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

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ABSTRACT

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

1. Introduction

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

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

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

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

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

2. Materials and methods

2.1. Sampling and samples collection and analysis

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

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

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

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

2.2. Data collection

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

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

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

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

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

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

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

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

2.3. Statistical analysis

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

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

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

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

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

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

The data were modeled in the following way:

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

where Y is the response variable.

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

The generic model used:

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

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

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

2.4. Univariable analyses

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

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

2.5. Multivariable analysis

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

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

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

3. Results

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

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

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

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

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

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

4. Discussion

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

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

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

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

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

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

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

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

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

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

5. Conclusion

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

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