of breeding pigs and number of pigs per pen), the characteristics of the pig (age of breeding sows), and the source of semen. On the other hand, the group "other serotypes" was associated with region of the herd, source of semen, control of rodents, breeding sector room and source of feed. The risk factors for serotype Typhimurium suggest a contagious pattern and the risk factors for other serotypes appeared to be related to environmental factors.

This valuable information can be incorporated in future control programmes for *Salmonella* spp. in breeding pigs in Portugal and other countries, and also highlight the areas where further studies should be done to explain the causality of some risk factors.

Modelling the dynamics of infection

To help policy makers, and the pig industry in general, in times of scarce resources, models are good approximations to the real transmission dynamics which allow the outcome of control measures to be tested, and gives an estimation of predicted prevalence for that outcome. An infection model that does not incorporate what is happening on a farm in terms of animal management could introduce bias in the final outcome. Among other events, the culling of infectious animals and the mixing of pigs from different litters could both interfere with transmission dynamics. For these reasons a production model linked to an infection model seemed sensible and more accurate. In Portugal a good published description of production parameters is lacking and companies do not share information between themselves or with external organizations. Therefore it was difficult to obtain production data, and as such, expert opinion was used to fill gaps in data/information. Even so, the production model reflects what is considered to occur in an average farrow-to-finish farm, which constitutes the dominant type of farms in Portugal. As the model was built in compartments for each of production phases, it can be easily adapted to different type of farms, such as breeding or finisher units. The model is flexible enough to accommodate changes in the various parameters values and distributions. For the infection part of the simulation model, estimates of the transmission parameters were required.
We found in the literature a gap in the knowledge about the values attributable to the transmission parameters for *Salmonella*, as majority of the simulations studies published in the literature used point estimates or fitted estimates to a final prevalence as inputs for the transmission parameters [140, 142, 143, 145]. Therefore, the available *Salmonella* Typhimurium transmission studies in field conditions were used to estimate the transmission parameters for the model. Several authors were contacted but only one was willing to share their data. Manuscript 4 describes the work done. The results showed that the transition rate found between susceptible and infectious (β) can be considered low compared with other infectious diseases (such as swine influenza) and reflects that in most of the herds, *Salmonella* does not cause outbreaks but maintains a residual level of infection represented by infectious animals and carrier animals that enable the infection to persist in the herds. The credible interval for the transition between infectious to carriers (α) shows the variability of shedding duration which is

known from experimental and field studies. The transition rate between carrier and infectious was small - as shown by the results from our model - and highly dependent on stress events (the authors of the Kranker *et al.* study [146] describe that two cohorts had a slurry overflow which is a stressful event). The variance of the cohort time dependent random effect was high which means that the different management procedures for each cohort influences the transmission data and in future studies this should be taken into consideration. For the spread to occur, R_0 should be above 1. In our study (Manuscript 4), the median R_0 value was estimated to be 1.91 which indicates that *Salmonella* Typhimurium was spreading in most of the cohorts. The value was not high (third quartile is less than 3) implying it would not spread rapidly through the susceptible populations under management systems similar to the ones used in these herds. In less frequent situations, however, R_0 was high enough to cause outbreaks.

Although the transmission parameters were estimated based on pig's growing cohorts, they were also applied to the sow-compartment in the simulation model, as it was assumed that they would behave in the same way for sows.

The results of the simulation model for the infectious pigs at fattening show the dynamic of the pigs' infection state over the time needed to raise a pig to slaughter, with an increase in the number of animals infected (infectious plus carriers).

The validity of the model was assessed by comparing the predicted results from the model with observed (epidemiological) results in the population of interest. The Portuguese results from the EU Baseline Survey on Salmonella Prevalence in Slaughter Pigs [36] and the EU Baseline Survey on the Prevalence of *Salmonella* in Breeding Pigs [37] were used as observed epidemiological results. The comparison was done by relating the magnitude of the predicted and observed value, and qualitatively assessing the degree of agreement/disagreement.

Our model predicted that in the sow-compartment the prevalence results for infectious sows at the end of farrowing stage would be 56.9% [16.7% - 100%]) whereas the infectious pig's prevalence at the end of fattening would be 30.9% [0.0% - 80%], predicting a reduction of prevalence among those two compartments. The same decreasing in prevalence was observed in the Baseline Studies where the prevalence of *Salmonella* Typhimurium was higher for breeding animals of positive holdings (13.4% [8.8% – 20.3%])[37] than for pigs for positive herds at slaughter (8.4% [6.1% - 11.5%])[36]. Even so the proportion of infectious sows at maternity can be considered relatively high while the proportion of carrier sows was considered low when compared to other simulation studies [140]. This lead us to suggest some assumptions about the transmission parameters, which where estimated using data from growing pigs, were

not suitable to be used in sows. Therefore care should be taken in the interpretation of the proportion of the infectious states at the sow compartment.
The infection state of sows at maternity room was identified as a critical step because it plays a major role in transmitting the infection to the offspring. It was influenced by several parameters; Manuscript 5 describes them in detail. Since only the infectious sows can infect the piglets it is desirable to reduce the proportion of infectious sows at farrowing. The results show that this can be done by promoting the increase of the transition rate from infectious to carrier. Although the majority of the control measures described in the literature are for pigs not for sows, it can be argued that they will also be effective if applied to sows. Therefore, increasing the transition from infectious to carriers can be achieved by promoting the use of organic acids in feed/water or fermented by products [171], which decreases the shedding duration. The super-shedders and long-shedders should also be reduced as they reduce the transition rate from infectious to carriers. To increase the farm immune status to other diseases, known to interfere with immunity (e.g. PRRS, parasitosis, etc.) [63], will help decreasing the number of super-shedders or long-time shedders. The increase of susceptible sows at maternity can be achieved by the reduction of the transition rate from susceptible to infectious. As suggested by other authors the use of "*Salmonella*-free" replacement stock [171], effective cleaning and disinfection procedures between batches [171], change in feed strategy from pelleted feed to non-pelleted feed, fermented wet feed or partially non-heat-treated feed [171] could help lower the exposure to *Salmonella* and increase the resistance to infection The reduction of the number of animals per pen, can also reduce the transmission of the infection [172]. In the model, the infection state of replacement gilts did not show a major influence in the infection state of sows at farrowing. Even so, recent reports [105] highlight the contribution of breeding animals to this infection in some countries such as Portugal.
The parameters which most influence the infection state of pigs at fattening were: the transition rates from susceptible to infectious (β) and from infectious to carriers (α), when changes applied to the pig-compartment; the transition rate from carriers to susceptible (θ) when changes applied to the sow-compartment, and the piglets' protective factor (pf). At the end of fattening the pigs are sent to the abattoir for slaughter. The transport to the abattoir and the lairage waiting time, are stressful events which potentially increase the transition rate from susceptible to infectious and from carrier to infectious (δ) in infected pigs. Therefore the aim should be to reduce the proportion of infectious and carrier animals. This can be achieved by reducing in the pig-compartment the transition rate β, reducing the piglets' protective factor value and the transition rate θ in the sow-compartment. To reduce the transition rate β, several

measures can be put in place such as adding organic acids to the feed or water [171], change the type of feed to a wet feed or non-pelleted feed [68, 173], cleaning frequently the pen floor [63], minimizing the mixture of litters at post-weaning and fattening [65, 93], and reducing stock density per pen [172]. The reduction of the pf value (which means increasing the piglets' protective factor) can be achieved by allowing the correct consumption of colostrum by the piglets [69], and by the reduction of all the stressful events [93] or concomitant diseases [84], which decrease the piglets' immunity.

The sensitivity analysis also showed that the parameters which depended on expert opinion have not caused a major change in the results of the simulation model. The most influential parameters were the transition rates and the piglets' protective factor. Further research on the transition rate parameters for sows and pigs (especially sows as described above), and the piglets' protective factor would be needed to improve the simulation model accuracy.

In Portugal, 25.6% of the production holdings with breeding animals (the majority farrow-to-finish farms) were considered to be positive to *S.* Typhimurium and strains similar to *S.* Typhimurium [172]. The infected holdings in Portugal have a higher number of animals per farm than the negative ones [172] and it can be assumed that they will contribute with a higher number of fattening pigs to slaughter and therefore with pork for human consumption. The simulation model can, then, be used to test control measures in terms of their cost-benefit, and the reduction of the prevalence in these farms will have an impact on human burden.

Conclusions

The work done in the PhD is valuable and can be applied to the Portuguese reality and other similar to this one. The risk factor analyses identified several risk factors some of which were never identified before within the relevant literature. The results of categorical (two groups of serotypes) risk factor analysis could be useful in future control programmes to adapt them to the country and farm status for *Salmonella* spp. The simulation model potentially allows estimation of cost-benefit control measures if linked to an economic model. The model is flexible enough to accommodate changes in the type of farm, parameters, and infra-structure, if future research and legislation so require. Some key parameters (transition rate from susceptible to infectious and the piglets' protective factor) have been identified by the model to influence, to a great extent, the infectious finishers, which are the target of the majority of the control programmes in place in other countries. This simulation model also explores the

dynamics of the infection in breeding animals and can be used to test control measures directed to this population.

REFERENCES

[1] EFSA, ECDC, (2012), The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010. EFSA Journal, 10:2597.

[2] Pires S.M., Vigre H., Makela P., Hald T., (2010), Using Outbreak Data for Source Attribution of Human Salmonellosis and Campylobacteriosis in Europe. Foodborne pathogens and disease, 7:1351-1361.

[3] Bahnson P.B., Fedorka-Cray P.J., Ladely S.R., Mateus-Pinilla N.E., (2006), Herd-level risk factors for Salmonella enterica subsp. enterica in U.S. market pigs. Prev Vet Med, 76:249-262.

[4] F L.P., Wierup M., (2006), Salmonella contamination: a significant challenge to the global marketing of animal food products. Revue scientifique et technique (International Office of Epizootics), 25:541-554.

[5] Hurd H.S., Enoe C., Sorensen L., Wachman H., Corns S.M., Bryden K.M., Grenier M., (2008), Risk-based analysis of the Danish pork Salmonella program: past and future. Risk Anal, 28:341-351.

[6] Consortium F., 2010, Analysis of the costs and benefits of setting a target for the reduction of *Salmonella* in slaughter pigs for European Commision Health and Consumers Directorate-General SANCO/2008/E2/036 Final Report, pp. 1-198.

[7] Hald T., 2001, *Salmonella* in pork. Epidemiology, control and the public health impact, Copenhagen.

[8] D'Aoust J.-Y., 2000, *Salmonella.* Chapter 45, in: Lund B.M., Baird-Parker T.C., Gould G.W. (Eds.), The Microbiological Safety and Quality of Food, An Aspen Publication, Gaithersburg, Maryland, pp. 1233-1279.

[9] Sousa J.C.F., 2000, Enterobacteriaceae, in: Ferreira W.F.C., Sousa J.C.F.d. (Eds.), Microbiologia, Lidel, Edições Técnicas, Lisboa, pp. 99-109.

[10] Grimont P.A.D., Weill F.X., 2007, Antigenic formulae of the *Salmonella* serovars, Report of the WHO Collaborating Centre for Reference and Research on *Salmonella*, Institute Pasteur, Paris.

[11] Grassl G.A., Finlay B.B., (2008), Pathogenesis of enteric Salmonella infections. Current opinion in gastroenterology, 24:22-26.

[12] Tietjen M., Fung D.Y., (1995), Salmonellae and food safety. Crit Rev Microbiol, 21:53-83.

[13] Fosse J., Seegers H., Magras C., (2008), Foodborne zoonoses due to meat: a quantitative approach for a comparative risk assessment applied to pig slaughtering in Europe. Veterinary research, 39:1.

[14] Hald T., Vose D., Wegener H.C., Koupeev T., (2004), A Bayesian approach to quantify the contribution of animal-food sources to human salmonellosis. Risk Anal, 24:255-269.

[15] Valkenburgh S., van Oosterom R., Stenvers O., Aalten M., Braks M., Schimmer B., van de Giessen A., van Pelt W., Langelaar M., 2007, Zoonoses and Zoonotic Agents in Humans, Food, Animals and Feed in the Netherlands 2003-2006, RIVM.

[16] Angulo F.J., Nargund V.N., Chiller T.C., (2004), Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. Journal of veterinary medicine, 51:374-379.

[17] Angulo F.J., Nunnery J.A., Bair H.D., (2004), Antimicrobial resistance in zoonotic enteric pathogens. Revue scientifique et technique (International Office of Epizootics), 23:485-496.

[18] Michael G.B., Butaye P., Cloeckaert A., Schwarz S., (2006), Genes and mutations conferring antimicrobial resistance in Salmonella: an update. Microbes and Infection, 8:1898-1914.

[19] Meakins S., Fisher I.S.T., Berghold C., Gerner-Smidt P., Tschape H., Cormican M., Luzzi I., Schneider F., Wannett W., Coia J., Echeita A., Threlfall E.J., (2008),

Antimicrobial Drug Resistance in Human Nontyphoidal Salmonella Isolates in Europe 2000-2004: A Report from the Enter-net International Surveillance Network. Microbial Drug Resistance, 14:31-35.

[20] Douris A., Fedorka-Cray P.J., Jackson C.R., (2008), Characterization of Salmonella enterica Serovar Agona Slaughter Isolates from the Animal Arm of the National Antimicrobial Resistance Monitoring System—Enteric Bacteria (NARMS): 1997 through 2003. Microbial Drug Resistance, 14:55-63.

[21] Khemtong S., Chuanchuen R., (2008), Class 1 Integrons and Salmonella Genomic Island 1 Among Salmonella enterica Isolated from Poultry and Swine. Microbial Drug Resistance, 14:65-70.

[22] Molla B., Berhanu A., Muckle A., Cole L., Wilkie E., Kleer J., Hildebrandt G., (2006), Multidrug resistance and distribution of Salmonella serovars in slaughtered pigs. Journal of veterinary medicine, 53:28-33.

[23] Schroeter A., Hoog B., Helmuth R., (2004), Resistance of Salmonella isolates in Germany. Journal of veterinary medicine, 51:389-392.

[24] García-Feliz C., Collazos J.A., Carvajal A., Herrera S., Echeita M.A., Rubio P., (2008), Antimicrobial Resistance of *Salmonella enterica* Isolates from Apparently Healthy and Clinically Ill Finishing Pigs in Spain. Zoonoses and public health, 55:195-205.

[25] Gebreyes W.A., Altier C., Thakur S., (2006), Molecular epidemiology and diversity of Salmonella serovar Typhimurium in pigs using phenotypic and genotypic approaches. Epidemiology and infection, 134:187-198.

[26] Mathew A.G., Beckmann M.A., Saxton A.M., (2001), A comparison of antibiotic resistance in bacteria isolated from swine herds in which antibiotics were used or excluded. Journal of Swine Health and Production, 9:125-129.

[27] Boyen F., Haesebrouck F., Maes D., Van Immerseel F., Ducatelle R., Pasmans F., (2008), Non-typhoidal Salmonella infections in pigs: a closer look at epidemiology, pathogenesis and control. Veterinary microbiology, 130:1-19.

[28] Oliveira C.J., Carvalho L.F., Garcia T.B., (2006), Experimental airborne transmission of Salmonella Agona and Salmonella Typhimurium in weaned pigs. Epidemiology and infection, 134:199-209.

[29] Oliveira C.J., Garcia T.B., Carvalho L.F., Givisiez P.E., (2007), Nose-to-nose transmission of Salmonella Typhimurium between weaned pigs. Veterinary microbiology, 125:355-361.

[30] Mikkelsen L.L., Naughton P.J., Hedemann M.S., Jensen B.B., (2004), Effects of physical properties of feed on microbial ecology and survival of Salmonella enterica serovar Typhimurium in the pig gastrointestinal tract. Applied and environmental microbiology, 70:3485-3492.

[31] Prouty A.M., Gunn J.S., (2000), *Salmonella enterica* serovar Typhimurium invasion is repressed in the presence of bile. Infect. Immun., 68:6763–6769.

[32] Nollet N., Maes D., Duchateau L., Hautekiet V., Houf K., Van Hoof J., De Zuttera L., De Kruif A., Geers R., (2005), Discrepancies between the isolation of Salmonella from mesenteric lymph nodes and the results of serological screening in slaughter pigs. Veterinary research, 36:545-555.

[33] Larsen S.T., McKean J.D., Hurd H.S., Rostagno M.H., Griffith R.W., Wesley I.V., (2003), Impact of commercial preharvest transportation and holding on the prevalence of Salmonella enterica in cull sows. Journal of food protection, 66:1134-1138.

[34] Boughton C., Egan J., Kelly G., Markey B., Leonard N., (2007), Quantitative examination of Salmonella spp. in the lairage environment of a pig abattoir. Foodborne pathogens and disease, 4:26-32.

[35] Beloeil P.A., Chauvin C., Proux K., Fablet C., Madec F., Alioum A., (2007), Risk factors for Salmonella seroconversion of fattening pigs in farrow-to-finish herds. Veterinary research, 38:835-848.

[36] EFSA, (2008), Report of the Task Force on Zoonoses Data Collection on the analysis of the baseline survey on the prevalence of *Salmonella* in slaughter pigs, PartA. The EFSA Journal, 135:111.
[37] EFSA, (2009), Analysis of the baseline survey on the prevalence of Salmonella in holdings with breeding pigs, in the EU, 2008, Part A: Salmonella prevalence estimates. EFSA Journal, 7:1377.
[38] Farzan A., Friendship R.M., Dewey C.E., (2007), Evaluation of enzyme-linked immunosorbent assay (ELISA) tests and culture for determining Salmonella status of a pig herd. Epidemiology and infection, 135:238-244.
[39] EFSA, (2006), Opinion of the Scientific Panel on Biological Hazards on "Risk assessment and mitigation options of *Salmonella* in pig production". *The EFSA Journal*, 341:1-131.
[40] Nielsen B., Baggesen D., Bager F., Haugegaard J., Lind P., (1995), The serological response to Salmonella serovars typhimurium and infantis in experimentally infected pigs. The time course followed with an indirect anti-LPS ELISA and bacteriological examinations. Veterinary microbiology, 47:205-218.
[41] Sorensen L.L., Alban L., Nielsen B., Dahl J., (2004), The correlation between Salmonella serology and isolation of Salmonella in Danish pigs at slaughter. Veterinary microbiology, 101:131-141.
[42] van Diemen P.M., Kreukniet M.B., Galina L., Bumstead N., Wallis T.S., (2002), Characterisation of a resource population of pigs screened for resistance to salmonellosis. Veterinary Immunology and Immunopathology, 88:183-196.
[43] Wigley P., (2004), Genetic resistance to Salmonella infection in domestic animals. Research in veterinary science, 76:165-169.
[44] Harris I.T., (2003), Serologic basis for assessment of subdinical Salmonella infection in swine: Part 1. Journal of Swine Health and Production, 11:247-251.
[45] Kich J.D., Schwarz P., Silva L.E., Coldebella A., Piffer I.A., Vizzoto R., Cardoso M., (2007), Development and application of an enzyme-linked immunosorbent assay to detect antibodies against prevalent Salmonella serovars in swine in southern Brazil. Journal of Veterinary Diagnostic Investigation, 19:510-517.
[46] Nowak B., von Muffling T., Chaunchom S., Hartung J., (2007), Salmonella contamination in pigs at slaughter and on the farm: a field study using an antibody ELISA test and a PCR technique. International journal of food microbiology, 115:259-267.
[47] Harris I.T., (2003), Serologic basis for assessment of subclinical Salmonella infection in swine: Part 2. Journal of Swine Health and Production, 11:300-303.
[48] Funk J., (2003), Pre-harvest food safety diagnostics for Salmonella serovars. Part 1: Microbiological culture. Journal of Swine Health and Production, 11:87-90.
[49] Sorensen L.L., Wachmann H., Alban L., (2007), Estimation of Salmonella prevalence on individual-level based upon pooled swab samples from swine carcasses. Veterinary microbiology, 119:213-220.
[50] Maciorowski K., Herrera P., Jones F., Pillai S., Ricke S., (2006), Cultural and Immunological Detection Methods for Salmonella spp. in Animal Feeds – A Review. Veterinary Research Communications, 30:127-137.
[51] Wu C.-C., Liu C.-I., Tsai C.-E., Yeh K.-S., (2003), Evaluation of a Polymerase Chain Reaction Based System for Detecting Salmonella Species from Pork Carcass Sponge Samples. Journal of food science, 68:992-995.
[52] Gebreyes W.A., (2003), Pre-harvest food safety diagnostics for Salmonella serovars. Part 2: Molecular diagnostics. Journal of Swine Health and Production, 11:141-145.
[53] Swanenburg M., Urlings H.A.P., Keuzenkamp D.A., Snijders J.M.A., (1998), Validation of ERIC PCR as a tool in epidemiologic research of Salmonella in slaughter pigs. Journal of Industrial Microbiology and Biotechnology, 21:141-144.
[54] Arnold T., Scholz H.C., Marg H., Rosler U., Hensel A., (2004), Impact of invA-PCR and culture detection methods on occurrence and survival of salmonella in the

flesh, internal organs and lymphoid tissues of experimentally infected pigs. Journal of veterinary medicine, 51:459-463.
[55] Moro C.V., Desloire S., Chauve C., Zenner L., (2007), Detection of Salmonella sp. in Dermanyssus gallinae using an FTA filter-based polymerase chain reaction. Med Vet Entomol, 21:148-152.
[56] Foley S.L., White D.G., McDermott P.F., Walker R.D., Rhodes B., Fedorka-Cray P.J., Simjee S., Zhao S.H., (2006), Comparison of subtyping methods for differentiating Salmonella enterica serovar Typhimurium isolates obtained from food animal sources. Journal of Clinical Microbiology, 44:3569-3577.
[57] Bessa M.C., Michael G.B., Canu N., Canal C.W., Cardoso M., Rabsch W., Rubino S., (2007), Phenotypic and genetic characterization of Salmonella enterica subsp enterica serovar Typhimurium isolated from pigs in Rio Grande do Sul, Brazil. Research in veterinary science, 83:302-310.
[58] Best E.L., Lindstedt B.-A., Cook A., Hadley F.A.C., Threlfall E.J., Liebana E., (2007), Multiple-locus variable-number tandem repeat analysis of Salmonella enterica subsp. enterica serovar Typhimurium: comparison of isolates from pigs, poultry and cases of human gastroenteritis. Journal of Applied Microbiology, 103:565-572.
[59] Al-Adhami B.H., Huby-Chilton F., Blais B.W., Martinez-Perez A., Chilton N.B., Gajadhar A.A., (2008), Rapid discrimination of Salmonella isolates by single-strand conformation polymorphism analysis. Journal of food protection, 71:1960-1966.
[60] Langvad B., Skov M.N., Rattenborg E., Olsen J.E., Baggesen D.L., (2006), Transmission routes of Salmonella Typhimurium DT 104 between 14 cattle and pig herds in Denmark demonstrated by molecular fingerprinting. J Appl Microbiol, 101:883-890.
[61] Funk J.A., Davies P.R., Gebreyes W., (2001), Risk factors associated with Salmonella enterica prevalence in three-site swine production systems in North Carolina, USA. Berliner und Munchener tierarztliche Wochenschrift, 114:335-338.
[62] Rajic A., Keenliside J., McFall M.E., Deckert A.E., Muckle A.C., O'Connor B.P., Manninen K., Dewey C.E., McEwen S.A., (2005), Longitudinal study of Salmonella species in 90 Alberta swine finishing farms. Veterinary microbiology, 105:47-56.
[63] Beloeil P.A., Fravalo P., Fablet C., Jolly J.P., Eveno E., Hascoet Y., Chauvin C., Salvat G., Madec F., (2004), Risk factors for Salmonella enterica subsp. enterica shedding by market-age pigs in French farrow-to-finish herds. Prev Vet Med, 63:103-120.
[64] Nollet N., Maes D., De Zutter L., Duchateau L., Houf K., Huysmans K., Imberechts H., Geers R., de Kruif A., Van Hoof J., (2004), Risk factors for the herd-level bacteriologic prevalence of Salmonella in Belgian slaughter pigs. Prev Vet Med, 65:63-75.
[65] Rossel R., Rouillier J., Beloeil P.A., Chauvin C., Basta F., Crabos J.P., Theau-Audin S., (2006), Salmonella en élevage de porcs du Sud-Ouest de la France: séroprévalence en fin d'engraissement et facteurs de risque associés. Journées de la Recherche Porcine, 38:371-378.
[66] Hautekiet V., Geert V., Marc V., Rony G., (2008), Development of a sanitary risk index for Salmonella seroprevalence in Belgian pig farms. Prev Vet Med, 86:75-92.
[67] van der Wolf P.J., Wolbers W.B., Elbers A.R.W., van der Heijden H.M.J.F., Koppen J.M.C.C., Hunneman W.A., van Schie F.W., Tielen M.J.M., (2001), Herd level husbandry factors associated with the serological Salmonella prevalence in finishing pig herds in The Netherlands. Veterinary microbiology, 78:205-219.
[68] Farzan A., Friendship R.M., Dewey C.E., Warriner K., Poppe C., Klotins K., (2006), Prevalence of Salmonella spp. on Canadian pig farms using liquid or dry-feeding. Preventive Veterinary Medicine, 73:241-254.
[69] Lo Fo Wong D.M., Dahl J., Stege H., van der Wolf P.J., Leontides L., von Altrock A., Thorberg B.M., (2004), Herd-level risk factors for subclinical Salmonella infection in European finishing-pig herds. Prev Vet Med, 62:253-266.

[70] Funk J.A., Davies P.R., Nichols M.A., (2001), Longitudinal study of Salmonella enterica in growing pigs reared in multiple-site swine production systems. Veterinary microbiology, 83:45-60.
[71] Meerburg B.G., Kijlstra A., (2007), Role of rodents in transmission of Salmonella and Campylobacter. Journal of the Science of Food and Agriculture, 87:2774-2781.
[72] Skov M.N., Madsen J.J., Rahbek C., Lodal J., Jespersen J.B., Jørgensen J.C., Dietz H.H., Chriél M., Baggesen D.L., (2008), Transmission of Salmonella between wildlife and meat-production animals in Denmark. Journal of Applied Microbiology, 105:1558-1568.
[73] Berends B.R., Urlings H.A., Snijders J.M., Van Knapen F., (1996), Identification and quantification of risk factors in animal management and transport regarding Salmonella spp. in pigs. International journal of food microbiology, 30:37-53.
[74] Leontides L.S., Grafanakis E., Genigeorgis C., (2003), Factors associated with the serological prevalence of Salmonella enterica in Greek finishing swine herds. Epidemiology and infection, 131:599-606.
[75] Poljak Z., Dewey C.E., Friendship R.M., Martin S.W., Christensen J., (2008), Multilevel analysis of risk factors for Salmonella shedding in Ontario finishing pigs. Epidemiology and infection, 136:1388-1400.
[76] Funk J., Gebreyes W.A., (2004), Risk factors associated with Salmonella prevalence on swine farms. Journal of Swine Health and Production, 12:246-251.
[77] Fosse J., Seegers H., Magras C., (2009), Prevalence and Risk Factors for Bacterial Food-Borne Zoonotic Hazards in Slaughter Pigs: A Review. Zoonoses and public health.
[78] Fablet C., Beloeil P.A., Fravalo P., Jolly J.P., Eveno E., Hascoet Y., Salvat G., Madec F., (2003), Etude des circonstances associées à l'excrétion de *Salmonella enterica* par les porcs en croissance. Journées de la Recherche Porcine, 35:401-408.
[79] Letellier A., Messier S., Paré J., Ménard J., Quessy S., (1999), Distribution of Salmonella in swine herds in Québec. Veterinary microbiology, 67:299-306.
[80] Amaechi N., Ezeronye O.U., (2006), Piggery Environment as a Source of *Salmonella* Contamination for Swine. Journal of Animal and Veterinary Advances, 5:102-107.
[81] Lo Fo Wong D.M., Dahl J., Wingstrand A., van der Wolf P.J., von Altrock A., Thorberg B.M., (2004), A European longitudinal study in Salmonella seronegative- and seropositive-classified finishing pig herds. Epidemiology and infection, 132:903-914.
[82] Bollaerts K., Aerts M., Ribbens S., Stede Y.V.d., Boone I., Mintiens K., (2008), Identification of Salmonella high risk pig-herds in Belgium by using semiparametric quantile regression. Journal of the Royal Statistical Society: Series A (Statistics in Society), 171:449-464.
[83] Benschop J., Stevenson M.A., Dahl J., French N.P., (2008), Towards incorporating spatial risk analysis for Salmonella sero-positivity into the Danish swine surveillance programme. Prev Vet Med, 83:347-359.
[84] Møller K., Jensen T.K., E. Jorsal S., Leser T.D., Carstensen B., (1998), Detection of Lawsonia intracellularis, Serpulina hyodysenteriae, weakly beta-haemolytic intestinal spirochaetes, Salmonella enterica, and haemolytic Escherichia coli from swine herds with and without diarrhoea among growing pigs. Veterinary microbiology, 62:59-72.
[85] Fablet C., Fravalo P., Jolly J.P., Eveno E., Madec F., Beloeil P.A., (2003), Recherche des facteurs de risque de l'excrétion de *Salmonella enterica* par les porcs en croissance. Enquête épidémiologique analytique en élevage naisseur-engraisseur. Epidémiol. Santé Animale, 43:61-73.
[86] Anderson R.C., Hume M.E., Genovese K.J., Callaway T.R., Jung Y.S., Edrington T.S., Poole T.L., Harvey R.B., Bischoff K.M., Nisbet D.J., (2004), Effect of Drinking-Water Administration of Experimental Chlorate Ion Preparations on Salmonella enterica serovar Typhimurium Colonization in Weaned and Finished Pigs. Veterinary Research Communications, 28:179-189.

[87] Taube V.A., Neu M.E., Hassan Y., Verspohl J., Beyerbach M., Kamphues J., (2009), Effects of dietary additives (potassium diformate/organic acids) as well as influences of grinding intensity (coarse/fine) of diets for weaned piglets experimentally infected with Salmonella Derby or Escherichia coli. J Anim Physiol Anim Nutr (Berl).
[88] Collado M., Grześkowiak Ł., Salminen S., (2007), Probiotic Strains and Their Combination Inhibit In Vitro Adhesion of Pathogens to Pig Intestinal Mucosa. Current Microbiology, 55:260-265.
[89] van der Wolf P.J., Bongers J.H., Elbers A.R., Franssen F.M., Hunneman W.A., van Exsel A.C., Tielen M.J., (1999), Salmonella infections in finishing pigs in The Netherlands: bacteriological herd prevalence, serogroup and antibiotic resistance of isolates and risk factors for infection. Veterinary microbiology, 67:263-275.
[90] Davies P.R., Funk J.A., Morrow W.E.M., (2000), Fecal shedding of *Salmonella* by gilts before and after introduction to a swine breeding farm. Swine Health Prod., 8:25-29.
[91] Zheng D.M., Bonde M., Sorensen J.T., (2007), Associations between the proportion of Salmonella seropositive slaughter pigs and the presence of herd level risk factors for introduction and transmission of Salmonella in 34 Danish organic, outdoor (non-organic) and indoor finishing-pig farms. Livestock Science, 106:189-199.
[92] Osterberg J., Vagsholm I., Boqvist S., Lewerin S.S., (2006), Feed-borne outbreak of Salmonella cubana in Swedish pig farms: risk factors and factors affecting the restriction period in infected farms. Acta veterinaria Scandinavica, 47:13-21.
[93] Callaway T.R., Morrow J.L., Edrington T.S., Genovese K.J., Dowd S., Carroll J., Dailey J.W., Harvey R.B., Poole T.L., Anderson R.C., Nisbet D.J., (2006), Social stress increases fecal shedding of Salmonella Typhimurium by early weaned piglets. Current Issues in Intestinal Microbiology, 7:65-71.
[94] Funk J., Wittum T.E., LeJeune J.T., Rajala-Schultz P.J., Bowman A., Mack A., (2007), Evaluation of stocking density and subtherapeutic chlortetracycline on Salmonella enterica subsp enterica shedding in growing swine. Veterinary microbiology, 124:202-208.
[95] Beloeil P.A., Chauvin C., Proux K., Madec F., Fravalo P., Alioum A., (2004), Impact of the Salmonella status of market-age pigs and the pre-slaughter process on Salmonella caecal contamination at slaughter. Veterinary research, 35:513-530.
[96] Rostagno M.H., Hurd H.S., McKean J.D., Ziemer C.J., Gailey J.K., Leite R.C., (2003), Preslaughter holding environment in pork plants is highly contaminated with Salmonella enterica. Applied and environmental microbiology, 69:4489-4494.
[97] Hald T., Wingstrand A., Swanenburg M., von Altrock A., Thorberg B.M., (2003), The occurrence and epidemiology of Salmonella in European pig slaughterhouses. Epidemiology and infection, 131:1187-1203.
[98] Delhalle L., De Sadeleer L., Bollaerts K., Farnir F., Saegerman C., Korsak N., Dewulf J., De Zutter L., Daube G., (2008), Risk factors for Salmonella and hygiene indicators in the 10 largest Belgian pig slaughterhouses. Journal of food protection, 71:1320-1329.
[99] Bolton D.J., Pearce R.A., Sheridan J.J., Blair I.S., McDowell D.A., Harrington D., (2002), Washing and chilling as critical control points in pork slaughter hazard analysis and critical control point (HACCP) systems. Journal of Applied Microbiology, 92:893-902.
[100] Pearce R.A., Bolton D.J., Sheridan J.J., McDowell D.A., Blair I.S., Harrington D., (2004), Studies to determine the critical control points in pork slaughter hazard analysis and critical control point systems. International journal of food microbiology, 90:331-339.
[101] Milnes A.S., Sayers A.R., Stewart I., Clifton-Hadley F.A., Davies R.H., Newell D.G., Cook A.J.C., Evans S.J., Smith R.P., Paiba G.A., (2006), Factors related to the carriage of Verocytotoxigenic *E. coli*, *Salmonella*, thermophilic *Campylobacter* and *Yersinia enterocolitica* in cattle, sheep and pigs at slaughter. Epidemiology and infection, Forthcoming:1-14.

[102] Berends B.R., van Knapen F., Snijders J.M., (1996), Suggestions for the construction, analysis and use of descriptive epidemiological models for the modernization of meat inspection. International journal of food microbiology, 30:27-36.
[103] Algino R.J., Badtram G.A., Ingham B.H., Ingham S.C., (2009), Factors associated with Salmonella prevalence on pork carcasses in very small abattoirs in Wisconsin. Journal of food protection, 72:714-721.
[104] Fablet C., Robinault C., Jolly J.P., Collet M., Chemaly M., Labbe A., Madec F., Fravalo P., (2006), Salmonella enterica level in French pig farms effluents: Experimental and field data. Livestock Science, 102:216-225.
[105] VLA-DTU-RIVM, 2010, EFSA Quantitative Microbiological Risk Assessment on Salmonella in Slaughter and Breeder Pigs: Final Report. Question No EFSA-Q-2007-00245. Grant number CFP/EFSA/BIOHAZ/2007/01, Veterinary Laboratories Agency., pp. 437.
[106] Sauli I., Danuser J., Geeraerd A.H., Van Impe J.F., Rufenacht J., Bissig-Choisat B., Wenk C., Stark K.D., (2005), Estimating the probability and level of contamination with Salmonella of feed for finishing pigs produced in Switzerland--the impact of the production pathway. International journal of food microbiology, 100:289-310.
[107] Beal J.D., Niven S.J., Campbell A., Brooks P.H., (2002), The effect of temperature on the growth and persistence of Salmonella in fermented liquid pig feed. International journal of food microbiology, 79:99-104.
[108] Creus E., Pérez J.F., Peralta B., Baucells F., Mateu E., (2007), Effect of Acidified Feed on the Prevalence of Salmonella in Market-age Pigs. Zoonoses and public health, 54:314-319.
[109] Busser E.V.D., Dewulf J., Nollet N., Houf K., Schwarzer K., Sadeleer L.D., Zutter L.D., Maes D., (2008), Effect of Organic Acids in Drinking Water During the Last 2 weeks Prior to Slaughter on Salmonella Shedding by Slaughter Pigs and Contamination of Carcasses. Zoonoses and public health, 9999.
[110] Patchanee P., Crenshaw T.D., Bahnson P.B., (2007), Oral sodium chlorate, topical disinfection, and younger weaning age reduce Salmonella enterica shedding in pigs. Journal of food protection, 70:1798-1803.
[111] Funk J.A., Lejeune J.T., Wittum T.E., Rajala-Schultz P.J., (2006), The Effect of Subtherapeutic Chlortetracycline on Antimicrobial Resistance in the Fecal Flora of Swine. Microbial Drug Resistance, 12:210-218.
[112] Denagamage T.N., O'Connor A.M., Sargeant J.M., Rajic A., McKean J.D., (2007), Efficacy of vaccination to reduce Salmonella prevalence in live and slaughtered swine: a systematic review of literature from 1979 to 2007. Foodborne pathogens and disease, 4:539-549.
[113] Okamura M., Tachizaki H., Kubo T., Kikuchi S., Suzuki A., Takehara K., Nakamura M., (2007), Comparative evaluation of a bivalent killed Salmonella vaccine to prevent egg contamination with Salmonella enterica serovars Enteritidis, Typhimurium, and Gallinarum biovar Pullorum, using 4 different challenge models. Vaccine, 25:4837-4844.
[114] Charles S.D., Abraham A.S., Trigo E.T., Jons G.F., Settje T.L., (2000), Reduced shedding and clinical signs of *Salmonella* Typhimurium in nursery pigs vaccinated with a *Salmonella* Choleraesuis vaccine. Swine Health and Production, 8:107-112.
[115] Roesler U., Heller P., Waldmann K.H., Truyen U., Hensel A., (2006), Immunization of sows in an integrated pig-breeding herd using a homologous inactivated Salmonella vaccine decreases the prevalence of Salmonella typhimurium infection in the offspring. Journal of veterinary medicine, 53:224-228.
[116] Selke M., Meens J., Springer S., Frank R., Gerlach G.F., (2007), Immunization of pigs to prevent disease in humans: Construction and protective efficacy of a Salmonella enterica serovar Typhimurium live negative-marker vaccine. Infection and Immunity, 75:2476-2483.
[117] Anderson R.C., Stanker L., Young C.R., Buckley S.A., Genovese K.J., Harvey R.B., DeLoach J.R., Keith N.K., Nisbet D.J., (1999), Effect of competitive exclusion

treatment on colonization of early-weaned pigs by *Salmonella* serovar Choleraesuis. Swine Health and Production, 7:155-160.
[118] Casey P.G., Gardiner G.E., Casey G., Bradshaw B., Lawlor P.G., Lynch P.B., Leonard F.C., Stanton C., Ross R.P., Fitzgerald G.F., Hill C., (2007), A five-strain probiotic combination reduces pathogen shedding and alleviates disease signs in pigs challenged with Salmonella enterica Serovar Typhimurium. Applied and environmental microbiology, 73:1858-1863.
[119] Vieira-Pinto M., Temudo P., Martins C., (2005), Occurrence of salmonella in the ileum, ileocolic lymph nodes, tonsils, mandibular lymph nodes and carcasses of pigs slaughtered for consumption. Journal of veterinary medicine, 52:476-481.
[120] Berends B.R., Van Knapen F., Snijders J.M.A., Mossel D.A.A., (1997), Identification and quantification of risk factors regarding Salmonella spp. on pork carcasses. International journal of food microbiology, 36:199-206.
[121] Botteldoorn N., Heyndrickx M., Rijpens N., Grijspeerdt K., Herman L., (2003), Salmonella on pig carcasses: positive pigs and cross contamination in the slaughterhouse. Journal of Applied Microbiology, 95:891-903.
[122] Giovannacci I., Queguiner S., Ragimbeau C., Salvat G., Vendeuvre J.L., Carlier V., Ermel G., (2000), Tracing of Salmonella spp. in two pork slaughter and cutting plants using serotyping and macrorestriction genotyping. Journal of Applied Microbiology, 90:131-147.
[123] Lo Fo Wong D.M.A., Hald T., 2000, *Salmonella* in Pork (SALINPORK): Pre-harvest and Harvest Control Options based on Epidemiologic, Diagnostic and Economic Research., Final Report to the Commission of the European Communities, Agriculture and Fisheries FAIR1 CT95-0400, Royal Veterinary and Agricultural University, Department of Animal Health and Animal Science and the Danish Veterinary Laboratory, Danish Zoonosis Centre, Copenhagen, pp. 132-155.
[124] Swanenburg M., van der Wolf P.J., Urlings H.A., Snijders J.M., van Knapen F., (2001), Salmonella in slaughter pigs: the effect of logistic slaughter procedures of pigs on the prevalence of Salmonella in pork. International journal of food microbiology, 70:231-242.
[125] Berends B.R., Van Knapen F., Mossel D.A., Burt S.A., Snijders J.M., (1998), Impact on human health of Salmonella spp. on pork in The Netherlands and the anticipated effects of some currently proposed control strategies. International journal of food microbiology, 44:219-229.
[126] Rajkowski K.T., Niebuhr S.E., Dickson J., (2006), Effect of gamma or beta radiation on Salmonella DT 104 in ground pork. Journal of food protection, 69:1430-1433.
[127] Morris C.A., Lucia L.M., Savell J.W., Acuff G.R., (1997), Trisodium Phosphate Treatment of Pork Carcasses. Journal of food science, 62:402-403.
[128] Sandberg M., Hopp P., Jarp J., Skjerve E., (2002), An evaluation of the Norwegian Salmonella surveillance and control program in live pig and pork. International journal of food microbiology, 72:1-11.
[129] Mousing J., Jensen P.T., Halgaard C., Bager F., Feld N., Nielsen B., Nielsen J.P., Bech-Nielsen S., (1997), Nation-wide Salmonella enterica surveillance and control in Danish slaughter swine herds. Prev Vet Med, 29:247-261.
[130] Alban L., Stege H., Dahl J., (2002), The new classification system for slaughter-pig herds in the Danish Salmonella surveillance-and-control program. Prev Vet Med, 53:133-146.
[131] Wegener H.C., Hald T., Lo Fo Wong D., Madsen M., Korsgaard H., Bager F., Gerner-Smidt P., Molbak K., (2003), Salmonella control programs in Denmark. Emerg Infect Dis, 9:774-780.
[132] Clough H.E., Fenton S.E., French N.P., Miller A.J., Cook A.J., (2009), Evidence from the UK Zoonoses Action Plan in favour of localised anomalies of Salmonella infection on United Kingdom pig farms. Prev Vet Med, 89:67-74.

[133] Frezen P.D., Riggs T.L., Buzby J.C., Breuer T., Roberts T., Voetsch D., Reddy S., (1999), *Salmonella* Cost estimate updated using FoodNet data. FoodReview, 22:10-15.
[134] Sockett P.N., (1991), The economic implications of human Salmonella infection. The Journal of applied bacteriology, 71:289-295.
[135] Roberts J.A., Cumberland P., Sockett P.N., Wheeler J., Rodrigues L.C., Sethi D., Roderick P.J., (2003), The study of infectious intestinal disease in England: socio-economic impact. Epidemiology and infection, 130:1-11.
[136] Kemmeren J.M., Mangen M.-J.J., van Duynhoven Y.T., Havelaar A.H., 2006, Priority setting of foodborne pathogens. Disease burden and costs of selected enteric pathogens., RIVM report 330080001/2006.
[137] van Lier E.A., Havelaar A.H., 2007, Disease burden of infectious diseases in Europe: a pilot study, RIVM report 215011001/2007.
[138] van der Gaag M.A., Saatkamp H.W., Backus G.B.C., van Beek P., Huirne R.B.M., (2004), Cost-effectiveness of controlling Salmonella in the pork chain. Food Control, 15:173-180.
[139] Goldbach S.G., Alban L., (2006), A cost-benefit analysis of Salmonella-control strategies in Danish pork production. Prev Vet Med, 77:1-14.
[140] Lurette A., Belloc C., Touzeau S., Hoch T., Ezanno P., Seegers H., Fourichon C., (2008), Modelling Salmonella spread within a farrow-to-finish pig herd. Veterinary research, 39:49.
[141] Krieter J., (2004), Evaluation of Salmonella surveillance in pigs using a stochastic simulation model. Arch. Tierz.-Arch. Anim. Breed., 47:337-349.
[142] van der Gaag M.A., Vos F., Saatkamp H.W., van Boven M., van Beek P., Huirne R.B.M., (2004), A state-transition simulation model for the spread of Salmonella in the pork supply chain. European Journal of Operational Research, 156:782-798.
[143] Hill A.A., Snary E.L., Arnold M.E., Alban L., Cook A.J., (2008), Dynamics of Salmonella transmission on a British pig grower-finisher farm: a stochastic model. Epidemiology and infection, 136:320-333.
[144] McNamara P.E., Miller G.Y., Liu X., Barber D.A., (2007), A farm-to-fork stochastic simulation model of pork-borne salmonellosis in humans: Lessons for risk ranking. Agribusiness, 23:157-172.
[145] Soumpasis I., Butler F., (2009), Development and Application of a Stochastic Epidemic Model for the Transmission of Salmonella Typhimurium at the Farm Level of the Pork Production Chain. Risk Anal.
[146] Kranker S., Alban L., Boes J., Dahl J., (2003), Longitudinal Study of Salmonella enterica Serotype Typhimurium Infection in Three Danish Farrow-to-Finish Swine Herds. J. Clin. Microbiol., 41:2282-2288.
[147] Nollet N., Houf K., Dewulf J., De Kruif A., De Zutter L., Maes D., (2005), Salmonella in sows: a longitudinal study in farrow-to-finish pig herds. Veterinary research, 36:645-656.
[148] Beloeil P.A., Chauvin C., Proux K., Rose N., Queguiner S., Eveno E., Houdayer C., Rose V., Fravalo P., Madec F., (2003), Longitudinal serological responses to Salmonella enterica of growing pigs in a subclinically infected herd. Prev Vet Med, 60:207-226.
[149] Pires AF, Funk JA, CA B., [on line] (2012), Longitudinal study of Salmonella shedding in naturally infected finishing pigs, Epidemiology and infection
[150] Sorensen J.T., Edwards S., Noordhuizen J., Gunnarsson S., (2006), Animal production systems in the industrialised world. Revue scientifique et technique (International Office of Epizootics), 25:493-503.
[151] FAS-USDA, 2011, Livestock and Poultry: World Markets and Trade, USDA.
[152] IACA, 2011, Anuário de 2011, Industriais da Alimentação para Consumo Animal.
[153] INE, 2012, Estatísticas Agricolas 2011, in: INE (Ed.), Estatísticas Oficiais, INE, Lisboa - Portugal, pp. 170.

[154] Steinfeld H., Wassenaar T., Jutzi S., (2006), Livestock production systems in developing countries: status, drivers, trends. Revue scientifique et technique (International Office of Epizootics), 25:505-516.
[155] DGV-SIPACE, 2012, SIPACE IS Ungulados – Análise exploratória de dados, Ministério da Agricultura, Mar, Ambiente e Ordenamento do Território, Lisboa-Portugal, pp. 28.
[156] Venables W.N., Ripley B.D., 2002, Modern Applied Statistics with S, Springer, New York.
[157] Eriksson E., Aspan A., (2007), Comparison of culture, ELISA and PCR techniques for salmonella detection in faecal samples for cattle, pig and poultry. BMC veterinary research, 3:21.
[158] Fravalo P., Cariolet R., Proux K., Salvat G., 2003, Le portage asymptomatique de *Salmonella enterica* par les porcs: résultats issues de la constituition d'un modèle en conditions expérimentales, 35èmes Journées de la Recherche Porcine, Paris, France.
[159] Gilks W.R., Richardson S., Spiegelhalter D.J., 1996, Markov chain Monte Carlo in practice, Chapman & Hall/CRC, Boca Raton, pp. 486.
[160] Lunn D.J., Thomas A., Best N., Spiegelhalter D., (2000), WinBUGS - a Bayesian modelling framework: concepts, structure, and extensibility. Statistics and Computing, 10:325-337.
[161] Spiegelhalter D., Thomas A., Best N., Lunn D., 2007, WinBUGS Version 1.4.3 User Manual., Medical Research Council Biostatistics Unit.
[162] Gelman A., Rubin D.B., (1992), Inference from iterative simulation using multiple sequences. Stat. Sci., 7:457–511.
[163] Kostoulas P., Leontides L., Enøe C., Billinis C., Florou M., Sofia M., (2006), Bayesian estimation of sensitivity and specificity of serum ELISA and faecal culture for diagnosis of paratuberculosis in Greek dairy sheep and goats. Preventive Veterinary Medicine, 76:56-73.
[164] Best N.G., Cowles M.K., Vines S.K., 1995, CODA Manual Version 0.30, MRC Biostatistics Unit, Cambridge, UK.
[165] Congdon P.D., 2010, Applied Bayesian Hierarchical Methods, Chapman and Hall/ CRC, pp. 604.
[166] Nielsen L.R., van den Borne B., van Schaik G., (2007), Salmonella Dublin infection in young dairy calves: transmission parameters estimated from field data and an SIR-model. Prev Vet Med, 79:46-58.
[167] Baptista F.M., Alban L., Nielsen L.R., Domingos I., Pomba C., Almeida V., (2010), Use of Herd Information for Predicting Salmonella Status in Pig Herds. Zoonoses and public health, 57:49-59.
[168] Benschop J., Hazelton M.L., Stevenson M.A., Dahl J., Morris R.S., French N.P., (2008), Descriptive spatial epidemiology of subclinical Salmonella infection in finisher pig herds: application of a novel method of spatially adaptive smoothing. Veterinary research, 39:2.
[169] Alban L., Olsen A.M., Nielsen B., Sorensen R., Jessen B., (2002), Qualitative and quantitative risk assessment for human salmonellosis due to multi-resistant Salmonella Typhimurium DT104 from consumption of Danish dry-cured pork sausages. Prev Vet Med, 52:251-265.
[170] (BIOHAZ) E.P.o.B.H., (2010), Scientific Opinion on monitoring and assessment of the public health risk of "Salmonella Typhimurium-like" strains. EFSA Journal, 8:48.
[171] Lo Fo Wong D.M.A., Hald T., van der Wolf P.J., Swanenburg M., (2002), Epidemiology and control measures for Salmonella in pigs and pork. Livestock Production Science, 76:215-222.
[172] Correia-Gomes C., Economou T., Mendonça D., Vieira-Pinto M., Niza-Ribeiro J., (2012), Assessing risk profiles for Salmonella serotypes in breeding pig operations in Portugal using a Bayesian hierarchical model. BMC veterinary research, 8:226.

[173] Bahnson P.B., Damman D.J., Isaacson R.E., Miller G.Y., Weigel R.M., Troutt F., (2006), Prevalence and serovars of Salmonella enterica isolated from ileocolic lymph nodes of market pigs reared in selected Midwest US swine herds. Journal of Swine Health and Production, 14:182-188.

U.PORTO

UNIVERSIDADE DO PORTO