

# Risk associations for presence of *Salmonella* sp. in pen samples of breeding pigs in Portugal using binomial multilevel models

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## Abstract

As *Salmonella* is one of the major causes of food-borne disease in the European Union (EU), EU approved legislation to achieve a reduction of the prevalence of this agent in the pig sector. To set the target for this reduction in each country it was decided to carry out baseline surveys in the EU to estimate the prevalence of the agent. The dataset analyzed in this work refers to the cross sectional baseline survey on the prevalence of *Salmonella* in breeding pigs in Portugal. A total of 1 670 pen fecal samples from 167 herds were submitted to fecal culture. Of these samples 170 were positive to *Salmonella*. Along with the collection of samples a questionnaire was applied to collect information about the herd management and potential risk factors. As data follows a multilevel structure, pen fecal samples (first level) nested within swine herds (second level) a multilevel analysis was performed using generalized linear mixed models (GLMM). The outcome variable was presence/absence of *Salmonella* in the pen sample. The results show significant associations ( $p < 0.05$ ) at herd level: North Region versus Alentejo Region (OR=3.86), rodents control (OR=0.23), more than 90% of boars home-raised or no boars versus more than 90% of boars from an external source (OR=0.54), semen bought from other herd versus semen bought at insemination centers (OR=4.47) and herds with 170 or more sows (OR=1.82); at pen level: maternity pens versus mating pens (OR=0.39), feed from external or mixed source versus home source (OR=2.81) and more than 10 animals in the pen versus 10 animals per pen (OR=2.02). This study gave valuable information that should be incorporated in future control plans for this agent in breeding pigs in Portugal.

## Introduction

*Salmonella* is one of the major causes of food-borne disease in the European Union (EU) in the past ten years (EFSA, 2010). Some cases are related to pork products. With the aim to control this agent the EU approved legislation (EU Regulation No 2160/2003) that imposed a reduction of 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 cross sectional studies also allowed the collection of information regarding management practices and potential risk factors.

Some of the known risk factors, summarized in systematic reviews (Fosse et al., 2009), are linked to: 1) biosecurity measures like potential biological vectors, as rodents, hygiene of hands, equipment and facilities, bought of animals from different suppliers, 2) herd management like herd size, batch production system, and housing - type of floor (partial slatted floor), type of pen partitions, 3) feeding practices like dry feed, purchasing feed, adding organic acids to feed, 4) health disorders like use of antibiotics, parasite infestations, health status of the herd among others.

The data were collected at the time of the baseline survey on the prevalence of *Salmonella* in breeding pigs in Portugal. The aim of this study was to search for risk factors of *Salmonella* in the pen fecal samples in this country.

## Material and Methods

### Sampling frame and collection of samples

The sampling frame, the diagnostic testing methods, 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 constitut-

ing 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 obtained 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 and sent to laboratory for detection of Salmonella (using method described by Annex D of ISO 6579). Positive samples were serotyped in the national Reference laboratory for Salmonella according to Kauffmann-White scheme.

### **Data collection**

During sample collection a questionnaire was applied to evaluate information about the herd management and potential risk factors. The variables collected concern pen data and herd data, such as: 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.

### **Statistical analysis**

Taking in consideration the questions about management of replacement breeding pigs and their source and also biosecurity measures it was created two new binary variables called "Good herd replacement policy" and "Good biosecurity measures". Some variables were recoded into new variables with fewer categories to have a reasonable sample size in each category. The outcome variable was presence/absence of Salmonella in the pen sample. As data follow a multilevel structure, pen fecal samples (first level) nested within swine herds (second level), were analyzed using a binomial generalized linear mixed model (GLMM) with a logit link function. The glmmPQL procedure of package MASS (Venables and Ripley, 2002) of R free software ([www.R-project.org](http://www.R-project.org)) was used. This type of model in this package is fit by the penalized quasi likelihood approximation. At first univariable models for each potential risk factor were considered. The variables with  $p < 0.15$  were selected to enter the multivariable model. The final multivariable model was found using backward and forward procedure to select the variables in the model and to control for confounding and interaction between variables. The regression coefficients were converted to odds ratio (OR) and the 95% OR confidence interval (CI) were calculated. The intra-class correlation factor (ICC) was calculated using the following formula (Twisk, 2006):  $ICC = \sigma^2_{\text{between}} / (\sigma^2_{\text{between}} + 2/3)$ , where  $\sigma^2_{\text{between}}$  is the between group variance (herd variance).

### **Results**

A total number of 1670 samples were tested (level 1), belonging to 167 herds (level 2) that filled the questionnaire. Out of 1670 samples collected 170 were positive to Salmonella detection, which belongs to 76 herds. Most of these positive samples were identified as Salmonella Typhimurium, followed by Salmonella Rissen. Results of the final multilevel multivariable model are shown in Table 1. The factors with higher risk of being positive to Salmonella with statistical significant differences ( $p < 0.05$ ) are: herds from North Region, herds with 170 or more sows, herds that use semen purchased in another herd, pens where feed is not exclusively from the producer, and pens with more than 10 animals/pen. On the other end factors with lower risk of being positive to Salmonella with statistical significant differences ( $p < 0.05$ ) are: herds which do rodent control, herds without boars or with >90% of boars homebred and maternity rooms pens. The ICC was estimated in 0.27, meaning that the herds account for 27% of the variation in pen samples.

Table 1: Binomial GLMM final multilevel multivariable OR and 95% CI for OR

Variable	Multivariable analysis			
	OR			
	Estimative	95% CI	p value	
<b>Region of the herd</b>				
	Alentejo	1.00		
	Center	1.97	0.75-5.22	0.17
	Lisbon and Tagus Valley	1.40	0.61-3.20	0.43
	North	3.86	1.08-13.75	<b>0.04</b>
<b>Number of sows</b>				
	<170	1.00		
	≥170	1.82	1.04-3.19	<b>0.04</b>
<b>Management of breeding boars</b>				
	more than 90% purchased	1.00		
	Without boars or >90% homebred	0.54	0.30-0.97	<b>0.04</b>
	10-90% purchased or homebred	0.93	0.38-2.30	0.88
<b>Source of semen</b>				
	Center for artificial insemination – CAI	1.00		
	Own boar+ CAI	1.84	0.97-3.46	0.06
	Boar from another herd	4.47	1.38-14.43	<b>0.01</b>
<b>Control of rodents</b>				
	No	1.00		
	Yes	0.23	0.09-0.59	<b>&lt;0.01</b>
<b>Number of animals per pen</b>				
	=10	1.00		
	>10	2.02	1.19-3.43	<b>&lt;0.01</b>
<b>Breeding sector room</b>				
	Mating	1.00		
	Gestating	0.78	0.53-1.15	0.21
	Mixture of animals of different sector	1.55	0.62-3.89	0.35
	Maternity	0.39	0.24-0.63	<b>&lt;0.01</b>
	Replacement breeders	0.81	0.26-1.81	0.61
<b>Source of feed<sup>a</sup></b>				
	Exclusively own	1.00		
	Not exclusively own	2.81	1.19-6.61	<b>0.02</b>
<b>Random effects<sup>b</sup></b>				
	Herd	Variance		
		1.50		

<sup>a</sup> although variable with  $p > 0.15$  the variable was forced into the multivariable model

<sup>b</sup> variance at pen fecal level constrained to be 1 (binomial variance)

## Discussion

In Portugal few studies about herd risk factors have so far been done (Baptista et al., 2010). Concerning the associations found with multivariable analysis in our study we will discuss the significant. Pens where the feed was purchased or mixture of purchased and own produced have higher risk (OR=2.81) of being Salmonella positive, this association was also found in another study ((Benschop et al., 2008); feed is a source of potential transmission of Salmonella and in the HACCP plan of commercial feed this hazard should be taken in consideration, but feed is mainly associated with exotic serotypes and not with more prevalent serotypes (like Typhimurium). The role of rodents in the transmission of this agent was also highlighted in various studies (Skov et al., 2008) and this study also found a protective association for the herds that control rodents (OR=0.23), rodents are biological vectors of this agent and if not controlled could play an important role in their transmission within herds and between nearby herds. The number of sows is a measure the herd size and in this study herds with equal or more sows than the median have higher risk of being positive (OR=1.82); this type of association was found for herds concerning the finishers (Poljak et al., 2008) and is mainly associated with practices of mixture of pigs that happens in big herds. Mating pens showed higher odds of being positive than the maternity pens (OR=2.6) which is similar to the results found in a longitudinal study (Nollet et al., 2005) where it was detected more Salmonella shedding at mating than in the others sector of breeding sows, and it is justified by the hormonal changes in the sow at this time which contribute to a higher shedding of the bacteria. The results concerning the region (North with higher risk than the South) were surprising and need further investigation, ideal spatial analysis, to see if there is another factor that influences this result. Using expert opinion, one explanation could be that in the north the herds are more close together than in the south where the distance between herds is higher. To purchase semen from another herd is a risk factor when compared to the purchase of semen from insemination centers, where the quality and safety of semen is controlled

and tested. This association is not found in literature, to the best of our knowledge, maybe because in the majority of the countries the semen comes from insemination centers. This is a practice that is beginning to be more prevalent in Portugal. The management of breeding boars is also a risk factor and using homebred boars is safer than using purchased boars. This happens because the majority (80%) of the herds with more than 90% of boars from purchased use the semen from own herd or another herd, while in the herds without boars or more than 90% homebred that happens just in 52% of the herds.

## Conclusion

This study allowed identifying significant risk associations as well as potential protective variables for the risk of *Salmonella* sp. presence in faeces in breeding pigs units in Portugal. So far, in Portugal, only another study about herd risk factors has been done (Baptista et al., 2010). The risk associations founded significant after this study should be evaluate in future longitudinal studies using these results as guidelines. To achieve a reduction on the prevalence of *Salmonella*, in the future, the control programs could take into consideration the information from both studies.

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