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Assessing pig farm biosecurity measures for the control of *Salmonella* on European farms

Richard P. Smith[1], Hannah E. May[1], Elke Burow[2,3], Marina Meester[4], Tijs J. Tobias[4], Elena-Lucia Sassu[5], Enrico Pavoni[6], Ilaria Di Bartolo[7], Christopher Prigge[5,8], Dariusz Wasyl[9], Jacek Zmudzki[9], Arvo Viltrop[10,11], Imbi Nurmoja[11], Veit Zoche-Golob[3], Giovanni L. Alborali[6], Romina Romantini[12], Arkadiusz Dors[9,13], Gergana Krumova-Valcheva[14], Ivana Koláčková[15,16], Guiseppe Aprea[12] and Hristo Daskalov[14]

[1]Department of Epidemiological Sciences, Animal and Plant Health Agency – Weybridge, Addlestone, UK; [2]Department of Biological Safety, German Federal Institute for Risk Assessment (BfR), Berlin, Germany; [3]Ministry of Agriculture, Environment and Climate Protection (MLUK), Potsdam, Germany; [4]Department Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands; [5]Division for Animal Health, Austrian Agency for Health and Food Safety (AGES), Mödling, Austria; [6]Istituto Zooprofilattico Sperimentale della Lombardia e Dell'Emilia Romagna, Brescia, Italy; [7]Emerging Zoonoses Unit, Istituto Superiore di Sanità, Rome, Italy; [8]University of Veterinary Medicine Vienna, Vienna, Austria; [9]National Veterinary Research Institute, Puławy, Poland; [10]Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia; [11]National Centre for Laboratory Research and Risk Assessment, Tartu, Estonia; [12]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise Giuseppe Caporale, Teramo, Italy; [13]Department of Preclinical Sciences and Infectious Diseases, Faculty of Veterinary Medicine and Animal Sciences, University of Life Sciences, Poznan, Poland; [14]National Diagnostic and Research Veterinary Medicine Institute, Sofia, Bulgaria; [15]Veterinary Research Institute, Brno, Czech Republic and [16]Department of Public Health, Masaryk University, Brno, Czech Republic

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Corresponding author:
Richard P. Smith;
Email: Richard.p.smith@apha.gov.uk

Abstract

Salmonella spp. is a common zoonotic pathogen, causing gastrointestinal infections in people. Pigs and pig meat are a major source of infection. Although farm biosecurity is believed to be important for controlling *Salmonella* transmission, robust evidence is lacking on which measures are most effective. This study enrolled 250 pig farms across nine European countries. From each farm, 20 pooled faecal samples (or similar information) were collected and analysed for *Salmonella* presence. Based on the proportion of positive results, farms were categorised as at higher or lower *Salmonella* risk, and associations with variables from a comprehensive questionnaire investigated. Multivariable analysis indicated that farms were less likely to be in the higher-risk category if they had '<400 sows'; used rodent baits close to pig enclosures; isolated stay-behind (sick) pigs; did not answer that the hygiene lock/ anteroom was easy to clean; did not have a full perimeter fence; did apply downtime of at least 3 days between farrowing batches; and had fully slatted flooring in all fattener buildings. A principal components analysis assessed the sources of variation between farms, and correlation between variables. The study results suggest simple control measures that could be prioritised on European pig farms to control *Salmonella*.

Introduction

Salmonella spp. are zoonotic pathogens that cause gastrointestinal infections, or more serious infections, in humans and are the second most common zoonotic organism reported in humans in the European Union after *Campylobacter* spp. [1]. *Salmonella* is typically transmitted from animals to humans through contaminated food of animal origin and via direct or indirect contact with animal faeces. Although poultry (and eggs) are typically identified as the most common source of zoonotic *Salmonella* infection in Europe, pigs are the second most common source [1, 2]. Controlling and limiting *Salmonella* on pig farms is deemed important for reducing the risk of zoonotic transmission, alongside proper slaughterhouse practices focusing on hygiene to reduce the risk of foodborne infection with *Salmonella.* One challenge for on-farm control is that infection in pigs is usually subclinical and so it is difficult for farmers to identify infected pigs, and target controls on these animals, to lower the probability of spread within a farm or stop infected pigs from entering a *Salmonella*-negative herd.

Successfully implemented biosecurity measures can help to control *Salmonella* on farms in two ways: by limiting the introduction of *Salmonella* onto a presumably infection-free farm, and by minimising the transmission of *Salmonella* between pens and buildings within an infected farm. Understanding which on-farm biosecurity measures have a substantial effect in limiting the transmission of pathogens is important to help advise farmers on how to control specific pathogens. Biosecurity audit protocols (e.g. Biocheck by the University of Ghent, https://

biocheckgent.com/en) are useful tools to identify sub-optimal practices on pig farms, and contribute to the awareness of farmers and farm advisors on the shortcomings of specific external and internal biosecurity measures [3]. External biosecurity affects the probability of transmission onto a farm, whereas internal biosecurity affects the probability of spread within a farm. Subsequently, farm-specific improvements can be defined that can contribute to reducing the risk associated with infectious pathogens.

There have been several previous studies on *Salmonella* in pig farms that have investigated different biosecurity measures. These include farms using a limited number of supplying herds or breeding their own replacements [4], rodent or wild bird control [5], staff using farm-specific clothing, and having fencing around the farm perimeter [6]. Reducing contact between pigs and faecal contamination of housing and equipment has been shown to be effective in some studies in which cleaning and disinfection of pens have reduced *Salmonella* contamination [7] and using slatted flooring systems to separate pigs from faeces has reduced *Salmonella* infections [8]. Reducing movement, contact and mixing between pig groups may also be important, and batch (all-in/ all-out) production systems reduce the probability of *Salmonella* infection in pigs when compared to continuous flow systems [9], as long as multiple sources of pigs are not used. Studies have also investigated the role of staff and visitor movements, the sharing of farm equipment and farm waste as routes of transmission that biosecurity measures can control [10]. However, these results are not always reproduced in other studies. Factors may not be consistently investigated, and sometimes contradictory or non-significant results are detected, for example, a study where the use of building-specific clothing and footwear was not found to be a significant protective factor [11] or where the effect of cleaning and disinfection between batches of pigs was inconclusive [12]. In general, pig farm biosecurity studies over the last decade have not attempted to progress these findings and test comprehensive lists of biosecurity measures.

The aim of this study was to conduct a structured and comprehensive European-wide risk factor analysis for the identification of farm biosecurity measures that are relevant for limiting the probability of introduction and transmission of *Salmonella* within pig farms. This was in order to determine which biosecurity measures should be prioritised for *Salmonella* control on-farm.

Methods

Study design

A cross-sectional study was performed on 250 farms from nine European countries between January 2020 and November 2021.

Study population and farm enrolment

Pig farms in nine European countries, participants of the BIOPIGEE consortium, were used for this study: Austria (AT), Bulgaria (BG), Czech Republic (CZ), Germany (DE), Estonia (EE), Italy (IT), the Netherlands (NL), Poland (PL) and the United Kingdom (UK). It was aimed to recruit 20–50 farms within each country for this study, targeting the most common commercial pig farm types present in each of the participating countries. Three farm types were selected and were defined as (1) fattening (farms that do not include breeding pigs), (2) breeding (farms that do not include growing or fattening pigs) and (3) farrow-to-finish units. To limit bias, it was agreed that small holdings (defined as those within the 5th percentile of herd size within countries based upon census data and European statistics) would not be enrolled in the study. Outdoor farms were included for countries where these farms represented a commercial enterprise and all the pigs for a pig type (e.g. breeding or fattening pigs) were kept outdoors. For breeding farms, only herds which supply pigs that go to farms to finish them for slaughter were targeted for inclusion, while nucleus/multiplier herds, which only produced replacement breeding pigs for breeding farms, were excluded. Finally, SPF (Specific Pathogen Free) farms were excluded from the study. Nucleus/multiplier and SPF herds were excluded because they are few in number and are special classes of farm, which are difficult for visitors to get permission to access and sample. None of the participating farms were using *Salmonella* vaccination.

The selection of farms in each country aimed (where possible) to try and select farms with both expected high and low *Salmonella* risk, based upon informed veterinary services or/and monitoring systems. However, in most instances the farm selection was simply a convenience sample of those farms willing to participate. To recruit the farms, an invitation letter and farmer consent form were prepared and translated into the language of each participating country. These documents covered the following parts: (1) introduction of the project, (2) benefits of the project, (3) description of participation and (4) agreement to participate.

Questionnaire design

The questions included in the questionnaire related to external and internal (primary and secondary) biosecurity measures applied on pig farms. Tertiary biosecurity measures which improve the resistance of animals to pathogens (e.g. probiotic treatment or vaccination) were not included. The selection of questions was guided by literature searches and expert opinions on *Salmonella* relevant measures. The questionnaire included questions on housing and flooring systems; presence of other animals; fencing; staff and visitor washing and clothing procedures; cleaning and disinfection of vehicles, equipment, feeding and drinking systems, and buildings; origin of pigs and semen; use of quarantine; wildlife problems; and the mixing of pigs. The questionnaire also included questions on farm characteristics, production performance and costs of veterinary services, which were used for other parts of the project and are not discussed further. In addition, questions were also included that were deemed relevant for the transmission of hepatitis E virus on farms, to be used in a parallel project. The completed questionnaire (see Supplementary Material) was translated into the native language of participating farmers.

The questionnaire was accessible in an electronic form through a mobile application (mobile Ingress Software, keyingress 5.0), or in paper format, and included mainly closed or semi-closed questions and two free-text comments fields at the end of the biosecurity and economic part. It was completed by project staff, subcontractors or veterinary practitioners, in an interview style, with the farmers or veterinarians taking care of the pigs. Although it was planned to complete the questionnaire face-to-face, due to COVID-19 restrictions, some of these were completed over phone conversations.

Sample collection

The optimal number of faecal samples, and which age groups were to be sampled, was determined from consultations with *Salmonella* experts. The sampling frame was chosen as a cost-efficient method to identify and stratify farms as either higher or lower risk for *Salmonella*. Ultimately, it was determined that 20 pooled faecal

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samples (with 10 pinches of separate faeces per pooled sample) were to be collected at each farm. Each pooled sample originated from a single pen, unless a pen contained less than six pigs, whereby it was collected from across two pens of similarly aged pigs. Sampled pens were selected representatively across the age groups and buildings present on each farm, following the specific protocol for each farm type. Twenty pooled samples provided sufficient sensitivity to detect at least one positive sample even if the within-herd prevalence was as low as 2% and would estimate an expected farm prevalence of 10% [13, 14] with 5.5% variance, 95% confidence and perfect test performance (Sample size to demonstrate freedom using pooled testing [15]).

The sampling protocol was adapted for the different farm types in the study. On fattening units, fresh faecal samples were collected from fattening pigs at around 4 months of age. In the case of breeding farms, the preferred target group was gilts (80% of samples taken) and, to a lesser extent, dry sows (20% of samples taken). On a farrow-to-finish farm, the number of samples to be collected was split between the fattening pigs around 4 months old (10 pooled faecal samples); dry sows (2 pooled faecal samples); and gilts (8 pooled faecal samples). This was agreed as optimal by the experts in the study team and according to the results from previously conducted studies on the occurrence of *Salmonella* in pig farms (unpublished results from [16]). On any farm with breeding pigs, where the number of gilts was too small to usefully collect the required number of samples (e.g. where the required number of samples was greater than the number of pens or number of gilts), replacement samples were collected from the dry sows.

In addition, it was recommended that faecal samples were best collected from as many different pens as possible. Samples were taken from fresh faeces, preferably immediately after defecation, with each sample containing a minimum of 10 g of faeces. Gloves and sampling material were changed between pooled samples. The sample was homogenised by mixing and then 25 g was collected from the sample to test for *Salmonella*.

Sample testing

The project experts expressed a common position regarding the transport of samples and their storage. It was determined that fresh faeces would be transported to the laboratory in a cool box (to stop temperatures exceeding 25°C) and tested as soon as possible (within 48 hours after collection) following guidance taken from ISO 13307:2013 standard. All laboratories from the project team (one per participating country, apart from two in IT) agreed that *Salmonella* detection, identification and serotyping would follow the ISO 6579-1 standard. After the completed tests, all laboratories entered their results into the sample record forms containing the following data: farm ID, sample collection date, farm type, sample ID number, pig type (dry sow, gilt, finisher), estimated age of finishers (months), barn/outdoor enclosure ID, pen/outdoor section ID, number of pinches collected per pooled sample, estimated number of pigs in pen, whether tested after 48 hours of collection (Y/N), sample frozen or not, final positive or negative *Salmonella* result, and *Salmonella* serovar.

Non-study Salmonella *results*

For NL and EE participating farms, which had completed the same study questionnaire, it was not possible to obtain faecal samples specific for this study, but information concerning *Salmonella* status was available from established routine *Salmonella* surveillance programmes or opinion and information from the farm's veterinary practitioner. In the NL, results from a serological *Salmonella* surveillance scheme were used. Scheme data was provided for 2019 as the biosecurity questionnaires from the selected farms were collected early in 2020. Every 4 months (quadrimester), serum from 12 fattening pigs was collected, either at the farm or at slaughter during exsanguination, and tested by ELISA (IDEXX Laboratories, Inc., Westbrook, ME) for *Salmonella* antibodies. When 20% or less of the samples had an optical density (OD) above 40 then the score was 1; >20% to 40% equalled a score of 2; and 40% or greater score was 3. The resulting scores of three quadrimesters (12 months) were summed to determine the farms' category. If the sum of three quadrimesters was 3 or 4 (so the farm had at least scored 1 on two occasions and not higher than a single score of 2), then the farm was in category 1. If the sum of three quadrimesters was 5, 6 or 7 (a maximum of one occasion of a score of 3), then the farm was in category 2. If the sum of three quadrimesters was 8 or 9, then the farm was in category 3.

In EE, 3 of the 32 farms were sampled and tested specifically for this study as described above. Whereas for nine farms, *Salmonella* test results from the National programme in 2021 were used. In that case, 3 to 14 faecal samples from each farm were collected, depending on farm size and type. For breeding and farrow-to-finish farms, individual faecal samples were taken from breeding pigs, whereas for fattening farms, pooled faeces samples (consisting of 5–10 pinches from faeces taken from the same pen) from the floor were collected from fattening pig pens. All samples were tested using method ISO 6579-1. The remaining 20 farms provided information on *Salmonella* status based upon interviews with the farm's veterinarian and their knowledge of the farm's historical *Salmonella* status.

Data analysis and risk categorisation

To generate a binary outcome response for *Salmonella* risk that would allow the analysis of all farms together whilst controlling for some known confounders, a cut-off value to define higher and lower-risk status was agreed upon by the project team after assessing a plot of the proportion of positive samples from all the farms. However, to account for deviations from the sampling protocol and differences between sampling the three farm types, the sample results were assessed against the variables of whether *Salmonella* samples were tested within 48 hours of collection, the age of finishers, number of pools collected per sample, types of pigs sampled (finishers, gilts and sows) and season of sample collection. These fixed effects variables were all entered into a single pooled sample-level mixed-effects multivariable logistic regression, with the outcome of whether *Salmonella* had been detected or not and Farm ID added as a random effect. The odds ratios of the significant variables ($P < 0.05$) were then assessed to weight their effect on the proportion of samples positive per farm. After weighting the sample results, a score was provided for each farm in order to determine whether it was higher or lower risk.

The questionnaire responses were reviewed and cleaned, with potentially incorrect information checked with the farmer or the local project team. Unanswered questionnaire data were reported as 'Missing', or 'Not Applicable' where appropriate, to allow these records to be retained in a multivariable model, whilst accounting for these missing records as separate from the other responses. Where possible, variables related to outdoor farms were incorporated into related variables for indoor farms (e.g. fencing of farm sites). Variables with multiple categories were condensed and

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recoded where it made biological sense and the number of observations in a category was below 20. The four continuous variables were plotted and assessed by eye to determine any trends or groupings within the data, and if relevant, categorical variables based on these variables were generated.

Multivariable analysis, using a forwards-stepwise logistic regression (logistic command) model, was used to evaluate the associations between the binary *Salmonella* risk status and the exposure variables of interest, whilst accounting for the presence of other variables included in the model. At the initial stage, a screening univariable stage was used and all variables with a *P*-value higher than 0.25 were excluded before the multivariable modelling phase. In subsequent steps, the significant variable (P-value < 0.05) that most improved the fit of the model (lowest Akaike Information Criterion (AIC)) was included until a step was reached where no further variables were significant or could improve the model fit. Collinearity within the final model was assessed using a correlation matrix of the model parameter estimates (estat vce, correlation command). If a variable was found to have collinearity with another variable of 0.75 or greater, then only the variable with the lowest *P*-value which had the largest effect on model fit was retained in the final model.

As it was expected that there would be some correlation between the variables which might be hard to control for in a typical logistic regression, Principal Components Analysis (PCA) was used to assess multicollinearity. PCA condenses the total number of variables and produces new, uncorrelated variables (principal components (PCs)). Each PC represents a proportion of variance from the explanatory variables. All variables that had a *P*-value under 0.25 at the univariable screening stage were used for PCA. The variables related to PCs with an Eigenvalue of at least two were assessed to provide more information on the correlation between variables entering the final multivariable logistic regression. All data analyses were conducted using Stata 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

Sensitivity analysis

To assess the effect of different sampling and test methodologies in EE and NL, the final logistic regression model was run without data from NL and EE and the direction and magnitude of effect estimates were evaluated.

Results

Farm population

A total of 250 questionnaires were received between January 2020 to November 2021 from farms that had corresponding *Salmonella* results, with between 18 and 38 questionnaires received from farms in each of the nine European countries (Table 1). Most farms were either farrow-to-finish (46.0%) or fattener farms (31.2%), and only five (2.0%) farms were outdoor units, and these came from two countries (IT and UK).

Salmonella results by farm

A total of 199 farms were sampled specifically for this study, whereas the remaining 51 farms (from EE and NL) were risk categorised based upon routine *Salmonella* surveillance programmes/veterinary advice. From the 199 study sampled farms, *Salmonella* was detected in pooled fresh faeces from 69 farms (34.7%). At pooled sample level, *Salmonella* was found in 305 out of 3,977 tested (7.7%). The number of positive samples per positive farm ranged from 1 to 18 (5–90%). The average percentage of positive samples was 21.9% for the positive farms. Results by farm type showed that farrow-to-finish farms had, on average, a significantly lower number of samples positive for *Salmonella* (5.8%, compared to 9.3% for breeding farms and 9.7% for fattener farms; Chi-squared $P < 0.001$), whereas a (non-significantly) higher number of breeding farms were positive for *Salmonella* (42.5% compared to 38.2% of fattener farms and 29.8% of farrow-to-finish farms; Chi-squared $P = 0.291$) (Supplementary Table 1). For the outdoor herds, sampled in UK and IT (4 and 1, respectively), three were positive with an average of 10.0% samples positive in total.

From the 305 positive samples, 297 provided serovar results, with 23 different serovars detected and 1–3 serovars detected per positive farm. The most commonly detected serovars were *S. Derby* (33.0%), monophasic *S. Typhimurium* (28.6%) and *S. Infantis* (10.4%). Five other serovars had 6–26 isolates detected, and the

Table 1. Population of 250 pig farms from nine European countries, summarised by farm production type and whether indoor or outdoor production

Country	Farrow-to-finish	Breeder	Fattener	Indoor	Outdoor	Total
Austria	14	4	2	20	0	20
Bulgaria	32	1	0	33	0	33
Czech Republic	23	3	4	30	0	30
Germany	9	9	12	30	0	30
Estonia	7	5	20	32	0	32[a]
Italy	5	17	16	37	1	38
Netherlands	7	0	12	19	0	19[a]
Poland	14	1	15	30	0	30
United Kingdom	9	4	5	14	4	18
Total	120	44	86	245	5	250
% of total	48.0	17.6	34.4	98.0	2.0	100.0

[a]Not all farms from these countries were sampled specifically for this study.

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remaining 15 serovars were represented by five or fewer isolates (Supplementary Figure 1 and Supplementary Table 2).

Risk factor model results and principal components analysis

To account for differences in sampling and testing between farms (e.g. deviations from the sample protocols, seasonality, different sampling regimes between farm types), an analysis was completed prior to the risk factor analysis. This initial sample-level multivariable analysis indicated that *Salmonella*-positive results were significantly more likely in samples from gilts (OR = 2.19) than other sampled pig types, and from fattening pigs aged 3 months old (OR = 4.03) in comparison to older fattening pigs. These results were used to weight sample results from these two categories to ensure a more consistent appraisal of risk from the various farm types included in the study. The results for each farm were adjusted so that a positive sample from a finisher of 3 months was scored as 0.25 and gilts were scored as 0.5, whereas all other positives were scored as 1.0. A cut-off of 20% (4 positives out of 20) of samples positive per farm was set by the project team to define a higher-risk farm status, whereas farms that were negative or had less than 20% of samples positive were defined as lower risk. Adjustment of the sample results affected only three farms that went from higher to lower-risk status.

From the 51 farms that used other information to determine risk category, 8 of the 19 NL farms had a surveillance scheme score of 2 and were classed as higher-risk (none had a score of 3). In EE, 6 of 32 farms were determined to be higher-risk based on four having 20% or more samples being *Salmonella* positive, and two being classed as higher-risk based on interviews (both reported recent *Salmonella* detection/ clinical issues).

In total, 41/250 (16%) farms were categorised as higher-risk, and these were from 7 of 9 participating countries (AT and DE only had lower-risk category farms). The higher-risk categorisation was subsequently used as the outcome in the stepwise multivariable logistic regression model-building process. The model-building results indicated that a relatively large number (97 out of 250) of the questionnaire variables were associated ($P < 0.25$) with farms being higher risk for *Salmonella* at the univariable screening step (Supplementary Table 3). Some groups of related questions on biosecurity were not significant even at the univariable screening stage ($P \geq 0.25$). These were the presence and location of hygiene locks, whether people shower before entering barns, and whether clothes and footwear are changed or cleaned between barn sections, whether external vehicles have access to clean areas within the farm perimeter, whether vehicles are cleaned and disinfected before collecting pigs, and whether specific ramps/loading areas are used when loading or unloading pigs. Other non-significant groups of variables included treatment of private water sources and cleaning and disinfection of the drinking system, and whether there were signs of the presence of rats/mice, whether bedding is protected from wildlife/pests and whether cats and dogs have access to barns, and factors related to carcase storage. The final group of non-significant questions was the use of cleaning and disinfection in the anteroom/hygiene lock (room at the entrance of the barn where work clothes and footwear are changed and stored), in the corridors of barns and on farm equipment.

PCA was completed on those variables which had *P*-value < 0.25 at the univariable screening stage. This produced 99 PCs, with nine PCs having an eigenvalue above two, and these explained 60% of the total variation (each individually explaining between 31.2% and 2.2%) (Table 2). When these nine PCs were added as explanatory variables into a single multivariable logistic regression model for the *Salmonella* higher-risk outcome, then three were found to be statistically significant (PC1, PC3 and PC6).

Each of the three significant PCs included small amounts of variance from a large number of explanatory variables. The results indicated the magnitude and direction of associations of each variable with the PC. For example, PC1 was positively associated with farms using boars as a semen source. Each variable in the PCs had a similarly small magnitude detected (range − 0.318 to 0.330, median 0.01; Table 3). Results with a magnitude of ≥0.17 or ≤−0.17, representing a value between the 3rd and 4th quartile of the absolute magnitude values for variables for all three PCs, were selected by the authors as the variables of greatest importance. PC1 was mainly representing six variables related to breeding farms, such as source of semen, use of all-in/ all-out management of the farrowing area and four variables related to breeding, farrowing or suckler pigs not being present. PC3 was mainly representing whether breeding pigs came from one or multiple sources within a year, four variables related to the *Salmonella* status of purchased pigs, and three variables related to the use and presence of a quarantine area. The other variables were whether feed storage areas are cleaned at least once a year and whether rodent baiting points are located at pig buildings. PC6 mainly represented whether public or private water sources were used, whether cleaning

Table 2. Results from Principal Components Analysis of the nine principal components with eigenvalues above two, which represented pig farm biosecurity data, and logistic regression results of these components against farm-level *Salmonella* risk status

							95% confidence intervals	
Component	Eigenvalue	Difference	Proportion	Cumulative	Odds ratio	*P*-value	Lower	Upper
PC1	31.22	25.329	0.312	0.312	1.095	0.005	1.028	1.167
PC2	5.89	0.124	0.059	0.371	1.070	0.420	0.908	1.259
PC3	5.77	1.409	0.058	0.429	0.831	0.028	0.704	0.980
PC4	4.36	1.088	0.044	0.472	1.008	0.943	0.821	1.236
PC5	3.27	0.272	0.033	0.505	1.227	0.121	0.947	1.590
PC6	3.00	0.499	0.030	0.535	0.762	0.008	0.623	0.932
PC7	2.50	0.209	0.025	0.560	0.930	0.578	0.720	1.201
PC8	2.29	0.143	0.023	0.583	0.912	0.457	0.715	1.163
PC9	2.15	0.173	0.022	0.604	1.033	0.805	0.796	1.341

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Table 3. Representation of individual biosecurity questions contribution to three principal components (PCs) that were significantly associated with higher risk of *Salmonella* on pig farms

Variable name	PC1	PC3	PC6
Where is semen sourced from? Boars on another farm	*0.170*	0.007	0.002
Do breeding pigs come from one or multiple sources within a year?	0.042	*−0.318*	0.127
If you purchase breeding pigs, do they have equal or higher Salmonella status than your own pigs?	0.025	*−0.264*	0.095
If you purchase weaning pigs, do they have equal or higher Salmonella status than your own pigs?	−0.034	*−0.262*	0.123
If you purchase fattening pigs, do they have equal or higher Salmonella status than your own pigs?	−0.055	*−0.179*	0.103
If you purchase weaning pigs, do they have equal or higher HEV status than your own pigs?	−0.042	*−0.275*	0.122
Are purchased breeding gilts moved to a quarantine area?	0.092	*−0.259*	0.107
Are purchased breeding boars moved to a quarantine area?	0.078	*−0.254*	0.079
Drinking water – Public network	−0.009	−0.022	*−0.311*
Drinking water – Private well/borehole	0.002	0.041	*0.330*
Feeding storage cleaned at least once a year?	−0.003	*0.172*	0.160
Are farrowing barn sections always managed all-in/all-out?	*0.170*	0.016	−0.031
C&D procedures – Breeding area not present at farm	*0.172*	0.007	−0.021
C&D procedures – Farrowing area not present at farm	*0.173*	0.010	−0.037
C&D procedures – Quarantine area checked by hygienogram	−0.050	−0.069	*−0.205*
C&D procedures – Quarantine area not present	0.114	*−0.172*	*0.176*
Flooring system is in the barn sections per age group? Breeding area not present	*0.171*	0.014	−0.018
Flooring system is in the barn sections per age group? Suckler area not present	*0.171*	0.017	−0.026
Flooring system is in the barn sections per age group? Fattener area part slats	0.027	−0.086	*−0.206*
Flooring system is in the barn sections per age group? Fattener area deep litter	0.029	−0.002	*−0.212*
Are disposable gloves worn when manipulating carcasses and/or are hands washed and disinfected	0.025	−0.023	*−0.175*
Is pest control against wild birds carried out by a professional company?	−0.053	−0.118	*−0.176*
Are rodent baits used in the surroundings of the farm enclosures?	−0.003	*0.172*	0.032

The results indicate the magnitude and direction of associations of the variable with the PC (e.g. PC1 was positively associated with farms using boars as a semen source). The results identify those variables with weightings of ≥0.17 or ≤−0.17 for each PC (in italics).

procedures within the quarantine area are checked by a hygienogram and whether the quarantine area is present (same variable as for PC3 but for the other (positive) direction), use of partly slatted or deep litter flooring in fattener areas, use of disposable gloves/ handwashing when manipulating carcasses and whether a professional company was used to control wild birds.

After stepwise selection for the risk factor analysis, the final logistic regression model included seven variables which were significant ($P < 0.05$), whilst also accounting for the effect of the other variables in the model (Table 4). The model was estimated to explain almost 30% of the variation (pseudo $R^2 = 0.287$). The results indicated that farms had lower odds of being in the higher-risk category for *Salmonella* if they were in the category of <400 sows present, used rodent baits, isolated stay-behind (sick) pigs from healthy ones, answered no to whether the hygiene lock/ anteroom was undamaged and easy to clean, did not have a full perimeter fence (either no fence or a partially interrupted fence), did apply downtime of at least 3 days in the farrowing rooms between batches and had fully slatted flooring in all fattener buildings. The largest correlation detected between these variables was between answers to the number of sows present and downtime in the farrowing rooms (−0.193).

Due to NL and EE using non-study *Salmonella* results, a sensitivity analysis was applied by running the final multivariable model without the farms from these two countries. The resulting model indicated that five variables produced similar results to the full model but two variables were no longer significant ($P > 0.05$): 'Are stay-behinds always isolated from the healthy ones' and 'What kind of flooring system is in the barn sections for fatteners' (-Supplementary Table 4). None of the variables in the model had a different direction of the Odds Ratio from that detected in the original model.

Discussion

This study utilised an extensive list of individual biosecurity measures, which had been selected based upon evidence of effect from published literature or expert opinion, and a robust population of pig farms from across Europe. The results have highlighted the scale of *Salmonella* infections in pigs and the importance of some biosecurity measures in controlling *Salmonella* on pig farms in Europe. *Salmonella* was present on over a third of the studied farms and the serovar information suggests that 40% of the positive samples were strains of human health concern (*S. Typhimurium* and its monophasic variants or *S. Enteritidis*). However, the most commonly detected serovar was *S. Derby,* which is not typically associated with human illness. This also highlights that maintaining focus on improving hygiene at slaughter is also needed to ensure any

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Table 4. Results of multivariable logistic regression of *Salmonella* risk categorisation on pig farms (n = 250)

Variable	Category	Higher risk	Lower risk	% Higher risk	Odds ratio	95% Confidence interval		*P*-value
						Lower	Upper	
No. of sows	0	18	47	27.7	1.000			
	<400	4	81	4.7	0.099	0.026	0.371	0.001
	401–1000	7	30	18.9	0.686	0.184	2.558	0.574
	1000+	4	19	17.4	1.407	0.278	7.124	0.680
	Not known	8	32	20.0	0.539	0.165	1.757	0.305
Are rodent baits used in the surroundings of the farm enclosures?	No	18	45	28.6	1.000			
	Yes	22	147	13.0	0.265	0.105	0.668	0.005
	NA	1	17	5.6	0.151	0.013	1.778	0.133
Are stay-behinds always isolated from the healthy ones (in physically separated hospital area / or by euthanasia)?	No	19	55	25.7	1.000			
	Yes	21	147	12.5	0.280	0.112	0.700	0.006
	N/A	1	6	14.3	0.338	0.030	3.815	0.381
Is the floor in each anteroom / hygiene lock even / without damages and thereby easy to clean and to disinfect?	Yes	37	164	18.4	1.000			
	No	4	45	8.2	0.156	0.040	0.601	0.007
Are all farm buildings/ fields surrounded by a perimeter fence?	Single or double fenced	31	123	20.1	1.000			
	No	9	64	12.3	0.191	0.062	0.589	0.004
	Partly interrupted	1	22	4.3	0.093	0.010	0.853	0.036
Which standard cleaning and disinfection procedures are used in the farrowing barns between batches? – Downtime (at least 3 days)	No	34	130	20.7	1.000			
	Yes	7	79	8.1	0.252	0.079	0.803	0.020
What kind of flooring system is in the barn sections for fatteners?	Any other flooring	23	66	25.8	1.000			
	Only solid floor	3	29	9.4	0.209	0.042	1.047	0.057
	Only full slats	9	69	11.5	0.293	0.103	0.833	0.021
	Not present/missing	6	45	11.8	0.352	0.102	1.214	0.098

improvements at farm are not compromised when the pigs are slaughtered [17]. A wide range of different biosecurity measures had a significant association, in the multivariable risk factor model, with farms being at higher risk from *Salmonella*.

The multivariable results indicated which of the studied biosecurity variables may be the most important, with the use of rodent bait, not mixing stay-behinds with healthy pigs, ensuring downtime of at least 3 days after cleaning and disinfection in the farrowing area, and only using fully slatted flooring in the fattener areas, being significantly associated with lower *Salmonella* risk and being retained in the final model. This agrees with previous research which has shown the importance of rodent control on pig farms in limiting *Salmonella*, although evidence of the specific effect of baiting was lacking [5, 18, 19]. Typical advice to farmers is not to mix recovered pigs from sick pens, or slow-growing pigs, with batches of healthy pigs, as these are expected to be more likely to excrete *Salmonella* and infect the healthy pigs [20]. Additionally, the effect of mixing pigs from different groups has also been studied and shown to be a risk factor. A small, controlled US study showed a significant increase in *S. Typhimurium* in weaned pigs that were mixed and described changes to their behaviour, with less eating or rooting [21].

Studies of cleaning and disinfection have typically focused on fattener pens and the selection and use of cleaning products, whereas in this study it was downtime in the farrowing room that was identified as associated with *Salmonella* risk in the final model. Downtime between batches allows for effective cleaning, disinfection and drying between batches. A study of hepatitis E virus in pigs identified that downtime of less than 4 days in the nursery was associated with increased seroprevalence, but published evidence for *Salmonella* is lacking [22].

The use of fully slatted flooring in the fattener buildings was found to be protective for *Salmonella* and that has been shown in a previous study [8]. Schwartz [23] suggested that with a fully slatted floor, the contaminated faeces would flow away much faster and therefore had a lower probability of infecting susceptible pigs in the pen than with a partly slatted floor. However, fully slatted flooring has been shown to have an impact on pig welfare, which is also a consideration to take into account, and the effectiveness of the slats is also reliant on regular emptying of the pit below the slats [6]. Interestingly, farms that used only solid flooring were also similarly protected, although this result was only approaching significance. As these types of flooring are very different, this might suggest that the association was more related to a combination of factors, such as the management and cleaning of the flooring, rather than just the flooring itself [24].

Some of the other identified variables may be proxies for combinations of other factors. The sow herd size question may be

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related to the higher risk of *Salmonella* on farms that do not have sows (i.e. fattener sites) and also represent the lower risk of *Salmonella* in farms provided by Austria which generally had smaller sow herd sizes in this study. The addition of sow herd size into the model resulted in the farm type being not significantly associated with *Salmonella* and appeared to account for the variation related to farm type. As indicated in this study, fattener farms had greater proportions of positive samples than the other two farm types which contained breeding pigs, and this concurs with other European studies [19, 25]. The sample results also indicated that *Salmonella* was more likely to be detected in younger pigs (3-month-old finishers and gilts) rather than older pigs which are likely to have been already exposed to *Salmonella,* and to have developed immunity, which agrees with previous research [26, 27].

The variable related to 'easy-to-clean' flooring in hygiene locks/anterooms may have been conflicted by those farms where a hygiene lock was not present but other similar types of control measures were used instead, and even if they were present it would have relied upon them being cleaned effectively to reduce the *Salmonella* risk.

The presence of a perimeter fence helps to prevent uncontrolled access to the farm, and stops incursions of large wildlife (e.g. wild boar), and it has previously been found to be a useful biosecurity practice for *Salmonella* control [28]. However, at the time of the study, it was not used as a standard in many major pig-producing countries in Europe. The identification in this study that lacking a complete perimeter fence was a protective factor may indicate that this variable was a proxy for other factors, such as those related to smaller or fattener-only farms (69% of farms that did not have perimeter fences either had no sows or had <400 sows).

Clusters of variables were detected as non-significant, even though they had been expected to be associated with *Salmonella* control from previous research or expert opinion. These mainly related to cleanliness procedures related to staff and external vehicles, water quality, pest presence and control, and cleaning and disinfection of the hygiene lock, corridors and farm equipment. The failure to detect associations could be related to an artefact of the study population, differences in effects on individual study farms, or a lack of statistical power to detect weaker associations with *Salmonella.* In other cases, it could be that the variables are heavily influenced by compliance (e.g. whether staff always comply with changing their clothes and boots before entering a pig building) which was not assessed in this study.

The PCA helped to identify the many sources of variation between the 250 farms, although (as would be expected) a large proportion of the PCs were not significantly associated with the higher-risk category for *Salmonella.* Due to the expected interconnection of biosecurity measures related to their control of *Salmonella*, PCA was selected as a useful, exploratory analysis to help infer the underlying correlation and clustering of the variables. This was complementary to the multivariable model, which could highlight the association of variables with *Salmonella* risk, but relies on highly-correlated variables being removed from the model. From the three PCs that were associated with *Salmonella*, the results suggest that the use of rodent baiting points around the farm enclosures and the flooring types in the fattener areas may have had a degree of correlation on the farms in which they were present. This was because both of these variables had a 'higher' degree of magnitude of explained variation within the same PC. The other variables were not likely to be correlated. It should also be noted that the variables within the final model did not have substantial covariance detected between them. This analysis demonstrates the usefulness of the PCA in defining the correlation in the variance structure of the dataset, which has helped inform the degree of correlation of variables within the final model. Another point of interest was that the PC that explained the most amount of farm variation (PC1), and was associated with *Salmonella* higher-risk, represented questions related to the presence/absence of breeding pigs, farrowing areas and sucklers (unweaned piglets). This may relate to the identification of the sow herd size variable in the multivariable model and that farms without sows had differences in biosecurity measures when compared to fattener farms.

There were a number of known limitations in the study which may have introduced bias. Only a relatively small number of samples was used to determine farm *Salmonella* status, although the sample size calculations and strategy should have allowed for the effective determination of categories of risk. Although the criteria for higher and lower-risk categorisation was selected by the study team (based upon examining the results and knowledge of typical farm prevalence) and this may have introduced bias, analytical methods were used to try and account for identified biases and apply a methodological approach to identify the cut-off for higher-risk farms. The inclusion of farms from NL and EE may also have introduced classification bias, due to these not being sampled and risk categorised in the same way as the others. Generally, the samples from these countries represented fewer individual pigs than those sampled specifically for the study, which may also have affected their categorisation. The use of serological samples as used in NL may have a different sensitivity of detecting evidence of *Salmonella* infection than a culture of faecal samples. Considering a higher probability of finding *Salmonella* infections by immunological tests, an *a priori* higher risk may have been attributed to participating farms. However, as the NL risk definition was based on results from three samplings from the previous year and the proportion with higher versus lower-risk status was more or less comparable to other countries, the authors believe the used risk categorisation can be defended. Moreover, the sensitivity analysis which excluded the NL and EE farms, did not result in any change in the direction of effect and so bias is assumed to be limited. Still, excluding the NL and EE farms resulted in a smaller study population and therefore reduced statistical power by which confidence intervals became wider and two variables appeared not to be significantly associated with *Salmonella* risk anymore.

Although the study population covered a large number of European commercial pig farms, it should be noted that the process of farm recruitment may have induced selection bias and there were differences in the farm types provided from each country and from some regions within countries, which may limit the comparison between countries and thus such comparisons were not made. Participating farms may have been more interested in *Salmonella* control and already applying good practice, or more likely to have a problem with *Salmonella*, than those that rejected to join the study thereby contributing to observation bias. Although the recruitment of farms aimed to have higher and lower-risk farms within each country, this was not achieved for two of the countries. Additionally, the *Salmonella* situation in each country may be very different and may have affected recruitment and the results of the study. For example, a farm in a country that has a relatively low prevalence of *Salmonella* may have been given a lower-risk status even though the biosecurity practices were sub-optimal, as transmission from other farms was less likely. The effect of country could have been controlled for by using a random effect. In initial testing, the addition of

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country into the model explained some of the variation in the outcome but reduced the ability to detect biosecurity measures with small effect sizes, and subsequently it was decided not to include it in the model. Moreover, it should be noted that the addition of country into the final model did not alter the results substantially. Another potential source of selection bias in farm type was through outdoor farms, although they provided a very small population in the study and the outdoor-specific biosecurity questions could not be analysed in the model due to data sparsity. The low number of included outdoor farms, from only two of the countries, limits the generalisability of the study results to mainly European indoor farms.

The study has indicated there are a number of biosecurity measures that could be prioritised on European pig farms to help control *Salmonella*. Although some represent structural changes and requirements (e.g. fully slatted flooring, which is typically applied to new buildings, or the potential need for additional farrowing barn space on commercial units to allow for greater downtime between batches in a barn), others should reflect lower cost options, like improving rodent control and restricting the mixing of stay-behind pigs with other groups of pigs. These control options are likely to also contribute to the control of other pathogens. It should be noted that the control options identified through the final model are likely to be the most important for the control of *Salmonella* on European pig farms.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0950268823001115.

Data availability statement. The questionnaire used in the study is available on request and the anonymised (without country identifiers) study data are also available on request.

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Competing interest. The authors declare no conflicting interests.

Ethical standard. No procedures were performed that fall within the definition of animal experimentation and thereby the study was exempt from ethical evaluation for use of animals in research. All participating farmers consented to participation and the sharing of anonymised data. Where required, this was reviewed by a local ethical review board.

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