

U. PORTO



INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR
UNIVERSIDADE DO PORTO

Relatório Final de Estágio
Mestrado Integrado em Medicina Veterinária

**EVALUATION OF RISK FACTORS FOR *SALMONELLA* INFECTION IN
DANISH PIG BREEDING HERDS**

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Foreword

This investigation study was carried as part of the four-month professional training period that ends the Integrated Veterinary Master of Instituto de Ciências Biomédicas Abel Salazar. The choice of this subject came from the disposition of being integrated in professional world and produce useful knowledge to scientific community at the same time.

Salmonella foodborne infections are of major concern and there is still much to do to prevent this problem. Working data from Denmark allowed me to contact with a new reality and expand my horizons. More than learning about *Salmonella* specific topic, I could exercise and learn more applying my statistical and epidemiological knowledge. Working with a diverse group of people, I experienced my weaknesses and had the opportunity to overcome them.

It is a fact that the final knowledge and wisdom achieved in this four months is not fully contained in this thesis, as its purpose is to approach the subject of risk factors for *Salmonella* infection in Danish breeding pigs. Concerning this, the following text is divided in to three major parts: an initial part with a brief revision of *Salmonella* impact in Public Health in Europe, Denmark and Portugal, specifically referring pigs as the source of the agent; a posterior part containing analyses targeting identification of risk factors in Denmark and analysis of possible comparisons with EU and Portugal; the last part, an Annex containing some information referred on the anterior section.

The more I work, the more I think I understand, the more I see how limited this text is. Still, it has the power to open windows for those who are disposed to try to see.

Wish you a good reading time!

Summary

Salmonella is one of the most important foodborne pathogens worldwide. It causes high incidence of disease in humans and it is an important source of costs for the Public Health Systems. Salmonella is ubiquitous, with more than 2500 serotypes, some host-adapted, and wide range host serotypes, able to infect all the farm animals. Control and eradication is complex and difficult as biology and epidemiology of Salmonella is not fully understood. Some countries have surveillance and control plans implemented and some are preparing their own. Pork and products thereof are known important sources of Salmonella to humans.

The main purpose of this study was to assess the risk profiles for Salmonella infection in Danish breeding pigs using data collected during EU baseline survey on prevalence of Salmonella in breeding pigs, 2008. Analyses performed targeted risk factors for Salmonella positivity and also serotype-dependent risk factors for Salmonella Typhimurium and Derby. The analyses were made using a similar methodology to the one used previously to analyze Portuguese data (risk factors for Salmonella positivity and serotype-dependent risk factors for Salmonella Typhimurium) and thus were possible of comparisons.

Significant risk factors for Salmonella positivity from this study are: total number of breeding pigs, boar replacement policy and type of feed; Typhimurium-dependent risk factors are boar replacement policy and type of feed; Derby-dependent risk factors are boar replacement policy, type of feed and source of feed. Comparison with risk factors from the EFSA and Portuguese study is made in the discussion.

Keywords: *Salmonella*, risk factor, breeding pig, serotype-specific

Summário

A *Salmonella* é um dos patogénios veiculados por alimentos mais importantes por todo o mundo. Causa elevada incidência de doença em humanos e é uma importante fonte de despesas para os Sistemas de Saúde Pública. A *Salmonella* é ubiqüitária, com mais de 2500 serotipos, alguns adaptados ao hospedeiro, outros com variado leque de hospedeiros, capazes de infetar todos os animais de pecuária. O controlo e erradicação são complexos e difíceis dada a biologia e epidemiologia da *Salmonella* não serem completamente compreendidos. Alguns países tem planos de controlo e vigilância implementados e alguns estão a preparar os seus. Carne de porco e produtos derivados são reconhecidas fontes importantes de *Salmonella* para os humanos.

O principal propósito deste estudo foi estimar os perfis de risco para infeção por *Salmonella* em suínos reprodutores dinamarqueses utilizando dados colhidos durante o estudo de base sobre a prevalência de *Salmonella* em porcos reprodutores, 2008. As análises efetuadas objetivavam fatores de risco para positividade a *Salmonella* e também fatores de risco serotipo dependentes para *Salmonella* Typhimurium e Derby. As análises foram efetuadas utilizando uma metodologia semelhante à utilizada anteriormente para analisar os dados portugueses (fatores de risco para positividade a *Salmonella* e fatores de risco serotipo dependentes para Typhimurium) possibilitando assim comparações.

Fatores de risco significativos para positividade a *Salmonella* deste estudo são: número total de porcos reprodutores, política de reposição de varrascos e tipo de alimento; fatores de risco Typhimurium-dependentes são política de reposição de varrascos e tipo de alimento; fatores de risco Derby-dependentes são política de reposição de varrascos, tipo de alimento e fonte de alimento. Comparação com fatores de risco do estudo da EFSA e Português são feitos na discussão.

Palavras chave: *Salmonella*, fator de risco, porco reprodutor, serotipo-específico

Acknowledgements

During one of my train trips between Mortágua and Porto, I met Mr. Dinis Resende. My travels usually are magnificent opportunities to meet randomly new people and until now, it's been very amusing to do. In that trip, Mr. Resende started telling me that I don't ever have the chance to be free. I am limited by everyone that I met until now, my family, friends, even those to whom I don't have any connection with, but made something that I noticed. Well, that was an argument that had an immediate impact on me: of course I am free, I can choose what I want to do, I have a built conscience behind but I can choose also to ignore or interpose it and bla, bla ,bla... The fact is that the argumentation went on inside myself after the end of that trip. Damn, I was wrong, he limited me! But wait, is that good or bad? What does that change? Well, I don't bother not to be free in the way Mr. Resende said: it attaches me to people, places, it gives me an identity and it gives me a road to make. And the truth is with that, I am pretty willing to be "limited": I have to be who I am, not more nor less than my capacities, but myself. That's my freedom!

This page is dedicated to everyone that ever crossed my days (or I crossed theirs). Some I know their names, some I don't. Other I didn't even realized they were there. To some I gave joy and hope, to others I caused suffering. I will never be an ideal "Clarisse" but I try every day: that's human perfection. I am very grateful for all the things that happened until this day. All that I could offer from myself, I received doubled and very often I feel I don't deserve it. All of you make me feel that deserve it or not, I have to make it! Each one of the names inscribed here mean that I am "limited" by you, in a good sense, of course, and I want to thank you! I want you to remember that because I think that the memories will be much more genuine than a few words that I could possible write here.

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To my village, my hometown.

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Train trip from Porto to Mortágua, 7th March, 2015

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EVALUATION OF RISK FACTORS FOR *SALMONELLA* IN DANISH PIG BREEDING HERDS

***A-Salmonella* infection in pigs and public health: brief review**

1. Public health and salmonellosis in pigs: dimensioning the concern

Worldwide, *Salmonella* has been a pathogen of major importance. The global number of non-typhoidal salmonellosis is estimated to be approximately 93.8 million cases (ranging from 61.8 to 131.6 million), of which an estimated 85.6% are foodborne (Wagenaar, Hendriksen, & Carrique-Mas, 2013).

Pork and pork products are considered to be one of the most important sources of human salmonellosis (Wong & Hald, 2000). *Salmonella* is a very diverse genus, there are host-adapted serotypes and others that have a wide range of hosts (Radostits, 2000). This means that infection can happen without apparent clinical signs, as host-adapted serotypes tend to cause more severe disease than non-adapted serotypes (Quinn et al., 2011), turning surveillance, control and eventually eradication in arduous tasks to complete. Serotypes with importance in human health are various, although usually only two or three are responsible for the majority of the cases (Wray & Wray, 2000). Other issue is related to the use of antimicrobials in intensive pig production and the potential that such practice has in development of multidrug resistant *Salmonella* strains; contamination through food chain allows those drug resistant bacteria to infect humans and compromise effective treatment of disease (Barton, 2014; Farzan, Friendship, Dewey, Poppe, & Funk, 2010; Garcia-Migura, Hendriksen, Fraile, & Aarestrup, 2014).

Foodborne *Salmonella* infection is responsible for substantial economic losses, associated with medical care (visiting a general physician, a hospital, hospitalization services, medications, premature dead), productivity loss (inability to work or being responsible for a sick person), among others (outbreak research). Infection can be fatal to old aged and immunocompromised people. The estimations of costs are difficult to make because most of affected people fail to go to the doctor and no report is produced (Forshell & Wierup, 2006).

1.1. EU situation

Salmonella is the second most reported cause of foodborne disease in EU, accounting for 91,034 confirmed human cases in 2012 (EFSA & ECDC, 2014). Although numbers have been showing a decreasing tendency, such a fact has been attributed to successful control measures applied in egg production sector. Pork is considered the second most important source of

Salmonella after table eggs and is closely related to *S. Typhimurium* infection. *Salmonella* is the most common cause of foodborne outbreak in EU, representing 28.6% of all analyzed cases, which sums 1,533 outbreaks; within these, 347 events are strongly considered to be caused by *Salmonella*. Pork and products thereof were the food vehicle in 5.8% of those 347 events (EFSA & ECDC, 2014). Foodborne salmonellosis is a reality that needs improved control efforts, tackling on its diverse sources. Pork sector is directing towards a risk-based control policy, similarly to the way followed by egg sector with good results. Regulation (EC) No 2160/2003 posed the legal framework for all MS to act on this issue: “proper and effective measures” should be “taken to detect and to control *Salmonella*”, requiring that all MS formulate their own control plans. To achieve harmonized information on prevalence of this agent in pigs, two baseline surveys were accomplished at EU level, as imposed by Commission Decision 2008/55/EC: one of them occurred from October, 2006 to September, 2007 and targeted slaughter pigs in order to assess *Salmonella* prevalence at this level; another occurred during 2008, targeting holdings with breeding pigs and collecting information on prevalence and potential risk factors for infection with *Salmonella* at holding and pen levels. In this last study, breeding population was selected to test its potential to maintain and disseminate *Salmonella* vertically and horizontally, through all their slaughter progeny and to the other pigs breeding pigs on the same farm. By separating breeding and production holdings, it was expected to observe the influence of *Salmonella* infection along the production chain (EFSA, 2007). Study on slaughter pigs yielded an observed prevalence of 10.3% of *Salmonella* in ileocaecal lymph nodes (MS prevalence ranging from 0.0% to 29.0%) and the most common serotypes found were *S. Typhimurium* (4.7%, ranging in MS from 0.0% to 16.1%) and *S. Derby* (2.1%, ranging in MS from 0.0% to 6.5%), but 87 different serotypes were found (EFSA, 2008). The study on breeding pigs yielded EU-level prevalence of *Salmonella* in holdings with breeding pigs of 31.8% (95% IC: 30.0 – 33.7%), being that breeding holdings prevalence was 28.7% (95% CI: 26.3 - 31.0%) and production holdings prevalence was 33.3% (95% IC: 30.9 - 35.7%) and the most frequent serotypes isolated were *S. Derby*, *S. Typhimurium*, *S. Infantis*, *S. Rissen*, *S. London* and a total of 99 different serotypes were found, 54 in breeding holdings and 88 in production holdings (EFSA, 2009). EFSA concluded that there is a correlation between *Salmonella* prevalence in breeding holdings and production holdings, not contradicted by serotype distribution from ones to another. This correlation indicates that the hypothesis that breeding holdings potentially contribute to maintain and disseminate *Salmonella* is supported (EFSA, 2011).

1.2. Portuguese situation

Currently, few data are available concerning *Salmonella* situation on pig production and pork in Portugal (Gomes-Neves et al., 2012; Xavier, Gonzales-Barron, Paula, Estevinho, & Cadavez, 2014). Information reported to EFSA on human salmonellosis during 2012 in Portugal shows occurrence of 190 cases, from which 185 were confirmed to be caused by *Salmonella*, resulting in a 1.8 rate of confirmed cases per 100,000 people. Portugal is among EU countries with lowest notification rates of salmonellosis but has one of the highest hospitalization rates, meaning that surveillance system is more suited to capture more severe cases (EFSA & ECDC, 2014).

Salmonella prevalence in primary pig production was assessed under European baseline study on *Salmonella* in breeding pigs in 2008. Prevalence of *Salmonella* in breeding holdings was 45.5% (95% CI: 38.5 – 53.8%) and in production holdings 43.3% (95% CI: 35.6 – 52.0%), both higher than European Union estimated prevalence (EFSA, 2009). Portugal is among the medium or high *Salmonella* prevalence countries in European Union (EFSA, 2006). Investigations in slaughterhouses have revealed high prevalence of the agent: 26.7% positive pigs and 12.9% positive carcasses from a sample of 101 pigs (Vieira-Pinto, Temudo, & Martins, 2005); 26.0% of positive ileocaecal lymph nodes, 16.0% of positive carcasses, and 14.0% of positive meat samples from a sample of 100 pigs, finding also clinically relevant multidrug resistant isolates and highlighting the role of meat handlers in spreading the agent inside facilities (Gomes-Neves et al., 2014; Gomes-Neves et al., 2012). In the 2006/2007 European baseline study on prevalence of *Salmonella* in slaughter pigs, EU-level observed prevalence in lymph nodes was 10.3% (95% CI: 9.2 – 11.5%) and in Portugal was 23.4% (95% CI: 19.4 – 28.0%) (EFSA, 2008). At the processing level, Portugal had the largest proportion of positive samples in a relatively small sample (17.5% of 40 samples) when comparing with data from other MS from 2012 and also the largest proportion of positive samples in cooked ready-to-eat meat preparations or meat products (3.3%) (EFSA & ECDC, 2014).

A recent meta-analysis of the incidence of foodborne pathogens in Portuguese meats and their products poses *Salmonella* as critical in Portuguese-produced pork, estimating a mean occurrence of 12.6% (95% CI: 8.0 – 19.3%) and classifying pig meat as the most important likely source of *Salmonella* infection (5%) despite of limited information available to support this (Xavier et al., 2014).

According to references abovementioned, serotypes that present major importance are *S. Typhimurium*, *S. 4,[5],12:i:-* (monophasic *S. Typhimurium*), *S. Derby*, *S. Rissen* and *S. London*.

1.3. Danish situation

Denmark has implemented a surveillance control plan for salmonellosis in pig production since 1993, in consequence of the high numbers of human affection (Alban et al., 2012). A considerable reduction has been achieved: the last data show a total of 1,136 human cases, showing a decreasing tendency. Still, pork is the most important food source for salmonellosis in this country: estimations account for 11.3% of all cases, meaning 128 human cases (DTU-Food, 2014).

In 1998, a bacteriological study was carried on in Danish pig herds in order to assess effects of *Salmonella* control plan implemented by comparing with data gathered in a reference study performed before that plan, in 1993/1994. Herd apparent prevalence for genetic herds was 11.7% (all herds sampled), for sow herds was 16.7% (95% CI: 13.2 – 20.9%) and for slaughter pig herds was 11.4% (95% CI: 10.2 – 12.7%). Total herd apparent prevalence in 1998 was 11.4% and this is a much better result when compared to an apparent herd prevalence of 22.2% in 1993/1994, despite of probable differences in methods sensitivity (Christensen, Baggesen, Nielsen, & Stryhn, 2002). According to (Dahl, 2013), proportion of *Salmonella* positive pig herds suffered an initial drop from 1995 to 1998 and doubled from 1998 to 2012, still the average prevalence in positive herd has been fairly constant over period. This is considered a result of the *Salmonella*-reduction strategy implemented in high prevalence breeding, multiplying and production herds, mainly through trade of pigs.

Results of 2006/2007 EU baseline study on slaughter pigs shows a prevalence of 7.7% (95% CI: 5.5 – 10.7%) in ileocaecal lymph nodes (EFSA, 2008). EU baseline study on breeding pigs of 2008 yielded prevalence of 41.1% (95% CI: 34.4 – 48.9%) on breeding holdings and 41.4% (95% CI: 35.2 - 48.4%) and the most common serotypes found were *S. Typhimurium*, *S. Derby*, *S. Infantis*, with a total of 17 different serotypes found (EFSA, 2009). Pork prevalence was 1.2% in 2011, with a reduction target set to reach less than 1% at the end of 2013 (Alban et al., 2012). Denmark is considered as a medium or high *Salmonella* prevalence country among European MS (EFSA, 2006).

Least referred, but of major importance, the context: pig production in Denmark originates from approximately 4,200 pig farms, delivering an impressive number of 29.1 million pigs per year and exporting more than 90% of whole production as live animals and pork (DAFC, 2013). Denmark is among the largest pig meat exporters and this turns to essential to Danish economy nowadays (DAFC).

2. *Salmonella enterica*: the bacteria and it's pathophysiology

Salmonella was first identified by Theobald Smith and Daniel Salmon in 1886 when they isolated *S. Cholerasuis* from pigs suffering from classical swine fever (Straw, 2006). Since then much more were identified. *Salmonella* belongs to the family *Enterobacteriaceae* and is divided in to two species: *S. enterica* and *S. bongori*. *Salmonella enterica* is divided into six subspecies: *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae* and *S. enterica* subsp. *indica*. Currently, over 2500 serotypes of *Salmonella* are recognised and number is constantly increasing. Strains are classified into serotypes on the basis of extensive diversity of lipopolysaccharide (LPS) antigens (O) and flagellar protein antigens (H) in accordance with the Kauffmann-White scheme. More than 99.5% of *Salmonella* belong to subspecies *enterica* (Grimont & Weill, 2007). Most of the *Salmonella* of veterinary importance belong to this subspecies (Quinn et al., 2011).

Salmonella is widespread in the environment, thus it appears to be more prevalent in areas of intensive animal husbandry. As a member of family *Enterobacteriaceae*, is usually found in any material subject to faecal contamination; the intestinal tract of warm and cold-blooded animals serves as reservoir for this bacteria. There are host specific and host adapted serotypes. (OIE, 2010).

Salmonella is a motile gram-negative bacteria, facultative anaerobic, non-spore forming, rod-shaped with fimbriae and flagella. They are hardy and ubiquitous bacteria, able to multiply at 7-45°C; they can survive freeze and desiccation well and can maintain for long periods in suitable organic substrates and water. When pH is lowered below 5.0 survival is greatly shortened. Bacteria are readily inactivated by heat and sunlight as well as by common phenolic, chlorine and iodine-based disinfectants (Straw, 2006).

The main route of infection is through ingestion although it can also occur through the mucosae of the upper respiratory tract and conjunctiva. Colonization and persistence in tonsils appears to be important in pigs (Quinn et al., 2011).

After passage through the stomach, the bacteria colonize the intestine, interacting with and translocate 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. The conditions faced by bacteria in stomach and intestinal tract form a stressful environment to

which bacteria reacts expressing genes essential for intestinal epithelium invasion and infection of the host (Forshell & Wierup, 2006).

Infection can happen without showing clinical signs (Kranker, Alban, Boes, & Dahl, 2003). Clinical expression of disease is more frequent in young animals and pregnant animals. The clinical signs possible englobe enteric disease, often presenting as a bloody or profuse watery diarrhea with pyrexia, acute septicemia, abortion, arthritis, necrosis of extremities and respiratory disease; signs and lesions are not pathognomonic (Wray & Wray, 2000).

3. Epidemiology

There are multiple ways for *Salmonella* to cause infection, not forgetting this duality that most of the times clinical signs are not present and those serotypes have potential to be a source of infection for pork products (Straw, 2006). From the moment a virulent *Salmonella* serotype gets in contact with a group of animals at a sufficient infective dose, there can be three outcomes: the animal will become clinically affected, he will be subclinically infected and become a shedder or, in a lower chance, a persistently infected. The bigger the group of animals, the bigger the possibility of having lower chances and get persistently infected animals. This dynamic allows an “invisible” and imperceptible dissemination of the agent, in the way that when clinical signs are seen they represent “the tip of the iceberg”. Factors that may activate a subclinical infection are connected to stress: intercurrent infections, transport, overcrowding, pregnancy, extreme ambient temperatures, water deprivation, oral antimicrobial therapy and sudden changes in rations altering the intestinal flora (Quinn et al., 2011).

3.1. Risk factors

Several studies and books describe factors that have been associated with *Salmonella* infection in primary production throughout the years. Fosse and colleagues published in 2009 a review of prevalence and risk factors for bacterial foodborne zoonotic hazards in slaughter pigs (Fosse, Seegers, & Magras, 2009). Mainly, they identified biosecurity measures (related to hygiene practices, like washing hands, changing clothes and boots before entering in the holding) as protective against infection; the type of floor was influent because if it allowed to decrease animals contact with faecal material, it would also reduce faecal-oral transmission between pigs; good hygiene practices towards building help to reduce residual contaminants than can maintain for long periods; instauration of all-in/all-out systems had potential to reduce infection, although no confirmatory information exists; vertical transmission between sows and piglets was a risk; existence of biological vectors (rodents, birds, insects and other animals) presented risk; feed could be contaminated, although the findings on feed were not greatly found on pork; physical structure of feed is seen to interfere in susceptibility to infection, with

dry/pellet in a risk position; acidification of feed with organic acids protected against infection; season of the year and environmental temperature; animal density increased risk; concurrent infections posed risk for *Salmonella* infection as well as use of antibiotics.

An analysis under Portuguese database from 2008 EU baseline study highlighted the following as risk factors for *Salmonella* infection: region of the herd, number of sows in herd, management of breeding boars, source of semen, rodents control in holding, number of animals per pen, the production stage of animal and source of feed (Correia-Gomes, Mendonca, Vieira-Pinto, & Niza-Ribeiro, 2013). The same database analyzed under perspective of serotype-dependent risk factors concerning (1) *S. Typhimurium* with *S. Typhimurium* monophasic strain and (2) other serotypes led to conclude that (1) supported a likely possibility of transmission between animals (number of breeding pigs in herd, source of semen, number of pigs per pen, age of breeding sows) and (2) an environmental relation (region of the herd, control of rodents, production stage of animals, source of feed) (Correia-Gomes, Economou, Mendonca, Vieira-Pinto, & Niza-Ribeiro, 2012).

4. Prevention and control measures: the EU Regulation no. 2160/2003

Eradication of *Salmonella* is difficult to accomplish for all the reasons abovementioned; despite all the knowledge available about the agent, it is not enough to easily clean a holding from infection. Still, it has to be done because of implications in human health. In 1980, the World Health Organization formulated three lines of defense against *Salmonella*, implicating firstly *Salmonella* control in primary production, secondly *Salmonella* control during slaughter and at last industry and consumers education on good-hygiene practices (Forshell & Wierup, 2006). This approach is a valid and valuable strategy as the combination of the three lines allow attainment of a safer final product. EU Regulation no. 2160/2003 follows this lines. There was the need of establishing targets for harmonized control through EU and the way of achieving that was making baseline studies EU-wide to collect that missing information. However, some measures are possible to be taken.

4.1. Surveillance programs and 2008 Baseline study

The 2008 baseline study was conducted to provide information enough for all MS's to create their own surveillance program for *Salmonella* in pigs as a useful tool for continuous evaluation of infection status.

4.1.1. EU

From all the participant MS, there were some that already control or surveillance plans: Sweden, Denmark, United Kingdom, Ireland, Germany and Netherlands, while there could be others with starting plans (EFSA, 2006). Exploring each MS surveillance system is beyond the scope of this brief review.

4.1.2. Portugal

Portugal has no control plan for *Salmonella* in pig production. Studies are available concerning the *Salmonella* situation in the country that can be useful for building a well fitted surveillance plan in terms that they state what is known by standardized methods and highlight gaps of knowledge that are important to unveil reality (Xavier et al., 2014). A study was performed in Portuguese herds by Baptista and colleagues using an innovative risk-based approach: departing from herd information, allows to predict the *Salmonella* status of the herd (Baptista et al., 2010). Results were encouraging and this can be the base of a cost-effective tool for future development of risk-based approaches to surveillance, targeting interventions to high-risk herds or differentiating sampling strategies in herds with different levels of infection. A stochastic model of the dynamics of *S. Typhimurium* in a farrow-to-finish farm in Portugal was developed (Correia-Gomes, Niza-Ribeiro, Vieira-Pinto, & Bradzil, 2014), as well as the estimation of transmission parameters of the agent (Correia-Gomes, Economou, et al., 2014). The model can be used to assess control measures and estimate cost-benefit control measures if linked to an economic model.

4.1.3. Denmark

Denmark has a surveillance and control plan for *Salmonella* in pig production since 1993, in response to the high number of *Salmonella* human cases in early 1990s. From there until now, different approaches were tried, tools were created and a paper was published aiming to present the main conclusions that can be useful for other countries (Alban et al., 2012). Current Danish *Salmonella* programme in finisher pigs is designed using both a pre-harvest and post-harvest approaches.

The first approach tried focused on pre-harvest measures, posing the hypothesis that safe pork would be produced if pigs slaughtered were *Salmonella*-free or present a low amount of the agent. A herd classification scheme was developed, methodologies for agent detection were tested: a serologic method was developed (Danish Mix-ELISA, able to detect 93% of *Salmonella* serotypes in pig production) and was compared to *Salmonella* cultivation (Kranker et al., 2003), revealing a lag between serology and bacteriology. Despite the fact that this lag can lead to misleading conclusions about *Salmonella* presence in pigs, it was considered

sufficient if the aim was to identify high-risk herds and follow a reducing *Salmonella* strategy (Sorensen, Alban, Nielsen, & Dahl, 2004). Based on this method, the herd classification scheme adopted comprised three levels: Level 1 was “acceptable, low” *Salmonella* prevalence, Level 2 was “moderate, still acceptable” *Salmonella* prevalence and Level 3 was “unacceptable high” proportion of seropositive samples. Herds were allocated monthly on these levels. From 1998 to 2001, a deduction and advisory system was introduced intending to act as an incentive for farmer in Levels 2 and 3. In 2001, enough data were collected to adjust control program. Sampling methodology was adjusted as well as analytical procedures. Herd level classification scheme was adjusted supported by the creation of a slaughter pig index for *Salmonella*, as described in (Alban et al., 2012): Level 1 had an index ranging from 0% to 39%, Level 2 had an index of 40% to 69% (with payment of a penalty) and Level 3 had an index equal or superior to 70% (with payment of a penalty and specific demands on slaughter procedures). Later on 2009, Level 2 higher limit was adjusted for 65% instead of 69%. Adjustments concerning sampling methodology were made in 2002. Risk-based sampling scheme was introduced in 2005 and allowed for sample and cost-reduction. In the end of 2010 fecal mandatory sampling stopped and in 2011, herd categorization changed: herds allocated at Levels 2 or 3 herds are considered positive for next five years unless they can prove they are negative by a specified program documenting freedom from infection.

Control points for *Salmonella* in primary production focus on mitigating between-herd spreading, with respect to control the origin of purchased animals in respect to *Salmonella* sanitary status, heat-treating feed, maintaining environment free of animals than can be potential reservoirs like birds and rodents and promoting feeding, management and hygiene practices that act as protective against *Salmonella* infection.

Cost-effective measures to obtain a lower prevalence of *Salmonella* in pork started to have importance due to financial difficulties that industry has been faced with. Hot water decontamination applied to pigs under sanitary slaughter prevent from herds with high levels of *Salmonella* proved to be economically efficient (Goldbach & Alban, 2006) and produces satisfactory results (Alban & Sorensen, 2010).

5. A new approach: serotype-dependent risk factors

There are risk factors related to the interaction of the agent itself with animals and factors related to environment, that predispose animal to be at a higher or lower level of exposure. In other words, attention can be paid to the interaction agent-animal, like biological “Trojan horses”, but firstly, agent has to be present, so environmental factors, primarily hygiene measures, are a major control point. How the agent makes himself available? Where should it be stopped? It is known that feed can carry *Salmonella*, water, rodents, birds, people’s boots

can move it from a contaminated to a clean area inside farm as well as dirty clothes, lack of an all-in/all-out system, hygiene wrongly made in empty pens leaving residual contamination and floors that allow pigs to contact with manure more frequently or low frequency of manure removal. It is also known that an infected pig doesn't necessarily show clinical signs and that he can act as a shedder or carrier despite of his clinical status. Moreover, he can be a persistently infected and will be very difficult to know that because of size of holdings and the testing work necessary to find such an animal. The animal itself can be suffering from a concurrent infection that will weaken animal's defenses, such as by *Lawsonia Intracelularis*, or even being prophylactically medicated with antibiotics that killed the commensal bacteria that would act as competitors against *Salmonella*, he can be subject to continuous social stress due to space constrains or stress for being mixed with a new group of "strange" pigs with all that it takes to build up again an healthy hierarchy between them, or even, in females, being under estrogen effect at estrous, that leads to an increase in circulating cortisol and a depression on immunity system. It is not possible to change all the existing holdings and turn them into the ideal. For the reasons exposed *Salmonella* is hard to eradicate.

The hypotheses that has already been posed is that the biological properties of some *Salmonella* serotypes can have different risk factors and also an important weight in infection patterns (Correia-Gomes, Niza-Ribeiro, et al., 2014); investigating the patterns of serotypes that cause major human and animal disease, better results can be achieved in disease control (Wagenaar et al., 2013).

B-Evaluation of risk factors for *Salmonella* infection in Danish pig breeding herds using multilevel hierarchical models

1. Objective

The main driver of this work was the current need of building knowledge concerning a risk-based *Salmonella* control approach in breeding pig production. *Salmonella* control and eradication in pig production is known to be hard to accomplish and our study represents a contribution for effectiveness and cost reduction. Considering the fact that this statistical methodology was used in Portuguese data and yielded interesting results, it is our purpose to apply it on Danish data and search for evidence of authenticity through repeatability in similar contexts.

The aim of this professional training period is to investigate serotype-dependent risk factors for *Salmonella* infection in breeding pigs in Denmark and compare them with those previously obtained to Portugal. Thus, objectives to be achieved are: (1) apply and extend knowledge acquired during course classes on a real-life environment; (2) learn about pig production in Denmark; (3) study *Salmonella* importance in Danish swine sector; (4) investigate serotype-dependent risk factors for *Salmonella* infection in breeding pigs in Denmark, concerning Veterinary Public Health; (5) compare Danish results with Portuguese results and draw conclusions.

2. Materials

2.1. Database

A database was received from Danish Veterinary Authorities containing information related to the routine and within-herd sampling from Danish breeding pig holdings, from the European baseline survey carried out in 2008. The database contains a total of 4030 registers and 79 variables. Danish sample is composed by 95 breeding holdings and 198 production holdings, totaling 293 unique holdings and matching 2930 rows, from what can be called routine sampling. The remaining 1100 rows show information from a within-holding study, from resampling 10 holdings: in each of the 10 pens of an holding, 10 individual samples were collected, yielding 100 individual samples per holding; at the lab, part of the 10 individual samples of a single pen were artificially pooled, yielding 10 artificial pooled samples per holding.

A Data Dictionary was also received, containing an explanation of variables included in the database produced by EFSA, as well as clarifying comments on it when needed. Variables were already coded and were related to routine sampling and within-herd sampling.

2.2. Aspects from study design, sampling and sample testing

The design and implementation of the study are published (EFSA, 2007) and legislated (Commission Decision 2008/55/EC). From those documents, relevant information will be exposed here. It is important to make some definitions: a “breeding pig” is defined as a pig that is over six months of age and is kept for breeding purposes; holdings with breeding pigs are divided on “breeding holdings” which sell gilts and/or boars for breeding purposes and “production holdings” which mainly sell pigs for fattening or slaughter. The word “holding” was chosen instead of “herd” because of the differences in definitions among MS. To be included in sampling frame are holdings: establishments, delimited production places with a unique identification where a group of pigs are held, kept or handled.

The target population of this survey includes 80% of the breeding pig population of a MS. Holdings with 50 breeding pigs or more were preferred for sampling. Holdings with breeding pigs were classified as “breeding holdings” or “production holdings”. Selection of holdings was made from two sampling independent frames: breeding holdings were randomly selected among admissible breeding holdings; the same procedure was made with production holdings. No strata were created. Holdings with outdoor production stages randomly entered the sample. Sampling was carried during 2008 and was divided through 12 consecutive months. When sampling inside holding, a random selection of pens was followed, fulfilling the requirement of representativeness of breeding pig production stages in holding. The sample size was calculated accounting for the total number of breeding and production holdings in the country, with an annual expected prevalence of 50%, confidence level of 95% and accuracy of 7.5%.

Collection of samples on holdings was performed by official veterinarians previously instructed by Danish Veterinary Authorities. Samples consisted of freshly voided faeces representing the whole holding, pooling material from at least 10 animals from the considered pen. This procedure should enable detection of at least one positive sample in an infected holding with 95% certainty if the true prevalence in the holding was 10% and with 80% certainty if the true prevalence was 5% instead. Sampling was preferentially made by swabbing faecal material from the ground and when that was not possible, individual pinches were collected from piles of faeces and artificial pooling was made.

An individual holding questionnaire was performed parallel to collection of samples to collect mandatory information on potential risk factors for *Salmonella* infection, both at holding and pen level. Questionnaires were composed mostly by closed answers and were applied by the same official veterinarians that were collecting samples and received training in order to minimize bias in collection of information. Mandatory information referred to information about holding management practices.

Laboratory analysis of samples was accomplished on National Reference Laboratory, following recommendations of the Community Reference Laboratory, constant in ISO 6579:2002/Amd 1:2007. Serotyping was made according to Kaufmann-White scheme. The sensitivity of this culture method is estimated to be above 85% (Kuijpers & Mooijman, 2013) and the specificity is considered to be 100% due to posterior execution of serotyping of positives (ISO, 2007).

3. Methods

3.1. Risk factor review

In order to compare the results obtained in this work with the results previously obtained with data from this baseline study at EU (EFSA, 2011) and Portugal (Correia-Gomes et al., 2012; Correia-Gomes et al., 2013), Table 1 was created to list the significant risk factors found in the analysis with respective ORs and 95% CI. In Portuguese data two different analysis were done: (a) one of them used a generalized linear mixed model with a logit link function for a dichotomous outcome variable of *Salmonella* positive or negative (the same type of model used at EU level) and (b) the other used a Bayesian hierarchical model using Monte Carlo Markov Chain for estimation considering a nominal outcome variable: *Salmonella* positive serotype Typhimurium and monophasic, *Salmonella* positive other serotypes and *Salmonella* negative. In Table 1, the associations found in one analysis can be classified in another as “Non evaluated” due to specificities at MS level, “Non comparable” due to the approach followed during the analysis, even if they yield conclusions generally in the same direction and “Non-significant” if association was found to be not significant on that particular case. In EU level analysis, ORs were adjusted for the factor “sample type”, also retained in the final model; variables “gilt replacement policy” and “floor type” interacted differently according to type of holding as signaled.

3.2. Database edition

In the database received, variables were all categorical with exception of the number of pigs per pen. A descriptive analysis of all variables was conducted to have an overview of the data. According to the aim of the analysis and to the results obtained in the exploratory analysis, it was necessary to recode variables in both levels of database in order to obtain meaningful variables. Table A1 in Annex 1 (pages *i* to *iv*) shows a variable dictionary, an overview of the edition work done on database as well as important information on variable meaning, while Table A2 in Annex 2 (page *v*) shows the reasons supporting variable recoding with respective references. Variables on Table A3 Annex 2 (page *v*) were excluded from the analysis. Given the

absence of information in at least one variable, some pens were excluded: fifteen pens with information “unknown” for supplements in feed or water, twenty five pens with information “unknown” for antibiotic administration. Four positive *Salmonella* pens had no information on serotype and were also excluded. Within these four cases, two belong to two different holdings and were the only positives found in each.

Table 1: Risk factor review - summary table (Legend on beginning of next page)

Risk/Protection factor			Factors for Salmonella infection		Serotype-dependent factors	
			EFSA	Correia-Gomes C. et al, 2013	Correia-Gomes C. et al, 2012	
			Salmonella positive [OR (95% CI)*]	Salmonella positive [OR (95% CI)]	Typhimurium and 1,4,5,12:i:- [OR (95% CI)]	Other Serotypes [OR (95% CI)]
Holding variables	Region of the herd	North	NE	3.86 (1.08 – 13.75)	NS	12.9 (2.97 – 64.33)
		Centre	NE	NS	NS	4.57 (1.33 – 17.57)
		Alentejo		1.0		1.0
	Number of sows	≥170	NE	1.82 (1.04 – 3.19)	NS	NS
		<170		1.0		
	Size of the herd (number of breeding pigs)	≥230	NC	NS	7.04 (1.46 – 60.04)	NS
		<230			1.0	
		>999	5.25 (3.48 – 7.93)	NC	NC	NC
		400-999	3.23 (2.26 – 4.62)	NC	NC	NC
		100-399	1.84 (1.34 - 2.53)	NC	NC	NC
	Gilt replacement policy	<100	1.0			
		10-90% Gilts homebred	0.32 (0.18 – 0.58)**	NS	NS	NS
	Boar replacement policy	>90% Gilts homebred	1.0			
		Without boars or > 90% homebred	NC	0.54 (0.30 – 0.97)	NS	NS
	Source of semen	More than 90% purchased		1.0		
		Boar from another herd	NE	4.47 (1.38 – 14.43)	41.22 (2.46 – 1392.7)	NS
Rodents control	Insemination center – IC		1.0	1.0		
	Yes	NE	0.23 (0.09 – 0.59)	NS	0.13 (0.03 – 0.45)	
	No		1.0		1.0	
Pen variables	Number of pigs in pen	Per 10 pigs	1.03 (1.00 – 1.06)	NC	NC	NC
		>10	NC	2.02 (1.19 – 3.43)	4.06 (1.03 – 19.73)	NS
		=10		1.0	1.0	
	Age of the breeding sows	Without gilts	NC	NS	0.17 (0.03 – 0.65)	NS
		Only gilts or gilts and others			1.0	
	Production stage	Pregnant	0.81 (0.67 – 0.96)	NC	NC	NC
		Farrowing and lactating	0.65 (0.53 – 0.80)	NC	NC	NC
		Maiden gilts	1.0			
		Farrowing and lactating	NC	0.39 (0.24 – 0.63)	NS	0.38 (0.17 – 0.80)
		Service area		1.0		1.0
	Floor type	Outdoors in fields or paddocks	3.32 (2.04 – 5.39)***	NC	NC	NC
		Solid floor other bedding	2.37 (1.08 – 5.19)***	NC	NC	NC
		Solid floor without bedding	2.07 (1.49 – 2.89)***	NC	NC	NC
		Partly slatted floor	1.43 (1.15 – 1.77)***	NC	NC	NC
		Other	3.82 (1.21 – 12.10)**	NC	NC	NC
		Slatted floor	1.0			
Source of feed	Other	0.51 (0.27 – 0.97)	NC	NC	NC	
	Home-mill	0.58 (0.45 – 0.74)	NC	NC	NC	
	Commercial compound	1.0				
	Not exclusively own	NC	2.81 (1.19 – 6.61)	NS	7.29 (2.25 – 29.46)	
Type of diet	Exclusively own		1.0		1.0	
	Meal/mash	0.52 (0.41 – 0.67)	NC	NC	NC	
	Porridge/liquids	0.46 (0.34 – 0.62)	NC	NC	NC	
	Cobbs/rolls/nuts/pellets	1.0				

Table 1 (cont): Risk factor review - summary table: [Legend](#)

NE: Non evaluated; NC: Non comparable; NS: Non-significant; *: All ORs adjusted for factor “sample type” also retained in final model; **: Different effect in breeding holdings; ***: Different effect in production holdings;

3.3. Outcome variables

The outcome variables were created in order to (1) identify risk factors for *Salmonella* positivity and (2) to identify serotype-specific risk factors. Inside (1) there is one outcome variable that has two levels: 0. *Salmonella* negative (reference category) or 1. *Salmonella* positive; within (2) there are two outcome variables aiming to compare Typhimurium and Derby with all other serotypes in separate approaches. Each of the two has three levels: Typhimurium-specific is 0. *Salmonella* negative (reference category), 1. *Salmonella* Typhimurium, 2. *Salmonella* remaining serotypes and Derby-specific is 0. *Salmonella* negative (reference category), 1. *Salmonella* Derby and 2. *Salmonella* remaining serotypes. Table 4 helps to clarify this approach.

Table 4: Outcome variables and *Salmonella* serotype distribution

<i>Salmonella</i> Serotype distribution		Outcome variables		
<i>Salmonella</i> Serotype	N=390 (100 %)	<i>Salmonella</i> positivity	S. Typhimurium serotype-specific	S. Derby serotype-specific
S. Agona	8 (2.1)	1	2	2
S. Derby	145 (37.2)	1	2	1
S. Enteritidis	2 (0.5)	1	2	2
S. Idikan	1 (0.3)	1	2	2
S. Infantis	62 (15.9)	1	2	2
S. Kedougou	1 (0.3)	1	2	2
S. Livingstone	38 (9.7)	1	2	2
S. London	13 (3.3)	1	2	2
S. London var. 15	1 (0.3)	1	2	2
S. Mbandaka	5 (1.3)	1	2	2
S. Meleagridis	6 (1.5)	1	2	2
S. Muenchen	9 (2.3)	1	2	2
S. Newport	2 (0.5)	1	2	2
S. Panama	5 (1.3)	1	2	2
S. Rissen	4 (1.0)	1	2	2
S. Typhimurium	82 (21.0)	1	1	2
S. Uganda	2 (0.5)	1	2	2
<i>Salmonella</i> untypeable	3 (0.8)	1	-	-
(without serotype)	1 (0.3)	1	-	-
<i>Salmonella</i> negative	-	0	0	0

3.4. Statistical analysis

Data collected follow a multilevel structure, with the pen level nested in holding level. According to this, multilevel logistic regression models were considered. For each of the three outcome variables, the Models 1, 2 and 3 in Annex 3 (pages *vi* and *vii*) were fitted. The random effects are in the form of a random intercept and this allows for the fact that the observations are nested in holdings. Treating the holding effect as random also allows for the fact that the number of holdings (293) is a sample of all existing holdings and not the whole population.

3.4.1. Univariable analyses

For an exploratory purpose, univariable analyses were performed for pen and holding level. Associations between variables were assessed using a chi-square test.

Multilevel univariable models were performed in order to identify candidates for the multilevel multivariate models and considering as significance level $\alpha=0.20$.

3.4.2. Multivariable analyses

For each outcome, multivariable logistic regression models were performed considering all significant variables retained in the univariable analysis. The final multivariable model was obtained using a forward and then backward selection process. The significance level was set at 0.05. Confounding was assessed inspecting changes in OR's magnitudes at each step when the least significant variables were excluded and by testing interactions.

After selection of variables, two-way interactions with biological meaning were investigated among those variables selected, considering significance at a level $\alpha=0.05$.

Relevance of the holding random effects was tested looking at the variance estimate: when close to 0, it indicates holding effect doesn't contribute to the dispersion of the outcome variable and a simpler model (without random effects) could be chosen.

4. Results

4.1. Descriptive

From the 293 holdings analyzed, 32.4% were breeding holdings and 67.6% were production holdings. Collection of samples was made throughout all year, more frequently on autumn (41.2% versus 17.1% collected on winter). Only 1.7% of total holdings are considered outdoor. More than 80% of holdings sampled housed between 100 and 999 breeding pigs at the time of sampling. More than an half of the holdings raise more than 90% of their replacement gilts on their own premises, as well as 48.5% do the same with their replacement boars. There are 21.2% of holdings that have no boars. Pens sampled contained between 10 and 320 animals

each, although 54.5% had only 10 pigs. Only 25 (0.9%) of the sampled pens have access to outside. The most frequent type of floor found in pens was partly slatted (76.2%). Sanitary gap between new breeders in pens was accomplished 19.2% of times. Meal/mash was the most frequently registered feed (37.4%). Home mill mixed feed was slightly more frequent than feed from a commercial origin or mixtures (51.7% versus 48.3%). Detailed descriptive analysis is available on Tables 6 and 7. The unique quantitative variable was “Number of pigs per pen” with a median value of 10 (the minimum 10 and maximum 320).

From the total faecal samples collected, 2540 (86.7%) were *Salmonella* negative and 390 (13.3%) were *Salmonella* positive. It was found only one *Salmonella* serotype in each positive sample. The three most frequent serotypes found were *S. Derby* (145 samples, 37.2%), *S. Typhimurium* (82 samples, 21%) and *S. Infantis* (62 samples, 15.9%). The remaining serotypes and their respective frequencies are listed in Table 4. From the 390 positive samples, 3 were untypeable and 1 had no information on serotype and they were considered as missing cases. The observed prevalence of *Salmonella* in breeding and production holdings was calculated with respective 95% confidence interval based on a finite population approach and is shown on Table 5 (EFSA, 2009).

Table 5: Observed *Salmonella* prevalence and respective 95% confidence interval

Observed prevalence	%	95% CI	
		Lower	Upper
<i>Salmonella</i> in breeding holdings	41.1	34.4	48.9
<i>Salmonella</i> in production holdings	41.4	35.2	48.4
<i>Salmonella</i> Typhimurium in breeding holdings	15.8	11.3	22.6
<i>Salmonella</i> Typhimurium in production holdings	12.6	8.6	17.9
<i>Salmonella</i> Derby in breeding holdings	12.6	9.1	18.8
<i>Salmonella</i> Derby in production holdings	14.6	10.6	20.2
Other <i>Salmonella</i> than Typhimurium and Derby in breeding holdings	17.9	13.4	24.7
Other <i>Salmonella</i> than Typhimurium and Derby in production holdings	18.7	14.1	24.7

4.2. Univariable analyses

Univariable analyses were performed for each of the three outcome variables in order to assess potential meaningful effects (Tables 6 and 7). In general, the assumption of independence between all pens sampled leads to misclassification of some variables as significant; those associations disappear when variables are fitted in the multilevel model (Table 8). A particular case of this is observed with outcome variable *Salmonella* positive/*Salmonella* negative: Table A4 in Annex 4 (page *viii*) shows differences observed.

Table 6: Holding level variables distribution and univariable analysis for outcome variables (bold: $p < 0.05$)

Variables and levels	Descriptive					Univariable analyses				
	Total	Salmonella positive				Salmonella positive OR (95% CI)	S. Typhimurium serotype-specific		S. Derby serotype-specific	
		ST+	SD+	SO+	Total		S. Typhimurium OR (95% CI)	Other Salmonella OR (95% CI)	S. Derby OR (95% CI)	Other Salmonella OR (95% CI)
Sample Season										
Winter	500	16	22	29	69	1.004 (0.739 – 1.363)	1.177 (0.641 – 2.160)	0.959 (0.681 – 1.352)	0.752 (0.460 – 1.229)	1.200 (0.825 – 1.745)
Spring	600	23	35	42	100	1.291 (0.985 – 1.693)	1.458 (0.847 – 2.509)	1.249 (0.923 – 1.688)	1.031 (0.679 – 1.567)	1.494 (1.068 – 2.090)
Summer	620	10	17	30	57	0.654 (0.475 – 0.899)	0.563 (0.275 – 1.151)	0.677 (0.477 – 0.960)	0.445 (0.259 – 0.763)	0.817 (0.555 – 1.201)
Autumn	1210	33	71	58	164	1.0	1.0	1.0	1.0	1.0
Type of holding										
Breeding holding	950	30	52	56	141	1.191 (0.951 – 1.490)	1.234 (0.782 – 1.950)	1.179 (0.919 – 1.513)	1.196 (0.843 – 1.697)	1.187 (0.900 – 1.565)
Production holding	1980	52	93	103	249	1.0	1.0	1.0	1.0	1.0
Type of breeding and production holding										
Nucleus	130	6	0	1	7	1.0	1.0	1.0	-	-
Multiplier or supplier	820	24	52	55	134	3.355 (1.532 – 7.351)	0.717 (0.287 – 1.791)	19.185 (2.653 – 138.735)	-	-
Farrow to weaner	750	13	54	38	106	2.865 (1.301 – 6.306)	0.414 (0.154 – 0.110)	17.571 (2.426 – 127.266)	-	-
Farrow to grower	520	18	26	20	64	2.466 (1.102 – 5.518)	0.809 (0.314 – 2.082)	12.408 (1.694 – 90.874)	-	-
Farrow to finish	710	21	13	45	79	2.200 (0.992 – 4.880)	0.682 (0.270 – 1.725)	11.306 (1.551 – 82.395)	-	-
Outdoor production										
No	2880	78	145	159	386	0.568 (0.203 – 1.586)	-	-	-	-
Yes	50	4	0	0	4	1.0	-	-	-	-
Total number of breeding pigs										
<100	150	3	3	1	8	0.240 (0.105 – 0.546)	0.338 (0.095 – 1.197)	0.197 (0.068 – 0.569)	0.278 (0.080 – 0.967)	0.218 (0.075 – 0.632)
100-399	1180	27	39	73	141	0.651 (0.453 – 0.937)	0.416 (0.215 – 0.806)	0.755 (0.497 – 1.147)	0.495 (0.275 – 0.890)	0.743 (0.480 – 1.152)
400-999	1330	38	86	70	195	0.832 (0.585 – 1.183)	0.536 (0.286 – 1.005)	0.962 (0.641 – 1.445)	0.998 (0.582 – 1.713)	0.735 (0.476 – 1.135)
>999	270	14	17	15	46	1.0	1.0	1.0	1.0	1.0
Gilt replacement policy										
>90% gilts homebred	1520	44	64	47	204	1.0	1.0	1.0	1.0	1.0
10-90% gilts homebred	340	8	11	19	38		0.792 (0.369 – 1.700)	0.833 (0.553 – 1.255)	0.749 (0.390 – 1.437)	0.859 (0.558 – 1.322)
>90% gilts purchased	1070	30	70	93	148	1.044 (0.830 – 1.312)	0.973 (0.607 – 1.560)	1.064 (0.825 – 1.371)	1.561 (1.101 – 2.214)	0.802 (0.599 – 1.074)
Boar replacement policy										
No boars on farm	620	22	52	56	130	1.0	1.0	1.0	1.0	1.0
>90% homebred	1420	38	53	76	169	0.503 (0.391 – 0.647)	0.677 (0.396 – 1.156)	0.468 (0.355 – 0.617)	0.399 (0.269 – 0.594)	0.572 (0.421 – 0.778)
10-90% purchased	140	5	9	3	18	0.525 (0.305 – 0.904)	0.913 (0.339 – 2.459)	0.446 (0.238 – 0.837)	0.695 (0.333 – 1.450)	0.412 (0.194 – 0.876)
>90% purchased	750	17	31	24	73	0.401 (0.294 – 0.547)	0.559 (0.294 – 1.064)	0.369 (0.261 – 0.520)	0.431 (0.272 – 0.683)	0.380 (0.256 – 0.565)

Table 7: Pen level variables distribution and univariable analysis for outcome variables (bold: $p < 0.05$)

Variables and levels	Descriptive					Univariable analyses				
	Total	Salmonella positive				Salmonella positive OR (95% CI)	S. Typhimurium serotype-specific		S. Derby serotype-specific	
		ST+	SD+	SO+	Total		S. Typhimurium OR (95% CI)	Other Salmonella OR (95% CI)	S. Derby OR (95% CI)	Other Salmonella OR (95% CI)
Number of animals per pen										
=10	1594	39	75	88	202	1.0	0.719 (0.432 – 1.199)	0.791 (0.604 – 1.036)	0.706 (0.487 – 1.024)	0.825 (0.608 – 1.119)
[11;15]	570	18	21	25	64	0.872 (0.646 – 1.175)	0.914 (0.493 – 1.693)	0.614 (0.424 – 0.890)	0.544 (0.322 – 0.919)	0.768 (0.517 – 1.142)
>15	762	25	49	46	120	1.288 (1.009 – 1.645)	1.0	1.0	1.0	1.0
The pen has direct access to outside										
No	2901	79	145	159	383	1.0	-	-	-	-
Yes	25	3	0	0	3	0.897 (0.267 – 3.010)	-	-	-	-
Individual pen										
No	1029	22	46	58	126	1.0	0.665 (0.405 – 1.091)	0.943 (0.734 – 1.211)	0.842 (0.588 – 1.206)	0.901 (0.681 – 1.192)
Yes	1897	60	99	101	260	1.138 (0.906 – 1.429)	1.0	1.0	1.0	1.0
Age of the breeding sows										
Only gilts or mixed age	1578	55	84	74	213	1.060 (0.855 – 1.314)	1.0	1.0	1.0	1.0
Without gilts	1348	27	61	85	173	1.0	0.570 (0.357 – 0.910)	1.073 (0.846 – 1.362)	0.844 (0.601 – 1.184)	1.009 (0.774 – 1.314)
Sex of the breeding pigs										
Only sows	2758	76	139	152	367	1.0	0.789 (0.338 – 1.842)	1.395 (0.781 – 2.491)	1.444 (0.627 – 3.324)	1.093 (0.610 – 1.958)
Boars or/and sows	168	6	6	7	19	0.831 (0.509 – 1.356)	1.0	1.0	1.0	1.0
Breeding sector room										
Replacement breeders	313	7	23	14	44	1.085 (0.748 – 1.574)	0.602 (0.256 – 1.414)	1.468 (0.940 – 2.293)	1.0	1.0
Gestation	290	15	13	17	45	1.218 (0.840 – 1.766)	1.416 (0.730 – 2.746)	1.307 (0.814 – 2.099)	0.621 (0.308 – 1.252)	1.673 (0.940 – 2.979)
Maternity	916	14	45	61	120	1.0	0.407 (0.209 – 0.793)	1.421 (1.002 – 2.015)	0.661 (0.393 – 1.113)	1.207 (0.730 – 1.996)
Mating	776	22	38	41	101	0.993 (0.747 – 1.318)	0.754 (0.418 – 1.359)	1.249 (0.856 – 1.804)	0.658 (0.385 – 1.126)	1.196 (0.715 – 1.998)
Mixture of animals of different sectors	631	24	26	26	76	0.908 (0.668 – 1.235)	1.0	1.0	0.548 (0.307 – 0.978)	1.154 (0.679 – 1.961)
Floor										
Partly slatted	2230	66	105	131	302	1.225 (0.841 – 1.787)	1.821 (0.727 – 4.561)	1.123 (0.748 – 1.686)	1.114 (0.617 – 2.011)	1.294 (0.811 – 2.066)
Fully slatted	396	11	27	12	50	1.131 (0.711 – 1.796)	1.691 (0.581 – 4.927)	1.034 (0.623 – 1.716)	1.597 (0.808 – 3.154)	0.842 (0.456 – 1.554)
Others	300	5	13	16	34	1.0	1.0	1.0	1.0	1.0
Sanitary gap before new breeders in the pen										
No	2363	63	116	129	308	1.0	0.783 (0.464 – 1.320)	0.980 (0.725 – 1.324)	0.944 (0.621 – 1.435)	0.925 (0.666 – 1.285)
Yes	563	19	29	30	78	1.073 (0.821 – 1.402)	1.0	1.0	1.0	1.0
Feed										
Cobbs/rolls/nuts/pellets	842	35	59	60	154	1.0	2.347 (1.270 – 4.337)	2.302 (1.634 – 3.242)	1.0	1.0
Others	231	6	3	12	21	0.447 (0.276 – 0.723)	1.318 (0.505 – 3.440)	0.951 (0.524 – 1.723)	0.167 (0.052 – 0.537)	0.621 (0.366 – 1.051)
Meal/mash	1094	26	53	65	144	0.677 (0.529 – 0.867)	1.263 (0.664 – 2.402)	1.653 (1.176 – 2.324)	0.651 (0.443 – 0.955)	0.694 (0.512 – 0.940)
Porridge/liquid	759	15	30	22	67	0.433 (0.319 – 0.587)	1.0	1.0	0.506 (0.322 – 0.794)	0.387 (0.261 – 0.574)
Source of feed										
Exclusively own	1513	22	83	64	169	1.0	0.326 (0.199 – 0.535)	0.833 (0.657 – 1.057)	1.0	1.0
Purchased + mixture	1413	60	62	95	217	1.443 (1.163 – 1.790)	1.0	1.0	0.839 (0.599 – 1.177)	2.025 (1.539 – 2.666)
Potential Salmonella control substances added to water										
Not added	2540	65	137	131	333	1.0	0.346 (0.161 – 0.742)	1.268 (0.633 – 2.543)	-	-
Organic acid	198	5	8	11	24	0.914 (0.588 – 1.422)	0.338 (0.107 – 1.061)	1.140 (0.496 – 2.620)	-	-
Probiotic	36	4	0	5	9	2.209 (1.030 – 4.739)	1.741 (0.487 – 6.225)	1.934 (0.598 – 6.257)	-	-
Other	111	8	0	9	17	1.199 (0.706 – 2.035)	1.0	1.0	-	-
Use of antibiotics in the last 4 weeks in breeders										
No	1724	36	81	78	195	1.0	0.558 (0.355 – 0.855)	0.745 (0.585 – 0.948)	0.824 (0.588 – 1.155)	0.635 (0.485 – 0.831)
Yes	1177	42	64	75	181	1.425 (1.146 – 1.771)	1.0	1.0	1.0	1.0

Table 8: Multilevel univariable analysis for outcome variables (bold: $p < 0.05$)

Variables	Salmonella positive OR (CI 95%)	S. Typhimurium serotype-specific		S. Derby serotype-specific	
		S. Typhimurium OR (95% CI)	Other Salmonella OR (95% CI)	S. Derby OR (95% CI)	Other Salmonella OR (95% CI)
Sample Season					
Winter	1.068 (0.532 – 2.143)	1.093 (0.415 – 2.873)	1.014 (0.456 – 2.256)	0.710 (0.245 – 2.057)	1.273 (0.601 – 2.695)
Spring	1.167 (0.604 – 2.255)	1.691 (0.726 – 3.938)	1.157 (0.546 – 2.451)	0.859 (0.324 – 2.281)	1.505 (0.748 – 3.032)
Summer	0.609 (0.307 – 1.210)	0.682 (0.254 – 1.833)	0.641 (0.292 – 1.404)	0.559 (0.203 – 1.540)	0.833 (0.399 – 1.738)
Autumn	1.0	1.0	1.0	1.0	1.0
Type of holding					
Breeding holding	1.137 (0.672 – 1.923)	1.329 (0.662 – 2.670)	1.070 (0.586 – 1.953)	1.048 (0.481 – 2.282)	1.067 (0.606 – 1.878)
Production holding	1.0	1.0	1.0	1.0	1.0
Type of breeding and production holding					
Nucleus	1.0	1.0	1.0	-	-
Multiplier or supplier	3.0 (0.725 – 12.414)	0.755 (0.169 – 3.380)	11.435 (1.0 – 130.742)	-	-
Farrow to weaner	2.508 (0.6 – 10.48)	0.444 (0.093 – 2.122)	10.401 (0.904 – 119.616)	-	-
Farrow to grower	2.447 (0.566 – 10.575)	0.636 (0.130 – 3.105)	8.826 (0.746 – 104.416)	-	-
Farrow to finish	2.057 (0.488 – 8.661)	0.722 (0.159 – 3.281)	7.281 (0.627 – 84.559)	-	-
Outdoor production					
No	1.511 (0.208 – 11.006)	-	-	-	-
Yes	1.0	-	-	-	-
Total number of breeding pigs					
<100	0.215 (0.047 – 0.981)	0.373 (0.052 – 2.673)	0.181 (0.028 – 1.152)	0.258 (0.026 – 2.525)	0.222 (0.039 – 1.247)
100-399	0.537 (0.228 – 1.265)	0.500 (0.161 – 1.551)	0.596 (0.224 – 1.582)	0.430 (0.124 – 1.491)	0.663 (0.265 – 1.662)
400-999	0.692 (0.298 – 1.606)	0.707 (0.237 – 2.108)	0.745 (0.285 – 1.944)	0.768 (0.233 – 2.529)	0.718 (0.290 – 1.776)
>999	1.0	1.0	1.0	1.0	1.0
Gilt replacement policy					
>90% gilts homebred	1.0	1.0	1.0	1.0	1.0
10-90% gilts homebred	0.704 (0.304 – 1.631)	0.884 (0.297 – 2.630)	0.696 (0.263 – 1.840)	0.672 (0.164 – 2.752)	0.831 (0.352 – 1.964)
>90% gilts purchased	1.040 (0.612 – 1.767)	0.872 (0.420 – 1.810)	1.102 (0.603 – 2.014)	1.958 (0.904 – 4.239)	0.724 (0.405 – 1.295)
Boar replacement policy					
No boars on farm	1.0	1.0	1.0	1.0	1.0
>90% homebred	0.429 (0.233 – 0.790)	0.702 (0.305 – 1.614)	0.413 (0.207 – 0.825)	0.358 (0.147 – 0.874)	0.495 (0.259 – 0.947)
10-90% purchased	0.564 (0.171 – 1.860)	0.908 (0.189 – 4.364)	0.491 (0.124 – 1.942)	0.745 (0.144 – 3.861)	0.360 (0.088 – 1.478)
>90% purchased	0.346 (0.170 – 0.703)	0.50 (0.183 – 1.361)	0.316 (0.140 – 0.712)	0.353 (0.125 – 0.998)	0.366 (0.171 – 0.786)
Number of animals per pen					
=10	0.698 (0.470 – 1.034)	0.866 (0.438 – 1.713)	0.655 (0.420 – 1.022)	0.601 (0.325 – 1.111)	0.855 (0.539 – 1.357)
[11;15]	0.872 (0.567 – 1.342)	1.185 (0.565 – 2.485)	0.781 (0.477 – 1.279)	0.529 (0.266 – 1.050)	1.180 (0.702 – 1.981)
>15	1.0	1.0	1.0	1.0	1.0
The pen has direct access to outside					
No	1.091 (0.094 – 12.628)	-	-	-	-
Yes	1.0	-	-	-	-
Individual pen					
No	1.026 (0.763 – 1.379)	0.708 (0.403 – 1.243)	1.109 (0.797 – 1.544)	0.824 (0.505 – 1.344)	1.070 (0.754 – 1.518)
Yes	1.0	1.0	1.0	1.0	1.0
Age of the breeding sows					
Only gilts or mixed age	1.0	1.0	1.0	1.0	1.0
Without gilts	0.946 (0.704 – 1.271)	0.730 (0.422 – 1.263)	0.987 (0.708 – 1.377)	0.762 (0.468 – 1.239)	1.038 (0.736 – 1.465)

Table 8 (cont.): Multilevel univariable analysis for outcome variables (bold: $p < 0.05$)

Variables	Salmonella positive OR (CI 95%)	S. Typhimurium serotype-specific		S. Derby serotype-specific	
		S. Typhimurium OR (95% CI)	Other Salmonella OR (95% CI)	S. Derby OR (95% CI)	Other Salmonella OR (95% CI)
Sex of the breeding pigs					
Only sows	0.875 (0.464 – 1.648)	0.816 (0.292 – 2.282)	0.966 (0.460 – 2.030)	0.658 (0.226 – 1.916)	1.114 (0.524 – 2.366)
Boars or/and sows	1.0	1.0	1.0	1.0	1.0
Breeding sector room					
Replacement breeders	1.0	1.0	1.0	1.0	1.0
Gestation	1.277 (0.692- 2.358)	2.396 (0.821 – 6.992)	0.983 (0.482 – 2.005)	0.629 (0.223 – 1.775)	1.796 (0.876 – 3.684)
Maternity	1.079 (0.656 – 1.774)	0.901 (0.328 – 2.471)	1.010 (0.579 – 1.760)	0.783 (0.353 – 1.736)	1.238 (0.680 – 2.252)
Mating	0.909 (0.548 – 1.506)	1.809 (0.689 – 4.748)	0.702 (0.397 – 1.240)	0.579 (0.263 – 1.275)	1.202 (0.651 – 2.220)
Mixture of animals of different sectors	0.912 (0.531 – 1.569)	1.987 (0.744 – 5.304)	0.659 (0.353 – 1.230)	0.712 (0.308 – 1.648)	1.068 (0.553 – 2.065)
Floor					
Partly slatted	0.809 (0.472 – 1.386)	1.629 (0.513 – 5.171)	0.810 (0.449 – 1.461)	0.957 (0.402 – 2.277)	0.937 (0.488 – 1.799)
Fully slatted	0.583 (0.299 – 1.135)	1.515 (0.409 – 5.614)	0.571 (0.271 – 1.205)	1.371 (0.485 – 3.871)	0.510 (0.227 – 1.145)
Others	1.0	1.0	1.0	1.0	1.0
Sanitary gap before new breeders in the pen					
No	0.980 (0.659 – 1.457)	0.618 (0.324 – 1.181)	1.181 (0.749 – 1.863)	1.327 (0.692 – 2.543)	0.793 (0.505 – 1.247)
Yes	1.0	1.0	1.0	1.0	1.0
Feed					
Cobbs/rolls/nuts/pellets	1.0	1.0	1.0	1.0	1.0
Others	0.317 (0.120 – 0.840)	0.361 (0.089 – 1.466)	0.319 (0.102 – 1.0)	0.238 (0.044 – 1.279)	0.432 (0.153 – 1.222)
Meal/mash	0.549 (0.322 – 0.934)	0.475 (0.215 – 1.047)	0.580 (0.321 – 1.051)	0.618 (0.281 – 1.356)	0.622 (0.347 – 1.116)
Porridge/liquid	0.366 (0.201 – 0.668)	0.386 (0.162 – 0.924)	0.369 (0.184 – 0.145)	0.482 (0.191 – 1.215)	0.346 (0.177 – 0.677)
Source of feed					
Exclusively own	1.0	1.0	1.0	1.0	1.0
Purchased + mixture	1.515 (0.947 – 2.424)	2.861 (1.416 – 5.780)	1.221 (0.718 – 2.076)	0.771 (0.385 – 1.545)	1.969 (1.174 – 3.303)
Potential Salmonella control substances added to water					
Not added	0.918 (0.270 – 3.128)	0.326 (0.088 – 1.205)	1.035 (0.250 – 4.289)	-	-
Organic acid	1.167 (0.275 – 4.961)	0.478 (0.088 – 2.606)	1.067 (0.198 – 5.738)	-	-
Probiotic	0.794 (0.101 – 6.239)	2.204 (0.213 – 22.824)	0.373 (0.035 – 3.920)	-	-
Other	1.0	1.0	1.0	-	-
Use of antibiotics in the last 4 weeks in breeders					
No	0.866 (0.628 – 1.194)	0.633 (0.364 – 1.099)	0.980 (0.679 – 1.415)	1.092 (0.654 – 1.822)	0.761 (0.524 – 1.105)
Yes	1.0	1.0	1.0	1.0	1.0

4.3. Multivariable analyses

Variables classified as candidates in multilevel univariable analyses were considered in respective adjusted models and removal of least significant variables yielded models aggregated in Tables 9 and 10. Total number of breeding pigs, boar replacement policy, number of animals per pen, feed and source of feed were considered for multivariable analysis with outcome variable for *Salmonella* positivity; boar replacement policy, breeding sector room, feed and source of feed were considered for multivariable analysis with outcome variable for *Salmonella* Typhimurium serotype-specific positivity; boar replacement policy, number of

animals per pen, floor, feed and source of feed were considered for multivariable analysis with outcome variable for *Salmonella* Derby serotype-specific positivity. Interactions were tested between feed and source of feed but they were not included in the final models ($p > 0.05$). Random effects were significant in all final models and help to validate the multilevel approach made to these data.

All the final adjusted models got an Akaike Information Criterion (AIC) slightly lower than the saturate model: for *Salmonella* positivity model, saturate model AIC was 15,736.509 and final model AIC was 15,716.005; for *Salmonella* Typhimurium serotype-specific positivity model, saturate model AIC was 30,167.415 and final model AIC was 29,734.849; for *Salmonella* Derby serotype-specific positivity, saturate model AIC was 30,070.282 and final model AIC was 29,909.526.

Final model for *Salmonella* positivity retained (1) total number of breeding pigs: holdings with more than 999 breeding pigs have higher odds of being infected than the ones with 399 or less, (2) boar replacement policy: purchasing or homebreeding more than 90% of boars has a protective effect for *Salmonella* positivity and (3) feed: all types of feed show a protective effect when compared to cobs/rolls/nuts/pellets.

Final model for *Salmonella* Typhimurium serotype-specific positivity retained (1) boar replacement policy: purchasing or homebreeding more than 90% of boars has a protective effect for *Salmonella* positivity to other serotypes than Typhimurium and (2) feed: porridge/liquid has a protective effect for *Salmonella* Typhimurium positivity and porridge/liquid and others show a protective effect for *Salmonella* positivity to other serotypes than Typhimurium, both when compared to cobs/rolls/nuts/pellets .

Final model for *Salmonella* Derby serotype-specific positivity retained (1) boar replacement policy: purchasing or homebreeding more than 90% of boars has a protective effect for both *Salmonella* Derby positivity and to other serotypes positivity, (2) feed: all types of feed show a protective effect when compared to cobs/rolls/nuts/pellets for *Salmonella* Derby positivity and (3) source of feed: purchased or mixed origin feed shows a lower odds of *Salmonella* Derby positivity when compared to exclusively own source feed.

The global p values of all retained variables are inferior to 0.05 except for variable boar replacement policy in *Salmonella* Typhimurium serotype-specific model, that has a global p value of 0.084.

Table 9: Multilevel adjusted models for outcomes variables: *Salmonella* positivity and *Salmonella* Derby serotype-specific (bold: $p < 0.05$)

Variables	Salmonella positive				Derby-specific positive							
					Derby				Other serotypes			
	Coef	OR	OR CI 95%	p-val	Coef	OR	OR CI 95%	p-val	Coef	OR	OR CI 95%	p-val
Total nr of breeding pigs												
<100	-1.889	0.151	0.033 – 0.687	0.014								
100-399	-1.036	0.355	0.146 – 0.864	0.022								
400-999	-0.677	0.508	0.215 – 1.202	0.123								
>999	0.0	1.0	-	-								
Boar replacement policy												
>90% purchased	-1.071	0.343	0.166 – 0.706	0.004	-1.068	0.344	0.199 – 0.991	0.048	-0.965	0.381	0.176 – 0.825	0.014
>90% homebred	-0.982	0.374	0.201 – 0.697	0.002	-1.037	0.355	0.143 – 0.882	0.026	-0.805	0.447	0.231 – 0.867	0.017
10-90% purchased	-0.761	0.467	0.139 – 1.569	0.218	-0.347	0.707	0.136 – 3.688	0.681	-1.208	0.299	0.070 – 1.275	0.103
No boars on farm	0.0	1.0	-	-	0.0	1.0	-	-	0.0	1.0	-	-
Feed												
Porridge/liquid	-1.121	0.326	0.176 – 0.603	<0.001	-1.436	0.238	0.073 – 0.774	0.017	-0.739	0.478	0.223 – 1.024	0.058
Others	-1.447	0.235	0.086 – 0.647	0.005	-2.054	0.128	0.020 – 0.835	0.032	-0.852	0.427	0.143 – 1.272	0.126
Meal/mash	-0.555	0.574	0.335 – 0.983	0.043	-1.334	0.263	0.084 – 0.825	0.022	-0.129	0.879	0.421 – 1.838	0.732
Cobbs/rolls/nuts/pellets	0.0	1.0	-	-	0.0	1.0	-	-	0.0	1.0	-	-
Source of feed												
Purchased + mixture					-1.105	0.331	0.116 – 0.945	0.039	0.550	1.734	0.879 – 3.421	0.112
Exclusively own					0.0	1.0	-	-	0.0	1.0	-	-
Random Effects	Variance Estimate	Standard Error	CI 95%	p-val	Variance Estimate	Standard Error	CI 95%	p-val	Variance Estimate	Standard Error	CI 95%	p-val
At herd level	2.704	0.344	2.107 – 3.471	<0.001	3.852	0.550	2.911 – 5.096	<0.001	2.598	0.357	1.984 – 3.402	<0.001

Table 10: Multilevel adjusted models for outcomes variables: *Salmonella* positivity and *Salmonella* Typhimurium serotype-specific (bold: $p < 0.05$)

Variables	Salmonella positive				Typhimurium-specific positive							
					Typhimurium				Other serotypes			
	Coef	OR	OR CI 95%	p-val	Coef	OR	OR CI 95%	p-val	Coef	OR	OR CI 95%	p-val
Total nr of breeding pigs												
<100	-1.889	0.151	0.033 – 0.687	0.014								
100-399	-1.036	0.355	0.146 – 0.864	0.022								
400-999	-0.677	0.508	0.215 – 1.202	0.123								
>999	0.0	1.0	-	-								
Boar replacement policy												
>90% purchased	-1.071	0.343	0.166 – 0.706	0.004	-0.701	0.496	0.179 – 1.375	0.178	-1.125	0.325	0.143 – 0.736	0.007
>90% homebred	-0.982	0.374	0.201 – 0.697	0.002	-0.421	0.656	0.279 – 1.543	0.334	-0.954	0.385	0.191 – 0.775	0.008
10-90% purchased	-0.761	0.467	0.139 – 1.569	0.218	-0.228	0.797	0.158 – 4.005	0.782	-0.862	0.422	0.105 – 1.697	0.224
No boars on farm	0.0	1.0	-	-	0.0	1.0	-	-	0.0	1.0	-	-
Feed												
Porridge/liquid	-1.121	0.326	0.176 – 0.603	<0.001	-0.935	0.392	0.163 – 0.947	0.037	-0.989	0.372	0.185 – 0.747	0.005
Others	-1.447	0.235	0.086 – 0.647	0.005	-1.103	0.332	0.078 – 1.419	0.137	-1.236	0.291	0.091 – 0.931	0.037
Meal/mash	-0.555	0.574	0.335 – 0.983	0.043	-0.747	0.474	0.214 – 1.051	0.066	-0.577	0.561	0.309 – 1.019	0.058
Cobbs/rolls/nuts/pellets	0.0	1.0	-	-	0.0	1.0	-	-	0.0	1.0	-	-
Random Effects	Variance Estimate	Standard Error	CI 95%	p-val	Variance Estimate	Standard Error	CI 95%	p-val	Variance Estimate	Standard Error	CI 95%	p-val
At herd level	2.704	0.344	2.107 – 3.471	<0.001	2.493	0.426	1.783 – 3.485	<0.001	3.250	0.413	2.532 – 4.170	<0.001

5. Discussion

5.1. Validity of the study

The main purpose of this EU survey was to assess *Salmonella* prevalence in herds of breeding pigs through all the Member States (MS). There was also the intention of supplying MSs with information that may help to perceive risk factors for *Salmonella* infection in their holdings, pose hypothesis, accounting for their specificities, and therefore to improve/design their control programs. Nevertheless, sample size was not estimated to optimize this topic: it was expected that observations were strongly clustered within holding level, as well as within MS level and this reduces the power of the study to detect statistically significant associations. The factors investigated are only a part of what are considered to be important points: there is no guarantee that unsearched factors wouldn't exert a major or even confounding effect with the investigated ones. Moreover, a cross-sectional study design doesn't provide much information on causality mechanism, as only a single measure in time was made. It is not possible to assess whether a significant factor is important concerning *Salmonella* dissemination or instead

its introduction in the holding. The results achieved concerning possible risk factors should be read regarding this information.

Denmark reported a total of 3,457 holdings with breeding pigs, from whom 198 are breeding holdings and 3,259 are production holdings. Targeting at least 80% of the breeding pig holdings, 293 holdings were selected.

Questionnaire used were designed mainly with closed questions following requirements from Commission Decision 2008/55/EC and instruction was given to official veterinarians that applied it on holdings.

Laboratory testing of samples was made accordingly to a standard methodology (ISO, 2007).

Model frameworks chosen to model data revealed to be adequate as the random variance estimates were significant in all cases. This result shows that a multilevel approach is required in order to retain the significant effect that the holding level produces in the pen level. Goodness-of-fit assessed through decreasing AICs while reducing model supports suitability of the whole methodology used.

5.2. Outcome variable for *Salmonella* positivity

The analysis made with outcome variable *Salmonella* positive/negative retained in the final model the total number of breeding pigs in the holding, showing a significant protection effect for 399 or less breeding pigs when compared to more than 999 animals. Similar and also neutral results were found the same country (Benschop et al., 2008) and in other countries (Fosse et al., 2009). The policy used to replace boars is significant and a protection effect exists both for more than 90% purchased or homebred when compared to no boars on farm. Although EFSA found a similar association (EFSA, 2011), it wasn't able to enter final model because when interaction with holding type was considered, boar policy lost significance from $p=0.028$ to $p=0.051$. The type of feed shows that all possibilities have a protective when compared to pelleted dry feed cobbles/rolls/nuts/pellets. This association is consistent to current knowledge on high risk swine feed concerning our subject (Arguello, Rubio, & Carvajal, 2012).

5.3. Outcome variable for *Salmonella* Typhimurium serotype-specific

The analysis made with the outcome variable *Salmonella* negative/*Salmonella* Typhimurium/Other *Salmonella* serotypes than Typhimurium retains as significant boar replacement policy and type of feed. Findings related to boar management may be connected to artificial insemination technique. Feed effect may be related to the digestibility of type of feed matrix and how it changes the gut environment, creating better conditions for *Salmonella* to infect. As Typhimurium is not host adapted as is a virulent strain, infection should be eased.

Comparing with risk factors for *Salmonella* positivity, boar replacement policy and feed are some of the global findings that thus could be explored.

5.4. Outcome variable for *Salmonella* Derby serotype-specific

The analysis made with the outcome variable *Salmonella* negative/*Salmonella* Derby/Other *Salmonella* serotypes than Derby retains as significant boar replacement policy and the type of feed. Remarks made to the same factors on Typhimurium model are also valid here. The source of feed is only relevant for *S. Derby*, presenting a protective effect for “purchased and mixture” when compared to “exclusively own”. This finding is contrary to what has been seen in analysis investigating risk factors for *Salmonella* positivity.

5.5. Comparing outcome variable for *Salmonella* positivity of Denmark level and EU level

Comparing results of the model for *Salmonella* positivity to EFSA’s, the biggest difference seen is that less variables were retained in final model. One explanation is that the amount of data analyzed enables EFSA to have a bigger power in their analysis, a bigger chance of having enough observations to find as significant smaller effects that would be able to be found in every place. Nevertheless, findings at Denmark level are directed towards the comparable ones at EU level. An interesting fact lies on behavior of variable boar replacement policy, excluded in EU analysis with a borderline *p*-value (0.051) due to interactions included in the model with type of holding: univariable analysis reveals association in the form of a protective effects when comparing >90% purchased and >90% homebred to no boars on farm, just the same found in the present results.

5.6. Comparing outcome variable for *Salmonella* positivity of Denmark level and Portugal level

Comparing results of the model for *Salmonella* positivity to Portuguese results in the same line, the first thing to note is that some of the significant factors found are MS-specific, are part of the non-mandatory information that MS could collect in their own interest. The second remark is that the recoding of the remaining variables was made based in dataset characteristics, therefore comparability is compromised. Conclusions are the main point to be compared, given that this two MS are considered both as *Salmonella* high-prevalent in swine. Upon this, the main focus is the boar replacement policy factor; a protective effect is seen when a holding “without boars or >90% homebred” is compared to a holding where “more than 90% are purchased” and there is a risk effect when semen comes from a “boar from another herd” comparing to semen sourced from an “insemination center”. There seems to be better to work with a closed herd or

controlled semen, leading to think that there is a great chance that the newcomer boars will bring *Salmonella* issues to sows in that holding. The novelty when comparing both analysis is that when “>90% are purchased” there is also a protective effect comparing with “no boars on farm”. Can the origin of semen explain that?

5.7. Comparing outcome variable for *Salmonella* Typhimurium serotype-specific of Denmark level and Portugal level

Comparing results of the model for serotype-specificity concerning *Salmonella* Typhimurium to Portuguese results in the same line, it should be noted that two different statistical models were used, as referred previously. This has implication, for instance, in the interpretation of the power of the analysis: the Bayesian priors and Markov Chain Monte Carlo methods are fundamental in enabling the possibility of making a robust analysis upon a database in which the casuistic is low enough to compromise the validity of conclusions. Still, present data were analyzable under the assumptions of a generalized linear mixed model and that was the approach followed as a first step. Another detail that should be noted is that Denmark didn't registered any monophasic *S. Typhimurium*, contrary to Portugal that summed 7% of all isolates, and those were joined with *S. Typhimurium*. The Portuguese findings relative to risk factors for *S. Typhimurium* and monophasic were size of the herd, source of semen, number of pigs per pen and age of breeding sows (Table 1). It is plausible to say that this factors are connected to animals, their biology has a major role in providing explanation for this findings. Concerning to risk factors for other *Salmonella* serotypes than *S. Typhimurium* and monophasic, association was found for region of the herd, rodents control, production stage and source of feed (Table 1). With exception of production stage, associations reflect environmental aspects of production, factors that can be changed in a distinct magnitude of the ones for *S. Typhimurium* and therefore have different implications. In other way, if following a risk-based control approach in holdings, the most important serotype in terms of public health is *S. Typhimurium* and is connected to a bunch of more delicate factors, perhaps even more expensive to change. In present analysis, Danish data showed association for factors boar replacement policy for other serotypes than *S. Typhimurium* and type of feed, both for *S. Typhimurium* and other serotypes than *S. Typhimurium*. Results can derive for other confounding factors not collected in questionnaires. Although countries are very similar at prevalence level, their production systems are quite different in dimension, perspectives of market, among others.

An extra datum is that in Portuguese analysis, *S. Derby* is included on other serotypes category: it is the fourth most frequent serotype, after *S. Typhimurium*, *S. Rissen* and *S. London*

and sums 11% of total isolates. Category other serotypes has different meanings in both countries and that can be important in knowing what is exactly being compared.

In a recent study (Matiasovic et al., 2014), it is suggested that *Salmonella* Derby epidemiological situation may be similar to *Salmonella* Typhimurium.

6. Conclusions

The analysis conducted on this work about risk factors for *Salmonella* positivity is directed towards the conclusions achieved by EFSA in EU-level analysis. The differences that occurred were that less associations were found in Danish data and variable boar replacement policy was retained in Denmark's final model. A similar association was found in Portugal, concerning variable source of semen. The reasons that may explain this associations could not be unveiled by authors and may need further research. In general, results from Denmark and Portugal retains similar risk factors but comparability is compromised due to methodology used in analysis.

Despite results from Denmark and Portugal don't conflict with EU level results, they reveal differences and reinforce the need that each MS conduct their own analyses on their data. EFSA report on risk factors states that results achieved "should be used as a guide to inform general control measures".

Serotype-specific risk factor investigation revealed different risk factors for *S. Typhimurium* in Portugal and Denmark. Statistical modelling used is different in both approaches and that can be relevant to reveal such differences. Serotype-specific risk factors for *S. Typhimurium* and *S. Derby* in Denmark were not the same, although not much different. This supports theory that risk factors for *Salmonella* can be different accounting for serotype and also different accounting for reality under analysis. The similarities found between *S. Derby* and *S. Typhimurium* may support the theory that these serotypes behave in similar ways as stated before (Matiasovic et al., 2014) and may be further explored.

Analysis findings fit inside the "Pre-harvest control of *Salmonella*" in Denmark referred by Alban et al., 2012, meaning that control measures in place are accounting or approaching the factors revealed.

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ANNEXES

Annex 1: Data Dictionary

Table A1: Data dictionary

Variable names as received	Variable codes as received	Frequencies (%)	Final Variable	Definition of final variable
V002_HoldingID	V002_C_HoldingID		V002_C_HoldingID	Holding unique identification number
alpha-numeric	alpha-numeric	2930 (100.0)	alpha-numeric	
V003_SampDate	V003_C_SampDate			
alpha-numeric: dates in 2008	alpha-numeric	2930 (100.0)		
V003b_MonthSampl			V003_C_Season	Season of year when sample was collected
12, 1, 2		500 (17.1)	1	Winter
3, 4, 5		600 (20.5)	2	Spring
6, 7, 8		620 (21.2)	3	Summer
9, 10, 11		1210 (41.3)	4	Autumn
V005_HoldType	V005_C_HoldType		V005_C_HoldType	Type of holding sampled
production holding	2	1980 (67.6)	2	Production holding
breeding holding	1	950 (32.4)	1	Breeding holding
V006a_HbreedType	V006a_C_HbreedType		V006ab_BPType	Type of breeding or production holding sampled
nucleus	1	130 (4.4)	1	Nucleus
multiplier or supplier , nucleus	2	820 (28.0)	2	Multiplier or supplier
multiplier or supplier	2		2	
V006b_HprodType	V006b_C_HprodType			
farrow to finish	1	710 (24.2)	3	Farrow to finish
farrow to finish, farrow to grower	1		3	
farrow to finish, farrow to grower, farrow to weaner	1		3	
farrow to finish , farrow to weaner	1		3	
farrow to grower	2	520 (17.7)	4	Farrow to grower
farrow to grower , farrow to weaner	2		4	
farrow to weaner	3	750 (25.6)	5	Farrow to weaner
V013_OutProd	V013_C_OutProd		V006c_C_Hprod_InOut door	Holdings with outdoor stages at some point during pig's life
no	0	2880 (98.3)	0	No
yes	1	50 (1.7)	1	Yes
V007_Hsize	V007_C_Hsize		VL007_C_Hsize	Number of breeding pigs in holding at sample time
<50	1	150 (5.1)	1	<100
50-99	2		1	
100-399	3	1180 (40.3)	2	100-399
400-999	4	1330 (45.4)	3	400-999
>999	5	270 (9.2)	4	>999
V009_GiltReplPol	V009_C_GiltReplPol		V009_C_GiltReplPol	Gilt Replacement Policy
>90% gilts homebred	1	1520 (51.9)	1	>90% gilts homebred
10-90% gilts homebred	2	340 (11.6)	2	10-90% gilts homebred
>90% gilts purchased	3	1070 (36.5)	3	>90% gilts purchased

Table A1 (cont.): Data dictionary

Variable names as received	Variable codes as received	Frequencies (%)	Final Variable	Definition of final variable
V010_BoarReplPol	V010_C_BoarReplPo		V010_C_BoarReplPo	Boar Replacement Policy
no boars on farm	0	620 (21.2)	0	no boars on farm
>90% homebred	1	1420 (48.5)	1	>90% homebred
10-90% purchased	2	140 (4.8)	2	10-90% purchased
>90% purchased	3	750 (25.6)	3	>90% purchased
V011_PenID	V011_C_PenID		V011_C_PenID	Pen unique identification number
alpha-numeric	alpha-numeric	2930 (100.0)	alpha-numeric	
V012_NPigPen			VL012_NPigPen	Number of breeding animals in the pen at the time of sampling
numeric: from 10 to 320		1596 (54.5)	0	10
		571 (19.5)	1	[11;15]
		763 (26.0)	2	>15
V013_OutProd	V013_C_OutProd		V013_C_OutProd	Pigs on the pen have access to outside
no	0	2905 (99.1)	0	No
yes	1	25 (0.9)	1	Yes
V013b_IndivHousing	V013b_C_IndivHousing		V013b_C_IndivHousing	Individual housing: animals can't contact with each other freely
no	0	1031 (35.2)	0	No
yes	1	1899 (64.8)	1	Yes
V015_PigAge	V015_C_PigAge		V015_C_PigAgeBinary	Age of pigs in pen sampled: intention to identify gilts (female intended for breeding until she has her first litter)
no gilts	0	1351 (46.1)	0	No gilts
mixed age (at least 1 gilt)	1	1579 (53.9)	1	Mixed age(at least one gilt)/ All gilts
all gilts	2		1	
V016_PigSex	V016_C_PigSex		V016_C_PigSexBinary	Sex of pigs in pen sampled
female	2	2761 (94.2)	0	Female
male	1	169 (5.8)		Male/Mixed
mixed	3		1	
V017_ProdStage	V017_C_ProdStage		V017_C_ProdStage	Productive stage of pigs in sampled pen
maiden gilts	1	313 (10.7)	1	Maiden gilts (has never been in service area before)
pregnant pigs	2	290 (9.9)	2	Pregnant pigs
farrowing and lactating pigs	3	918 (31.3)	3	Farrowing and lactating pigs
service area	4	777 (26.5)	4	Service area
mixed	5	632 (21.6)	5	Mixed

Table A1 (cont.): Data dictionary

Variable names as received	Variable codes as received	Frequencies (%)	Final Variable	Definition of final variable
V018_Floor	V018_C_Floor		VL018_C_Floor	Type of floor in pen sampled
outdoors in fields or paddocks	1	302 (10.3)	2	Others
solid floor with wood shavings	3		2	
solid floor with compost	4		2	
solid floor with straw	5		2	
solid floor with deep straw	6		2	
solid floor without bedding	7		2	
other	10		2	
partly slatted floor	8	2232 (76.2)	0	Partly slatted floor
fully slatted floor	9	396 (13.5)	1	Fully slatted floor
V018b_ALLinOut	V018b_C_ALLinOut		V018b_C_ALLinOut	Sanitary gap: new pigs enter an empty and cleaned pen
no	0	2367 (80.8)	0	No
yes	1	563 (19.2)	1	Yes
V019_Diet	V019_C_Diet		V019_C_DietSimpler	Feed given to pigs on sampled pen
cobbs/rolls/nuts/pellets	1	843 (28.8)	1	Cobbs/rolls/nuts/pellets
cobbs/rolls/nuts/pellets, meal/mash	3	231 (7.9)	2	Others
cobbs/rolls/nuts/pellets, other	4		2	
cobbs/rolls/nuts/pellets, porridge/liquid	6		2	
meal/mash, other	11		2	
meal/mash, porridge/liquid	12		2	
other	13		2	
other, porridge/liquid	14	2	2	
meal/mash	8	1095 (37.4)	3	Meal/mash
porridge/liquid	15	761 (26.0)	4	Porridge/liquid
V019b_FeedOrigin	V019b_C_FeedOrigin		V019b_C_FeedOriginBinary	Source of feed given to pigs on sampled pen
commercial compound	1	1415 (48.3)	1	Purchased and mixed
commercial compound, home mill mixed	3		1	
commercial compound, home mill mixed, maize+commercial supplement	4		1	
home mill mixed, maize+commercial supplement	10		1	
home mill mixed, other	12		1	
maize+commercial supplement	13		1	
maize+commercial supplement, other	15		1	
other	16		1	
home mill mixed	7	1515 (51.7)	0	Home mill mixed

Table A1 (cont.): Data dictionary

Variable names as received	Variable codes as received	Frequencies (%)	Final Variable	Definition of final variable
V020_FeedWatSuppl	V020_C_FeedWatSuppl		V020_C_FeedWatSuppl_NA	Potentially Salmonella reducing substance systematically added to the feed or water of the animals in the pen
not added	1	2544 (86.8)	1	Not added
organic acid	3	198 (6.8)	3	Organic acid
other	8	111 (3.8)	8	Other
probiotic	11	36 (1.2)	11	Probiotic
unknown	15	41 (1.4)	NA	NA
V021_Antibiotic	V021_C_Antibiotic		V021_C_Antibiotic_NA	Use of antibiotics in feed, water or by injections during the last 4 weeks in the animals in the pen
"no treatment"	0	1727 (58.9)	0	No
"treatment"	1	1178 (40.2)	1	Yes
"unknown"	2	25 (0.9)	NA	NA
V023b_SampleType	V023b_C_SampleType			
swab	1	2879 (98.3)		
composite of at least 10 individual droppings	2	51 (1.7)		
V027_V039_V046_TestRes	V027_V039_V046_C_TestRes		V027_V039_V046_C_TestRes	Faecal sample testing result
negative	0	2540 (86.7)	0	Negative
positive	1	390 (13.3)	1	Positive
V031_040_048_SalmSerov	V031_040_048_C_SalmSerov		V054_ternary_ST	Salmonella negative against S. Typhimurium and other serotypes
S. Agona	18	8 (0.3)	0	Salmonella negative
S. Derby	34	145 (4.9)	1	Salmonella Typhimurium
S. Enteritidis	39	2 (0.1)	2	Salmonella other serotypes
S. Idikan	49	1 (0.0)	V056_ternary_SD	Salmonella negative against S. Derby and other serotypes
S. Infantis	53	62 (2.1)	0	Salmonella negative
S. Kedougou	56	1 (0.0)	1	Salmonella Derby
S. Livingstone	63	38 (1.3)	2	Salmonella other serotypes
S. London	66	13 (0.4)		
S. London var. 15	67	1 (0.0)		
S. Mbandaka	69	5 (0.2)		
S. Meleagridis	70	6 (0.2)		
S. Muenchen	74	9 (0.3)		
S. Newport	76	2 (0.1)		
S. Panama	82	5 (0.2)		
S. Rissen	85	4 (0.1)		
S. Typhimurium	95	82 (2.8)		
S. Uganda	96	2 (0.1)		
Salmonella untypeable	102	3 (0.1)		
(without serotype)	(no code)	1 (0.0)		

Annex 2: Database edition

Table A2: Variable recoding framework

Variable Recoding (as received → final variable)	Reference	Rationale
V003b_MonthSampl → V003_C_Season	(EFSA, 2011)	Assess <i>Salmonella</i> seasonality
V013_OutProd → V006c_C_Hprod_InOutdoor	(DTU-Food, 2010) page 16	Eventual importance of outdoor in infection at holding level
V006a_HbreedType + V006b_HprodType → V006ab_BPType	(EFSA, 2011)	Avoid data sparsity
V007_Hsize → VL007_C_Hsize	(EFSA, 2011)	Avoid low frequency categories
V012_NPigPen → VL012_NPigPen	Author's opinion	Compare increases in number of animal; avoid creation of low frequency categories
V015_PigAge → V015_C_PigAgeBinary	(Correia-Gomes et al., 2013)	Compare to absence of gilts; avoid creation of low frequency categories
V016_PigSex → V016_C_PigSexBinary	(Correia-Gomes et al., 2013; EFSA, 2011)	Avoid creation of low frequency categories
V018_Floor → VL018_C_Floor	Author's opinion	Avoid creation of low frequency categories by grouping for general faecal draining capacity
V019_Diet → V019_C_DietSimpler	(EFSA, 2011)	Consider all multiple uses of feed as "Others"
V019b_FeedOrigin → V019b_C_FeedOriginBinary	(Correia-Gomes et al., 2013)	Compare all options with home sourced feed

Table A3: Variables excluded from analysis

Variables excluded from analysis
V001_Country
V003_SampDate
V004_comment
V008_HLoc/ V008_C_HLoc
V012b_H_excl_pensize
V014_Diarrhoea/ V014_C_Diarrhoea
V022b_DaysAMadmsamp
V023_V037_V043_SampleID/ V023_V037_V043_C_SampleID
V023b_SampleType/ V023b_C_SampleType
V023c_SampleSchema/ V023c_C_SampleSchema
V024_V044_SampleWght/ V024_V044_C_SampleWght
V026_V038_V045_TestDate/ V026_V038_V045_C_TestDate
V026b_V038b_V045b_DelayBactSamp
V033_041_049_SEntPhType/
V033_041_049_C_SEntPhType
V034_042_050_STypPhType/
V034_042_050_C_STypPhType
V035_V051_PhTypMeth/ V035_V051_C_PhTypMeth

Annex 3: Models

Model 1: Outcome variable for *Salmonella* positivity

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

$$\Pr(Y) = p_{ih};$$

$i = 1, \dots, 2930$ for samples; $h = 1, \dots, 293$ for holdings

$$\text{logit}(p_{ih}) = \alpha + \beta_k \text{ herd variables}_h + \beta_k \text{ pen variables}_{hi} + \beta_k \text{ herd variables}_h * \text{herd variables}_h + \beta_k \text{ pen variables}_{hi} * \text{pen variables}_{hi} + \beta_k \text{ herd variables}_h * \text{pen variables}_{hi} + b_h$$

Model 2: Outcome variable for *Salmonella* Typhimurium serotype-specific positivity

$$Y_{khi} = \begin{cases} 0 & (\text{no } Salmonella) \\ 1 & (S. Typhimurium) \\ 2 & (\text{Other Serotypes}) \end{cases}$$

where

$$\Pr(Y_{khi}) = p_{khi};$$

$k = 0, 1, 2$ for outcome variable; $h = 1, \dots, 2930$ for samples; $i = 1, \dots, 293$ for holdings and

$$\sum_{k=0}^3 p_{khi}$$

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

$$\text{logit}(p_{khi}) = \alpha_k + \beta_{jk} \text{ herd variables}_{ih} + \beta_{jk} \text{ pen variables}_h + \beta_{jk} \text{ herd variables}_{ih} * \text{herd variables}_{ih} + \beta_{jk} \text{ pen variables}_h * \text{pen variables}_h + \beta_{jk} \text{ herd variables}_{ih} * \text{pen variables}_h + b_{2ik}$$

where j is the number of explanatory variables.

Note that with the use of random effects, the probabilities of $Y = 0, 1$ or 2 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 $Y=0$ (no *Salmonella*) and all the results from each of the categories $Y=1$ and 2 are compared to the reference category.

Model 3: Outcome variable for *Salmonella* Derby serotype-specific positivity

$$Y_{khi} = \begin{cases} 0 & (\text{no } Salmonella) \\ 1 & (S. \text{ Derby}) \\ 2 & (\text{Other Serotypes}) \end{cases}$$

where

$$\Pr(Y_{khi}) = p_{khi};$$

$k = 0, 1, 2$ for outcome variable; $h = 1, \dots, 2930$ for samples; $i = 1, \dots, 293$ for holdings and

$$\sum_{k=0}^3 p_{khi}$$

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

$$\text{logit}(p_{khi}) = \alpha_k + \beta_{jk} \text{ herd variables}_{ih} + \beta_{jk} \text{ pen variables}_h + \beta_{jk} \text{ herd variables}_{ih} * \text{herd variables}_{ih} + \beta_{jk} \text{ pen variables}_h * \text{pen variables}_h + \beta_{jk} \text{ herd variables}_{ih} * \text{pen variables}_h + b_{2ik}$$

where j is the number of explanatory variables.

Note that with the use of random effects, the probabilities of $Y = 0, 1$ or 2 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 $Y=0$ (no *Salmonella*) and all the results from each of the categories $Y=1$ and 2 are compared to the reference category.

Annex 4: Univariable analyses

Table A4: Variable significant at a 0.05 level in a univariable analysis and in a multilevel univariable analysis using outcome variable for *Salmonella* positivity

Univariable analysis	Multilevel univariable analysis
Season	Non-significant
Type of breeding and production holding	Non-significant
Total numbers of breeding pigs in holding	Non-significant
Boar replacement policy	Boar replacement policy
Number of animals per pen	Non-significant
Feed	Feed
Source of feed	Non-significant
Use of antibiotics in the last 4 weeks in breeders	Non-significant