Differences in risk factors for Salmonella serotypes in breeding pigs in Portugal

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Abstract

The EU Regulation No 2160/2003 imposes a reduction of the prevalence of Salmonella in food producing animals, like pigs. The Member States Salmonella serotypes prevalence varies. So could also vary the risk factors association with different Salmonella serotypes. The aim of this study is to assess if these differences are present in the Portuguese pig production system. The data used in the study refers to the baseline survey for the prevalence of Salmonella in breeding pigs in Portugal. A total of 1670 pen fecal samples, from 167 herds, were tested. Of these 170 samples were positive to Salmonella. The serotypes found were grouped under two groups for the purpose of this study, as follows: 27% S. Typhimurium or serotype 4,5,12:i:-, 73% other serotypes. Along the samples collection a questionnaire about the herd management and potential risk factors was applied. As data follows a hierarchical structure (pen samples – first level - nested in herds – second level) a multinomial multilevel analysis of the dataset was carried out using generalized linear mixed models (GLMM) with Markov chain Monte Carlo methods. Three categories for the outcome variable were specified: i) no Salmonella, ii) serotype Typhimurium or serotype 4,5,12:i:-, iii) other serotypes. Comparing to "no Salmonella" as reference the significant associations ($p<0.05$) found for "serotypes Typhimurium or serotype 4,5,12:i:-" were: mixed age in the pen, herds with 203 or more breeding pigs, pen samples with more than 10 animals/pen. For the "other serotypes": control of rodents, region of the country, semen from other sources than insemination centers, maternity pens versus mating pens, and feed from external or mixed source. A control plan design to reduce the prevalence of Salmonella should take these results in consideration to improve effectiveness.

Introduction

Salmonella is one of the major causes of food-borne disease in the European Union (EU) in the past years (EFSA, 2010). With the aim to control this agent the EU approved legislation (EU Regulation No 2160/2003) that imposed a reduction on the prevalence of this agent in food production animals, like pigs. To set the target of this reduction for each country it was decided to carry on baseline surveys in the EU to estimate the prevalence of Salmonella sp. in some food production animals. In pigs the baseline study was done at abattoir level (collection of lymph nodes of pigs slaughtered) and at herd level (collection of pen fecal samples of breeding pigs). These studies show that the prevalence of Salmonella positive holdings with breeding pigs in the European Union was 31.8% (28.7% for breeding holding and 33.3% for production holdings). Also the results showed that the prevalence of different serotypes varies between countries. For instance in Portugal 9.1% of the breeding holdings were positive to Salmonella Typhimurium and 33.3% were positive to other serotypes than Typhimurium and Derby, while in Ireland these numbers were 17.5% of prevalence for the both cases (EFSA, 2009). So we have different countries with different profiles in terms of serotype prevalence. This should be taken in consideration in control programs at herd level as they could improve the effectiveness of these programs because we might have differences in risk factors for different serotypes. Along the sample collection it was also collected information regarding management practices and potential risk factors. Some of the known risk factors in the literature are linked to: 1) biosecurity measures, 2) herd management practices, 3) feeding practices, 4) health disorders among others (Fosse et al., 2009). But all these known risk factors did not take in consideration possible differences between serotypes. We wonder if there are differences between risk factors for different serotypes or groups of serotypes. The aim of the study was to search for potential risk factors for the shedding among two different groups of serotypes of Salmonella sp. using pen fecal samples from breeding pig farms representative of the Portuguese reality.

Material and Methods

The sampling frame, the diagnostic testing methods, the sample collection procedures, and the timelines of this cross sectional study were specified in the Commission Decision 2008/55/EC. The target population was holdings constituting at least 80% of the breeding pig population in the Member State. The target population was 4522 herds with a total of 204 584 breeding pigs and 1 827 533 pigs in total (known population in 2007). These herds were divided by Regions. In each region herds with 50 or more breeding pigs, breeding holdings and production holdings were identified. The sample was calculated using expected prevalence of 50%, desired confidence level of 95%, accuracy of 7.5% and then applied a finite population correction factor, with an increase in 10% for each group. The sample size was formed by 174 swine herds. The choice of herds to sample was random and proportional to the distribution of herds along the regions of the country. The samples were collected between November 2008 and January 2009 by the herd veterinary assistant. The samples were sent to laboratory for detection of Salmonella (using method described by Annex D of ISO 6579). The Salmonella strains isolated from positive samples were serotyped by the national Reference laboratory for Salmonella according to Kaulfmann-White scheme Along the collection of the sample a questionnaire was applied to collect information about the herd management and potential risk factors. The variables collected concern pen and herd data, like for example: type of housing, number of animals that contributed to the sample, if it was detected diarrhea in the last three months, production phase, floor type, region of the country, number of breeding pigs, biosecurity measures among others.

Before statistical treatment some variables were recoded into new variables with lesser categories to have a reasonable sample size in each category and other variables were merged when biological arguments allowed this procedure. The outcome variable is the result to the presence of Salmonella in each sample, and was classified in three categories: i) no Salmonella, ii) serotype Typhimurium or serotype 4,5,12:i:-, and iii) other serotype. Given the similar characteristics between serotype Typhimurium and serotype 4,5,12:i- one group was created; the remaining serotypes were merged together because we could not analyze each serotype individually given the low number of cases per serotype. After this treatment we found that, among the 170 positive pen fecal samples, 27% were positive to S. Typhimurium or serotype 4,5,12:i:-, and 73% were positive to the remaining serotypes. As the data follows a multilevel structure, pen fecal samples (first level) nested in swine herds (second level) and the data were analyzed using a generalized linear mixed model (GLMM). We used a Monte Carlo Markov Chain (MCMC) method applied to GLMM described in the package MCMCglmm (Hadfield, 2010) of R free software (R Development Core Team, 2010). The outcome variable follows a categorical distribution with a logit link function. The regression slopes of fixed effects and the random effects were assessed for each separated category in the outcome variable. The final adjusted multivariable models were manually built using a backward and forward elimination process. The results then were converted to odds ratio (OR) and the 95% OR credible interval (OR CI) were calculated. The first 5000 samples were discarded as the burn-in period and the following 500 000 samples were used for posterior inference, with a thin interval of 10. The convergence was assessed by visual inspection of time series plots. Priors for Bayesian multinomial multilevel regression were expressed for fixed effects as a multivariate normal distribution with zero mean vector and a diagonal variance matrix with large variances (1e+10), for residuals the priors matrix were constraint to one for variance and 0.5 to the covariance as is recommended in bibliography for a categorical outcome (Hadfield, 2010). For random effects the prior was 0.5 to all the (co)variance matrix. The fit of the model was assessed by calculating the deviance information criterion (DIC) (Hadfield, 2010).

Results

A total number of 1670 samples were tested, belonging to 167 herds. Out of these samples 170 were positive to Salmonella detection, which belongs to 76 herds. Among the positive samples 23% were Salmonella Typhimurium positive followed by Salmonella Rissen (19%). The final results of the multilevel multivariable model are shown in Table 1 for the different outcomes. The coefficients and the corresponding OR are adjusted for all variables in the model. The DIC of the final model was 950.

Table 1: Final multinomial multilevel model, with coefficient, standard deviation (SD), OR and 95% credible interval (CI) for outcome serotypes Typhimurium or 4,5,12:i- and other serotypes.

Variable	Typhimurium or 4,5,12:i-				Other serotypes			
	Coefficient	SD	OR	95% OR CI	Coefficient	SD	OR	95% OR CI
Region of the herd								
Alentejo	Reference	-		.	Reference	-		.
Center	-1.2	1.6	0.3	0.01-6.5	1.6	0.7	4.9	1.2-22.6
Lisbon and Tagus Valley	-0.4	1.2	0.7	0.06-7.8	1.0	0.6	2.8	0.8-10.7
North	0.4	1.9	1.5	0.03-66.7	2.8	0.9	16.4	3.1-104.6
Number of breeding pigs								
<203	Reference	-		.	Reference	-		.
≥203	2.3	1.0	10.0	1.6-109.9	0.5	0.4	1.7	0.8-3.9
Source of semen								
Center for artificial insemination - CAI	Reference	-		-	Reference	-		.
Own boar+CAI	0.4	0.9	1.4	0.2-8.4	1.2	0.5	3.3	1.3-8.4
Boar from another herd	4.2	1.8	69.4	3.0-3904	1.7	0.9	5.5	1.0-33.1
Control of rodents								
No	Reference	-		.	Reference	-		.
Yes	-2.7	1.9	0.06	0.001-1.7	-2.2	0.7	0.1	0.02-0.4
Number of animals/pen								
=10	Reference	-		.	Reference	-		.
>10	1.7	0.8	5.4	1.1-30.6	0.7	0.4	2.0	0.9-4.6
Age of the breeding sows								
Only gilts or gilts and others	Reference	-		.	Reference	-		.
Without gilts	-2.0	0.8	0.1	0.03-0.57	0.2	0.3	1.3	0.7-2.5
Breeding sector room								
Mating	Reference	-		.	Reference	-		.
Gestating	0.1	0.6	1.1	0.4-3.5	-0.2	0.4	0.8	0.4-1.6
Mixture of animals of different sector	-0.08	1.8	0.9	0.02-31.8	1.0	0.8	2.6	0.5-11.7
Maternity	-1.2	0.7	0.3	0.1-1.2	-1.1	0.4	0.3	0.2-0.8
Replacement breeders	-1.2	1.2	0.3	0.02-2.8	0.2	0.7	1.2	0.3-4.8
Source of feed								
Exclusively own	Reference	-		.	Reference	-		.
Not exclusively own	0.8	1.3	2.2	0.2-33.1	2.2	0.7	9.3	2.5-44.7

Discussion

It can be seen from the analysis of Table 1 that there are different risk profiles for the two groups of Salmonella created. Concerning the category "other serotype" the risk associations were the following: region of the herd (samples from herds in the North and Center Region have higher odds of being positive than samples from herds in the Alentejo Region, probably because herds in the Center and North regions are more closed together); purchase semen from another herd or to use semen from own boar are risk factors when compared to the purchase of semen from insemination centers (where the quality and safety of semen is higher), this association has not been reported in literature, probably because that in the majority of the countries the semen comes from insemination centers; and pens where the pigs feed are not exclusively home produced (linked to exotic serotypes like the ones that are isolated in commercial feed), similar association was also found in other study ((Benschop et al., 2008). The protective associations found for "other serotype" were: control of rodents (the role of rodents in the transmission of this agent was also highlighted in others studies) (Skov et al., 2008) and maternity pens compared to mating pens (this could be justified by the hormonal changes in the sow at mating) (Nollet et al., 2005). For category "Typhimurium or 4,5,12:i-" the risk associations found were linked to the size of the herd: 203 or more breeding pigs in the herd (similar association was found for Salmonella sp. in finishers) (Poljak et al., 2008) and the number of animals/pen (the greater the number of animals in the pen the easier is the transmission of infection between pigs). A protective association, pens without gilts, was found (older pigs with more resistance to infection). In this

category "Typhimurium or 4,5,12:i:-" the purchase of semen from another herd was also considered a risk factor but the wide credible interval (probably because of the high odds ratio and also the relatively small number of positive pen fecal samples in this variable category) indicates that this association should be a matter of further studies. Furthermore when this variable was removed from the analysis the other risk associations found remained statistically significant.

Conclusion

The majority of risk associations found were different between the groups of serotypes and as we had 13.8% herds with at least one sample positive to serotype Typhimurium or serotype 4,5,12:i:-, and the rest of the 31.7% herds positive to other serotypes, these should be taken in consideration when implementing a control program to Salmonella sp. as we have different herd risk profiles. To achieve a reduction on the prevalence, the measures of future control program should be cost-effective and adapted to country characteristics and serotypes. In this context this study gave valuable information that should be incorporated in future control plans for this agent in breeding pigs in Portugal.

Acknowledgments

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