Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar
http://dx.doi.org/10.5424/sjar/2012102-446-11

Spanish Journal of Agricultural Research 2012 10(2), 372-382
ISSN: 1695-971-X
eISSN: 2171-9292

Serological survey of *Salmonella* spp. infection in finishing pigs from northeastern Spain and associated risk factors

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Abstract

The results of a serological survey carried out in northeastern Spain to estimate the seroprevalence to *Salmonella* spp. and to determine potential risk factors are presented. Sera were obtained from farms submitting serum samples to the Regional Diagnostic Laboratory (RDL) for the diagnosis of other infectious diseases included within official eradication/surveillance programs, and farm data collected through a questionnaire. Out of 6,182 pig sera (217 farms) analyzed 1,219 (19.7%) were positive (optical density, OD ≥ 40%). More than 70% of the herds presented ≥ 1 seropositive animal indicating that salmonellosis was widespread. In a multivariable logistic mixed regression model seroprevalence was associated with farms where birds were visible inside the fattening units (OR = 2.1; 95% CI: 1.3-3.2) or that shared workers with other pig farms (OR = 2.5; 95% CI: 1.4-4.7). Seropositivity also increased when farmers used footwear exclusive for the farm (OR = 3.1; 95% CI: 1.3-7.3) or pigs were fed mostly with pellets (OR = 1.7; 95% CI: 1.1-2.6). It was also higher during the fall and winter months. The presence of a changing room and shower in the farm was negatively associated with it (OR = 0.5; 95% CI: 0.3-0.8). Most risk factors could be mitigated through strict hygiene and biosecurity measures, but the high (> 40%) within-herd seroprevalence observed in many herds may challenge the capacity of intervention of animal health authorities.

Additional key words: ELISA test; risk factors; salmonellosis; seroprevalence; swine.

Resumen

Estudio serológico de la infección por *Salmonella* spp. en cerdos de engorde del Noreste de España y factores de riesgo asociados

Se presentan los resultados de un estudio serológico realizado en explotaciones porcinas del Noreste de España para estimar la seroprevalencia frente a *Salmonella* spp. y determinar posibles factores de riesgo asociados. Los sueros se obtuvieron a partir de aquellos enviados por las explotaciones al Laboratorio Regional de Diagnóstico para el diagnóstico de otras enfermedades infecciosas sometidas a erradicación/vigilancia oficial, y los datos sobre las explotaciones se recogieron mediante un cuestionario. De un total de 6.182 sueros de cerdo (217 granjas) analizados 1.219 (19,7%) resultaron positivos (densidad óptica, DO ≥ 40%). Más del 70% de los rebaños presentaron al menos un animal positivo, indicando que la salmonelosis está ampliamente distribuida en la región. Según un modelo multivariable mixto de regresión logística, la seroprevalencia se asoció con granjas en las cuales se veían aves en el interior de las naves (OR = 2,1; IC 95%: 1,3-3,2) y también con granjas que compartían trabajadores (OR = 2,5; IC 95%: 1,4-4,7). Se observó que la seropositividad se incrementaba cuando los trabajadores usaban botas exclusivas en la explotación (OR = 3,1; IC 95%: 1,3-7,3) o al alimentar a los cerdos principalmente con alimento granulado (OR = 1,7; IC 95%: 1,1-2,6). La seroprevalencia también fue superior durante los meses de otoño e invierno. La existencia en las granjas de vestuarios y duchas para el personal se asoció negativamente con la seroprevalencia (OR = 0,5; IC 95%: 0,3-0,8). La mayoría de los factores de riesgo podrían controlarse a través de estrictas medidas de higiene y bioseguridad, pero el gran número de rebaños que presentaron alta seroprevalencia (> 40%) puede poner a prueba la capacidad de intervención de las autoridades sanitarias oficiales.

Palabras clave adicionales: factores de riesgo; porcino; prueba de ELISA; salmonelosis; seroprevalencia.

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Received: 06-09-11. Accepted: 12-04-12

Abbreviations used: CI (confidence interval); EFSA (European Food Safety Authority); EU (European Union); NN (nearest neighbour); OD (optical density); OR (odds ratio); RDL (Regional Diagnostic Laboratory); REGA (Registro General de Explotaciones Ganaderas); S/P (sample-to-positive ratio).

Introduction

In Europe human salmonellosis is mostly associated to consumption of contaminated eggs and chicken products (Anonymous, 2008), but the implementation during last years of strict control and surveillance programs on eggs and poultry has contributed to a significant declining of the number of cases of salmonellosis in people (Collard *et al*., 2008; Anonymous, 2011a). Pork meat is now considered the second most important source of *Salmonella* infection for humans in Europe. A recent European report highlighted the increasing importance of this animal species as pig meat was associated with more *Salmonella* outbreaks than broiler meat, particularly in case of *S*. Typhimurium outbreaks (Anonymous, 2010). The reduction of the prevalence of *Salmonella* serovars with public health significance in pig herds is considered a major objective in Europe (OJ, 2003).

A recent report showed that the mean *Salmonella* prevalence in slaughter pigs in the European Union (EU) was 10%, with wide variations (from 0% to 29%) among Member States (Anonymous, 2007). Given the lack of effective vaccines to protect animals from infection with non-typhoidal *Salmonella* (Farzan & Friendship, 2009; Malcolm, 2010), the success of any large-scale control program should be based on the knowledge of its epidemiology and cost effective of control measures. The availability of reliable and accurate diagnostic tests to identify infected farms and levels of infection/exposure and the identification of potential risk factors are also basic pillars of any *Salmonella* control scheme.

Pig salmonellosis is mostly asymptomatic and the detection of *Salmonella*-infected pigs is not straightforward. Bacteriology, the method of reference, is expensive, tedious, time-consuming and less sensitivity, particularly when performed on feces (Hurd *et al*., 2004; Mainar-Jaime *et al*., 2008a), making this approach unfeasible for large-scale control programs. Serology is thus foreseen as one of the best alternatives. Although the detection of antibodies against *Salmonella* spp. is not the best indicator of infection at the pig level (Nollet *et al.*, 2005; Mainar-Jaime *et al.*, 2008b), most national programs against pig salmonellosis are based on serology as this technique is considered useful to establish the level of exposure of a herd and, therefore, some sort of "level of risk" (British Pig Executive, 2002; Alban *et al.*, 2011; Anonymous, 2011b).

Spain is, after Germany, the second largest pig-producing country in Europe, producing > 15% of the total production in the EU-27 (MARM, 2009), and also shows the highest level of *Salmonella* infection in slaughtered pigs in Europe (Anonymous, 2007). More than 40% of the Spanish pig census is concentrated in the northeastern part of the country. The results of a serological survey carried out in this area are presented. The presence of spatial clusters of herd infection is analyzed and the potential risk factors associated with seroprevalence in finishing-pig farms determined. The knowledge of these factors will contribute to a better understanding of the epidemiology of this infection in one of the largest European pig production areas and will help to set up feasible *Salmonella* control programs in Spain.

Material and methods

Farm population and samples

The study population was composed of finishing pig farms within the region of Aragon submitting a minimum of 30 individual serum samples to the Regional Diagnostic Laboratory (RDL) for the diagnosis of other infectious diseases included within official eradication/surveillance programs (*i.e*. Aujezsky's disease). This group of farms included mostly exporting farms, farrow-to-finish farms, and farms in the last stages of the Aujezsky's disease eradication program. Thirty animals per farm were considered sufficient to detect, with 95% confidence (Win Episcope 2.0), at least one seropositive animal if seroprevalence was ≥ 10%. Pig farms where Aujezsky's disease had been eradicated were excluded if the number of individual samples submitted to the RDL was less than 30.

Along the year 2008, farms were randomly selected weekly from the total of entry forms filed at the RDL, and proportionally to the official census by councils. Once sera had been used for official purposes, they were set aside and stored at –20°C until use. Since herd seroprevalence to *Salmonella* spp. was expected to be around 90% (Vico *et al.*, 2011a), a sample size of 200 farms was considered appropriate to estimate the seroprevalence with a precision of ±4% (Win Episcope 2.0).

Questionnaires

A questionnaire (available in Spanish upon request) was designed to obtain information regarding specific

aspects of the fattening units from which pigs had been bled. An initial draft of the questionnaire was pre-tested on two pig veterinarians to ensure that the questions would be easily understood by pig producers. Suggested modifications were included in the final form. The questionnaire was designed as a mail questionnaire following the Total Design Method (Dillman, 1978). The questionnaire was divided in four main sections: a) farm general characteristics, including question such as type of farm (finishing or farrow-to-finish), Aujezsky's disease status, number of fattening units, herd size, percentage of pen floor covered by slats, type of separation between pens (solid walls *vs* bars-like), pen stocking density, length of the fattening period, number of full-time workers in the farm; b) farm biosecurity, with questions on proper maintenance and use of the outside fence, the use of sanitizing wheel and boot baths, the wearing of specific clothes before entering the facilities, presence of changing rooms and shower in the farm, restrictions on people visiting the farm, sharing workers with other farms, presence of other domestic animals, wild birds and rodents inside the fattening units, etc.; c) feeding management, with questions regarding the way animals were fed (automatic or manual), type of feed (pelleted *vs* meal), number of different diets during the fattening period; and d) farmer's characteristics, namely, age, educational degree (no studies, primary, secondary or university degree) and further training on pig production. The questionnaire was submitted to the farmers through their corresponding veterinarians.

Serology

Serum samples were analyzed by the ELISA Swine *Salmonella* Antibody Test Kit (HerdCheck, IDEXX Laboratories, Westbrook, USA). According to manufacturers this assay was designed to detect antibodies to the lipopolysaccharide (LPS) antigen of *Salmonella* B, C_1 and D serogroups. To perform the test the manufacturer's instructions were followed. Results were calculated as sample-to-positive ratio (S/P) and a correlation factor of 2.5 was used to obtain the results as Optical Density percentage (OD%).

Although manufacturer claims high sensitivity (Se) and specificity (Sp) (>99%) in their commercial brochures, after testing a control panel of positive and negative sera at a cut-off $\geq$ 10% (http://www.idexx.com/pubwebresources/pdf/en_us/livestock-poultry/swine-salmonella-ab-test-sheet.pdf), test's sensitivity and specificity on field samples were estimated as 73% and 95%, respectively, in an independent study (Mainar-Jaime *et al.,* 2008b). Nevertheless, in order to minimize the number of false-positive individual results a cut-off value OD% $\geq$ 40% (equivalent to a S/P $\geq$ 1) was used to deem an animal as positive.

Statistical analysis

Before any analysis data were checked for inconsistencies, data entry mistakes and missing values, and corrected when possible. A herd was considered positive when at least one of the sampled animals yielded a positive result (OD% $\geq$ 40%). Individual and herd seroprevalences with their corresponding 95% confidence intervals (95% CI) were calculated first. Data were analyzed using STATA software (StataCorp LP, College Station, TX, USA).

Exact geographical location (longitude and latitude) of the pig farms were retrieved from REGA (*Registro General de Explotaciones Ganaderas,* the farms official register, Spanish Ministry of Environmental, Rural and Marine Affairs). The Cuzick and Edwards' test (Cuzick & Edwards, 1990) was used to determine whether or not geographical clustering of *Salmonella*-infected herds existed. The test identified spatial patterns in the data, regardless of location, that were unlikely to have arisen by chance, from individual (herd)-level case-control data (Ward & Carpenter, 2000). Cuzick & Edwards' test is a nearest neighbour (NN) test that, for each case, counts the number of other cases in k NNs among all remaining cases and controls. The NN cases observed (Tk statistic) were then compared to the NN cases expected if cases and controls were randomly distributed in the area (E[Tk]). When cases were clustered, the NN to a case would tend to be another case and Tk would be large. The significance of the Cuzick & Edwards' test was assessed using a Z-score that calculated a standardized difference between Tk and E[Tk]; p-values were assessed for several Tk statistics (k = 1–10). A multiple comparison analysis (Simes & Bonferroni tests) to determine the proper level of significance for all comparisons was also performed. The analysis was carried out using Clusterseer (TerraSeer Inc, Ann Arbor, MI, USA).

The identification and location of significant clusters of pig salmonellosis was assessed further using a spatial scan statistic (Kulldorff & Nagarwalla, 1995). It tests the hypothesis that herds within a particular window had the same risk of being seropositive to *Salmonella*

spp. than herds outside the window, and the primary cluster was that with the largest likelihood ratio. Secondary clusters could be also identified. The distribution of a seropositive herd was assumed to be Bernouilli, *i.e.* the total population was expressed as the sum of all the cases and controls. This analysis was carried out using SatScan software (http://www.satscan.org).

Farm-level information was used to assess whether *Salmonella* seropositivity was related to each of the variables that characterized the herd. Farm-level variables were defined as exposures to the individual animals in the farm that might influence serological results. Since animals were grouped by farm, a logistic mixed regression model was fitted in which the outcome variable was the number of seropositive animals out of total tested in the herd, herd was included as a random effect and variables from the questionnaire were the explanatory variables included in the model as fixed effect. Because the large number of variables collected, univariable logistic mixed regression analysis assessing the relationship between each factor and the outcome variable were performed first as a screening step. Variables with a significant relationship ($p < 0.25$) with the infection were tested in the multivariable model. If required, quantitative variables were log transformed or categorized according to percentiles. The multivariable regression model was constructed using a stepwise approach in which variables were entered into the model and all that showed a $p \leq 0.05$ were finally retained. Biologically plausible two-way interactions between variables in the model were assessed as well.

Results

Descriptive data

Two hundred and seventy six finishing farms were initially selected from the RDL files, their sera collected and stored and the corresponding questionnaires sent out. Two hundred and seventeen (78.6%) of the questionnaires were returned. Data from RDL entry forms were used to compare basic data from respondent and non-respondent farms through χ^2 analysis. No significant differences were observed regarding herd size ($p = 0.85$), farm type ($p = 0.21$), Aujeszky's disease status ($p = 0.97$) and province ($p = 0.41$). Only sera from respondent farms were analyzed.

A total of 6,182 serum samples were tested from these herds, an average of 28.5 pigs/herd, as for some animals there was not enough serum to perform serology. The geographical distribution of the sampled herds is shown in Figure 1. Areas on the map of greater concentration of sampled herds corresponded to areas of higher pig farm density. A total of 1,219 (19.7%; 95% CI = 18.7%, 20.7%) animals were seropositive when the cut-off used was OD% ≥ 40%. At least one seropositive animal was found in 71.4% (155) herds from which 18% (39) presented high seroprevalence (> 40% seropositivity). The median within-herd seroprevalence among the 155 seropositive herds was 20% (Q1 = 8%, Q3 = 40.6%). The distribution of the within-herd seroprevalence is shown in Figure 2.

Spatial analysis

The Cuzick & Edwards' test did not find overall clustering among *Salmonella*-infected herds in the region (combined Simes $p = 0.51$; combined Bonfer-

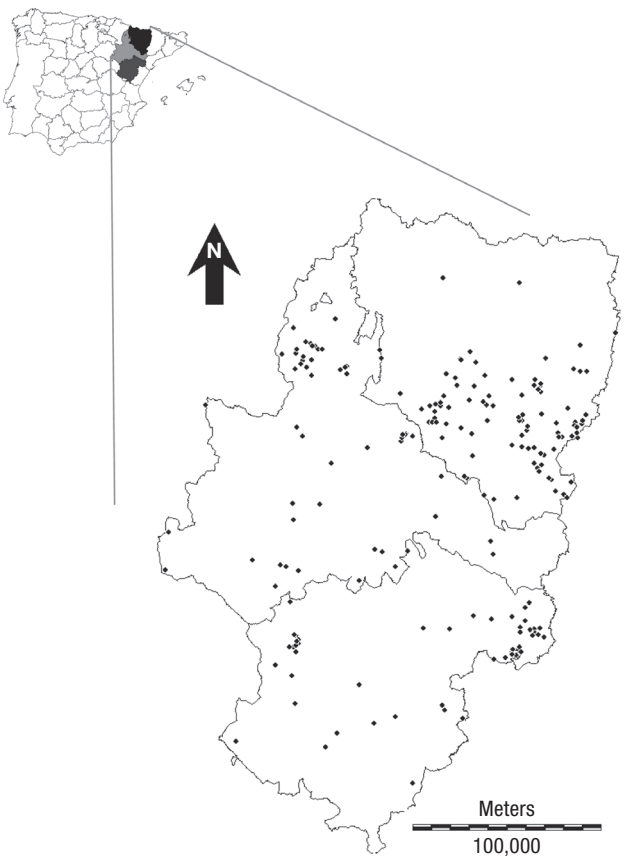

Figure 1. Map of the region of Aragon, NE of Spain, and geographical distribution of the 217 pig farms sampled.

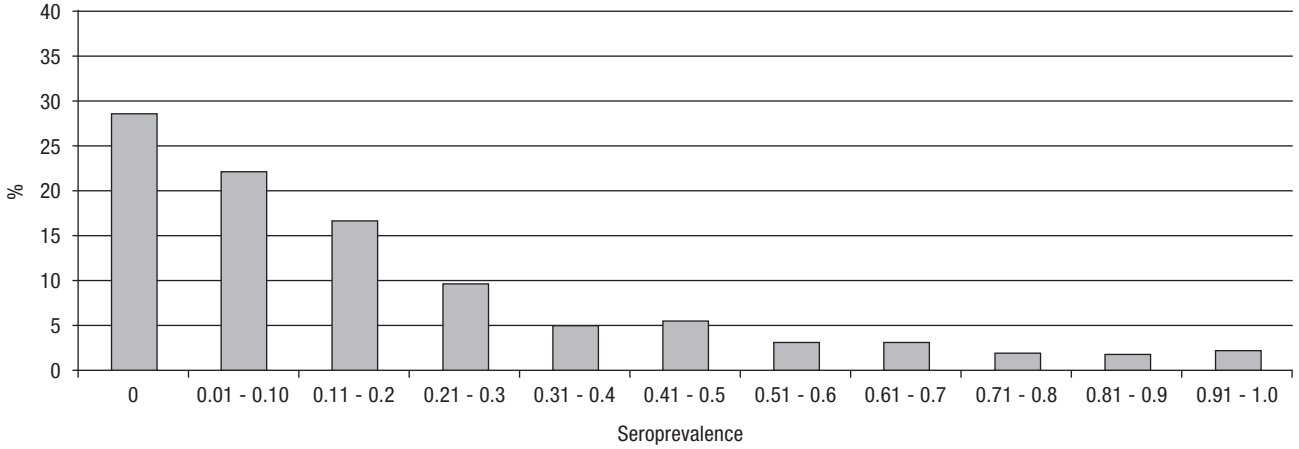

Figure 2. Frequency distribution of within-herd *Salmonella* seroprevalence of 217 finishing-pig herds in NE of Spain in 2008 (cut-off: OD% ≥ 40).

roni $p = 1$). Statistical significance was neither achieved for any of the $k = 1$-10 NN analyzed. The spatial scan statistic supported the results obtained by the Cuzick and Edwards' test as no significant clusters of *Salmonella*-infected herds were detected within the herd population analyzed.

Risk factors analysis

Fifteen variables were significant at $p \leq 0.25$ in the univariable analysis and further tested in the multivariable logistic mixed regression model (Table 1). Seven variables were significant in the final multivariable model (Table 2). Since animals had been bled at different ages, the variable "age at sampling" was kept in the model as confounder. Seroprevalence was positively associated with pigs coming from farms where birds were visible inside the fattening units. Sharing workers with other pig farms was common practice in almost 20% of the surveyed farms and was related to higher seropositivity, as well as the use of footwear (boots) exclusive for the farm. Likewise the odds of being seropositive increased for pigs fed mostly with pellets compared to those fed with meal feed. Seroprevalence was also higher in the provinces of Teruel and Zaragoza (Central and South of the region) compared to Huesca (North).

Salmonella seropositivity was significantly lower during the spring and summer months compared to the fall-winter ones, and in those pigs fattened in farms where there was a changing room and shower. No significant two-way interactions were found.

Discussion

To the authors' knowledge this is the largest serological survey published so far in Spain. Although both serum or meat juice are accepted as matrices for serological diagnosis of *Salmonella* spp. in pig (Nielsen *et al*., 1998; Nollet *et al*., 2005; Szabó *et al*., 2008), in this study serum was used given its availability from the RDL. Serum would have the additional advantage of yielding better diagnostic sensitivity (Wilhelm *et al*., 2007; Vico & Mainar-Jaime, 2011).

Reasons why farmers did not answer were unknown, but the response bias was considered negligible as 78% of the farmers responded to the questionnaire and no significant differences were observed between respondent and non-respondent farms regarding basic variables (herd size, farm type, Aujeszky's disease status, and province). Likewise, a selection bias was possible since some finishing and farrow-to-finish farms were excluded from the survey as they submitted less than 30 samples to the RDL. The number of samples analyzed was, in part, a function of the Aujezsky's disease status. Many farms free of this infection only sampled 15 animals. Since Aujezsky's disease status was not a factor related to the seroprevalence to *Salmonella* spp. within the farms selected ($p = 0.26$; data not shown), no significant bias was expected from this selection.

The overall individual seroprevalence observed (19.7%) was much lower than the prevalence found in a study on the same region carried out between 2008-2009 using microbiology on mesenteric lymph nodes (30%; Vico *et al*., 2011a). This discrepancy should not be related to the *Salmonella* serotypes present in the

Table 1. Variables univariably associated ($p < 0.25$) with Salmonella seroprevalence in pigs by logistic mixed regression analysis in a study in NE of Spain

Variable	Total herds	Positive herds	%	Total animals	Positive animals	%	*p*
Province							
Huesca	97	65	67.0	2,780	447	16.0	
Teruel	66	49	74.2	1,825	391	21.4	0.27
Zaragoza	54	41	75.9	1,577	351	22.3	0.21
Season							
Summer	48	33	68.7	1,399	242	17.3	
Spring	63	40	63.5	1,794	291	16.2	0.80
Fall	39	33	84.6	1,087	270	24.8	0.14
Winter	67	49	73.1	1,902	416	21.9	0.31
Pen stocking density (animals m^{-2})							
≤ 1.3	49	32	65.3	1,377	199	14.5	
> 1.3 ≤ 1.4	43	28	65.1	1,231	215	17.5	0.46
> 1.4	89	66	74.5	2,535	511	20.2	0.11
Pen separation							
Solid walls	187	134	71.7	5,319	999	18.8	
Bars-like	28	20	71.4	803	209	26.0	0.20
Length of the fattening period							
≤ 5 months	131	98	74.8	3,732	798	21.4	
> 5 months	86	57	66.3	2,450	421	17.2	0.23
Number of full time workers							
≤1	143	104	72.7	4,058	884	21.8	
>1	38	25	65.8	1,099	165	15.0	0.09
Presence of perimetral fence deficiencies							
No	157	113	71.9	4,483	814	18.2	
Yes	60	42	70.0	1,699	405	23.8	0.15
Use of exclusive footwear in the farm							
No	27	15	55.6	750	102	13.6	
Yes	190	140	73.7	5,432	1117	20.6	0.13
Availability of changing room and shower in the farm							
No	128	96	75.0	3,622	853	23.6	
Yes	89	59	66.3	2,560	366	14.3	< 0.01
Sharing workers with other pig farms							
No	177	124	70.1	5,018	921	18.4	
Yes	40	31	77.5	1,164	298	25.6	0.14
Presence of dogs in the farm							
No	177	131	74.0	5,059	1050	20.8	
Yes	40	24	60.0	1,123	169	15.1	0.18
Presence of rodents inside the fattening units							
No	66	49	74.2	1,873	304	16.2	
Yes	151	106	70.2	4,309	915	21.2	0.12
Presence of birds inside the fattening units							
No	110	79	71.8	3,116	465	14.9	
Yes	106	75	70.8	3,044	750	24.6	< 0.01
Feed type							
Meal	79	51	64.6	2,264	373	16.5	
Pelleted	137	103	75.2	3,896	842	21.6	0.14
Age at the time of bleeding							
≥ 5 months old	33	23	69.7	4,605	993	21.5	
< 5 months old	161	117	72.1	927	139	14.9	0.06

region as more than 80% of them would belong to the serogroups theoretically detected by the ELISA used (B, C1 and D) (Vico *et al*., 2011a).

The difference may be explained for other reasons. One may have to do with animal age. While this survey was based on serum samples from pigs of different ages (mostly between 3 and 6 months old), the previous work was performed on slaughtered pigs, which implied, on average, older animals than those included in the present survey and therefore a higher opportunity to get the infection as they were exposed to potential *Salmonella*-contaminated environments longer.

Differences could also be due to the lack of agreement showed between serology and microbiology at the pig level, especially when cut-off values of 10% or 20% were used (Nollet *et al*., 2005; Farzan *et al*., 2007; Mainar-Jaime *et al*., 2008b). We used a higher cut-off value (OD%≥40) that might not improve overall diagnostic accuracy but allowed higher confidence with regard to test specificity. Indeed, this cutoff was similar to that used in the *Salmonella* German QS system (Quality and Safety). Studies based on results from this monitoring system found a positive correlation between herds of higher risk (*i.e.* >40% seropositivity) and residual *Salmonella* in the environment, supporting the usefulness of the serological monitoring at this cut-off as a means for estimating the *Salmonella* risk that herds pose (Gotter *et al*., 2011).

The herd seroprevalence reported in this study was high (>70%) and similar to that reported in the neighboring region of Catalonia (77%) using a cut-off value similar to the one used here (Mejia *et al*., 2006). The

Table 2. Variables associated with *Salmonella* serprevalence in finishing pigs by multivariable logistic mixed regression model in a study in NE of Spain

Variables	β	SE (β)	OR	95% CI (OR)	*p* value
Age at the time of bleeding					
≥ 5 months old			1		
< 5 months old	–0.30	0.29	0.74	0.41-1.30	0.29
Season					
Summer			1		
Spring	0.18	0.34	1.19	0.61-2.33	0.59
Fall	0.66	0.33	1.93	1.00-3.73	0.05
Winter	0.64	0.31	1.90	1.02-3.52	0.04
Province					
Huesca			1		
Teruel	0.96	0.29	2.62	1.47-2.70	< 0.01
Zaragoza	0.46	0.27	1.59	0.93-4.67	0.08
Presence of birds inside of the fattening units					
No			1		
Yes	0.72	0.22	2.06	1.33-3.18	< 0.01
Availability of changing room and shower in the farm					
No			1		
Yes	–0.69	0.23	0.5	0.31-0.78	< 0.01
Use of exclusive footwear in the farm					
No			1		
Yes	1.12	0.43	3.09	1.30-7.28	0.01
Feed type					
Meal			1		
Pelleted	0.50	0.22	1.67	1.06-2.59	0.03
Sharing workers with other pig farms					
No			1		
Yes	0.93	0.31	2.55	1.38-4.72	< 0.01

SE = standard error; OR = odds ratio; 95% CI = 95% confidence interval.

high herd seroprevalence observed would support the idea that *Salmonella* infection is widespread among pig farms in NE Spain (Vico *et al*., 2011a), and higher than that of many other European countries (Grafanakis *et al*., 2001; van der Wolf *et al*., 2001, Merle *et al.*, 2011). These observations are in agreement with the reports from the European Food Safety Agency (EFSA) indicating that the prevalence of *Salmonella* infection in finishing pigs in Spain is the highest (Anonymous, 2007).

Prevention of *Salmonella* exposure to humans requires both the reduction of *Salmonella* prevalence in the primary production and in the abattoir. The high seroprevalence (>40%) observed in almost 20% of the seropositive herds (Figure 2) would likely make them the target of official interventions in a first step. Therefore large farm intervention capacity will be required from the animal health authorities to enable a strict implementation of control measures against *Salmonella* infection on such proportion of the pig farm population. In addition, logistic slaughter should be implemented to prevent contamination of carcasses at abattoir.

Although seroprevalence was significantly higher in the provinces of Teruel and Zaragoza (Table 2), it seemed not to be related to particular areas but widespread all over the region, as suggested by the lack of spatial clusters of seropositivity among herds. No clustering was observed even when >1 seropositive animal were considered in order to classify a herd as positive (data not shown). This result contrasts with results from previous studies in Denmark that found some spatial dependency between seropositive farms (Benschop *et al*., 2008). Differences may lie in part on the overall level of infection found in each country at the time studies were performed. In Denmark swine *Salmonella* control programs had been implemented for several years and seroprevalence was very low (< 5%) (Bager & Halgaard, 2002) while in Spain no control program is in place and prevalence seems to be very high (Anonymous, 2007; Garcia-Feliz *et al*., 2007; Vico *et al*., 2011a).

Most factors associated to *Salmonella* seroprevalence were related to a lack of proper hygienic measures (Table 2). For instance, sharing workers with other pig farms, which was common in 20% of the herds, was associated with an important increase of seroprevalence of *Salmonella* spp. (odd ratio-OR = 2.55). If workers do not maintain correct attitudes about biosecurity when moving from one pig farm to another (*i.e.* hygienic practices such as change of clothes and footwear, hand washing, etc.), this "human traffic" will surely increase the pig's risk of infection (Funk *et al*., 2001). Surprisingly, the use of specific footwear (boots) while working on the farm was related to higher seroprevalence, being the magnitude of this association large (OR = 3.1). It could be an indicator of a lack of overall farm hygiene or simply the farmer could act as a source of continuous reintroduction of contaminated feces among pens or units due to improper cleaning and disinfection of boots, thus maintaining the infection within the farms. But also this result could be the consequence of a spurious relationship caused by a hidden variable. In any case boots should be cleaned and disinfected often since they are one of the environmental samples on which *Salmonella* spp. is more frequently recovered (Rajic *et al*., 2005). On the contrary, the availability of a changing room and shower in the farm showed a protective effect (OR = 0.5), which could be considered a reflection of the farmer's level of awareness on farm hygienic practices, as other authors pointed out after finding similar results (Funk *et al*., 2001; van der Wolf *et al*., 2001; Lo Fo Wong *et al*., 2004; Hautekiet *et al*., 2008).

A risk factor associated with biosecurity breaches was also identified. When birds had access to the fattening units (they were visible inside the units) there was higher odds of *Salmonella* seropositivity (OR = 2.06). Wild birds may harbor *Salmonella* organisms and can contaminate the environment through their droppings (Funk & Gebreyes, 2004; Tizard, 2004). We have found phenotypic and genetic correlations between *Salmonella* isolates from pig feces and from birds captured in the surroundings of the corresponding farm in this region (Vico *et al*., 2011b), supporting the important role that wild birds may have in the maintenance of this infection within the farms.

Feeding animals mostly with pellets also increased significantly the odds of being seropositive compared to meal feed (OR = 1.67). A higher risk of *Salmonella* seroprevalence in pigs fed with pelleted rations compared to those fed with meal feed was previously found (Lo Fo Wong *et al*., 2004; Hautekiet *et al*., 2008; García-Feliz *et al*., 2009). Meal feeds may benefit the natural gut flora of pigs by increasing the concentration of lactobacillus and the acidification at gut level through fermentative processes, creating a hostile environment for *Salmonella* spp. (Jorgensen *et al*., 1999). Pellet contamination in trucks or even in the farm could be another explanation for this finding, although these

authors cannot find a reason why it will happen more frequently in pelleted feed than in meal feed. The fact that this same finding has been observed in different regions highlights the importance that some feeding strategies might have on helping to reduce *Salmonella* infection at the farm level.

Seasonal variations of seroprevalence similar to those observed in this study were reported in other studies (Christensen & Rudemo, 1998; Vonnahme *et al*., 2007) but contrasts somewhat with results by Hautekiet *et al*. (2008) who found higher S/P-values in sera from animals bled in summer compared to those bled in winter. Seasonal effects on *Salmonella* seroprevalences are difficult to detect as most pigs are raised indoors and many herd factors may have a stronger influence on seroprevalence (indoor heating, forced ventilation, stocking density, etc.). Our results may be explained partially by the lag time expected between serology and microbiology. Some studies suggest that seroprevalence peaks approximately 60 days after the peak prevalence (Kranker *et al*., 2003), thus in our population the highest risk of infection would have occurred during the warmest months of the year for this region (July to September). Peaks of *Salmonella* incidence during these months would be more likely due to the more favorable climatic conditions for its survival and multiplication, as it occurs in humans (Anonymous, 2010).

Concluding, this survey emphasizes the need to initiate *Salmonella* control programs to reduce the overall level of infection in the region. Since major factors associated with *Salmonella* seroprevalence were related to farm hygiene and biosecurity practices, educational programs to increase farmers' awareness on this infection are foreseen as one of the main strategies. Despite the high cut-off value used the within-herd seroprevalence was high in a significant proportion of herds, the authorities should bear in mind that the capacity of official interventions may therefore be challenged.

Acknowledgements

This study has been fully funded by the *Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria* (INIA) of Spain (research grant no. RTA2007-65). Collaboration of the Regional Diagnostic Laboratory, Agriculture Department, *Gobierno de Aragón,* is also acknowledged.

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