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Research Article

Serological Evaluation of *Salmonella* Status in Greek Swine Herds

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Received: September 20, 2018; **Published:** October 31, 2018

Abstract

Subclinical *Salmonella* infection, attributable to any of the more than 2600 Salmonella serovars is of zoonotic interest due to human food safety concerns regarding pork production. Serology has been used to determine the prevalence of salmonellae on pig farms and has been adopted by several countries into national control programmes designed to reduce occurrence of *Salmonella* on the farm and in pork. The aim of this study was firstly to examine *Salmonella* seroprevalence in sows and slaughtered fattening pigs from 39 farrow to-finish swine herds of unknown *Salmonella* status in Greece, using an ELISA test, and secondly to investigate the possible discrepancies between the isolation of *Salmonella* in different pig tissues and the results of serological screening. The serum samples were analysed using an indirect mix-ELISA for the presence of *Salmonella* antibodies and evaluated at 3 cut-off values namely 10, 20, and 40% Optical Density (OD). All tissue samples were submitted to *Salmonella* isolation culture and the isolates were serotyped.

The seroprevalence comparison in sows and slaughtered fattening pigs suggests that sows play an important role in the epidemiology of *Salmonella* infection, being carriers which harbor infections in a herd for an extended period of time, thus being a long-lasting, but substantial risk for *Salmonella* transmission to pig fattening. However, a weak agreement was found between bacteriology and serology for *Salmonella* diagnosis only at a cut off of OD 40%, which had more discriminatory power in detecting the *Salmonella* harbouring pigs in comparison with lower cut-off values (OR (95% CI) 2.456 (1.004 - 6.007). Therefore, serological surveillance of *Salmonella* infections in swine herds allows the detection of high risk herds and assessment of the efficiency of the control measures implemented into the primary production.

Keywords: *Slaughter Pig; Sow; Salmonella; Seroprevalence; Bacteriology*

Introduction

Pig salmonellosis is typically subclinical [1], making pigs a reservoir of the pathogen and causing difficulties in the evaluation of the overall prevalence of the disease. Subclinically infected animals, especially those remaining in the farm for long time as sows, maintain the microorganism in the farm, contaminating their environment, infecting susceptible animals and contaminating carcasses during slaughtering, eventually passing virulent serovars to consumers, having also adverse consequences in their productivity [2]. Thus, the pig has proved to be an important contributor to human salmonellosis, responsible, in some states, for up to 56% [3] of the confirmed human cases, with an overall estimated contribution between 10 - 20% [4]. With this in mind, EU authorities issued Regulation EC-2160/2003, recognizing the importance of protecting human health from food borne zoonotic agents. Thus, member states are responsible in formulating effective national control programs, adjusted to each country's needs, taking into account the burden of disease and the economic impact to pork production [5,6]. The timetable for setting targets for pig production has been March/April 2008 for herds of slaughter

pigs and March/April 2009 for breeding herds of pigs, while the national control programmes had to be sent to the European Union until 31 December 2008 [7].

For these reasons, studies undertaken to estimate herd prevalence are necessary in order to predict the risk of contaminated food products entering the food chain and to estimate the success of surveillance and control programmes [8]. The objective of these programmes is to lower the *Salmonella* seroprevalence to acceptable levels, rather than eradicate the pathogen. Elisa tests, based on serology and showing to have a satisfactory sensitivity and specificity in the past [9], are a useful tool for this purpose, enabling the evaluation of the prevalence at the herd level and pointing out high-prevalence herds. However, they cannot be used as an indication of the potential consumer risk of any individual animal [10,11]. Moreover, in chronically infected herds, the individual-animal sensitivity and specificity of a serological test (using bacteriology as the gold standard) may be difficult to define. Thus, the proportion of animals positive on serology may or may not correlate closely to the microbiological burden at the time of sampling, reflecting differences in the sensitivities of the two techniques [8]. Furthermore, the cut-off value used to evaluate a sample as serologically positive, having also the best correlation to bacteriology, is of major importance, depending on the prevalence of infection in a region and on the country-specific distribution of *Salmonella* serovars.

Aim of the Study

The aim of the present study was firstly to examine *Salmonella* seroprevalence in sows and slaughtered fattening pigs from farrow -to-finish swine herds of unknown *Salmonella* status in Greece and secondly to determine how many of the animals that were *Salmonella* culture positive, as obtained by *Salmonella* isolation in different pig tissues collected at the slaughterhouse, were also serologically positive.

Materials and Methods

First part: *Salmonella* seroprevalence in sows and slaughtered fattening pigs

Blood samples and sampling procedure

Thirty nine farrow -to- finish herds were randomly selected from five distinct geographical regions of Greece. Herds with less than 20 sows were excluded. Finally 688 blood serum samples were collected coming from 314 dry sows and 374 finishers 1 - 2 days before slaughtering.

Blood serum samples were stored at -80°C until their testing. They were examined for antibodies to *Salmonella* spp. using a commercial indirect mix-ELISA Test Kit (Idexx Laboratories, Inc., Maine, USA), which detects antibodies against the commonest serovars (serogroups B, C1, D) isolated in Europe, Asia and America. Tests were performed following the manufacturer's instructions.

The presence in each sample of antibodies against *Salmonella* was determined by relating the absorbance value at 650 nm of the unknown sample to the positive control of the test and calculating the ratio Sample-to-Positive (S/P). S/P sample values were associated with the percentages of the Optical Density (OD%) by a factor of 2.5, which has been experimentally determined previously, using German and Dutch reference samples and an international ring trial [12]. The OD% was calculated using the formula: OD% = (S/P)/2.5 x 100.

Three different cut-off values have been used for the interpretation of the results: OD 10%, recommended by the manufacturer, OD 40% experimentally determined as the optimal value for the Danish *Salmonella* surveillance and control program [10,13] and OD 20% consequently recommended for epidemiological investigations [14,15]. Values lower than the cut-off value were regarded negative and those equal or higher positive.

Statistical analyses

Statistical analyses to estimate the relation between the serological *Salmonella* status of sows and the *Salmonella* status of finishers were performed using Pearson's correlation coefficient and ANOVA (one factor) with the statistical package excel 2007 [16].

Second part: Comparing *Salmonella* isolation and serology in slaughtered pigs

Six hundred fifteen samples were collected, after several visits to two abattoirs in Central Greece (September 2013 - March 2014). In each visit, a randomly selected group of pigs was sampled during slaughtering. A total of 123 pigs were sampled. Samples were collected from 15 of the above swine finishing farms. From each selected pig, blood samples were collected at exsanguinations and, after evisceration of the same pig, samples were collected from the colon, ileum, mesenteric lymph nodes and gall-bladder. All samples were immediately transported to the laboratory for further serologic testing and bacteriological culturing.

Laboratory examination of samples

Blood serum samples were examined as mentioned in the first part of the study.

Isolation and serotyping of *Salmonella* spp.

Samples of feces, ileum tissue, lymph nodes and swabs from gall-bladder were cultured by standard culture methods following the *Salmonella* ISO 6579:2002, Annex D for food and animal feeding stuffs [17], as described elsewhere [18].

Statistical analyses

Quantitative data were presented as the mean and standard deviation and qualitative as frequency and percentage. Fitting of the data in the normal distribution was assessed with the Kolmogorov-Smirnov test. Comparisons between groups of qualitative data were conducted by using Chi-square test, whereas for quantitative Mann-Whitney U test was used.

The correlation between Elisa, Microgen, API and serotyping was examined with the use of Spearman's rho test.

All statistical analyses were performed with the Statistical Package for Social Sciences (SPSS) version 21 program. Statistical significance for all tests was set as $p < 0.05$ [19].

Results

First part: *Salmonella* seroprevalence in sows and slaughtered fattening pigs

Salmonella seroprevalence ranging from 0% to 10% was found in 169 animals (58 sows-111 fattening pigs). Low seroprevalence (OD ≤ 40%) was observed in 566 animals (250 sows-316 fattening pigs) from nine herds, 111 animals (58 sows-53 fattening pigs) from 23 herds showed middle seroprevalence (40% < OD ≤ 70%), while only eleven animals coming from seven herds (6 sows-five fattening pigs) showed high seroprevalence (OD > 70%) (Table 1).

The total results show strong positive correlation between the five geographical areas under consideration. All geographical regions show the same pattern infection rates, regardless of the age of the animals (Tables 1, 2). Similarly, ANOVA one factor (a = 0.05) showed that among sows and finishers there is no statistically significant difference in infection rates among the geographical areas for all OD values (P-value = 0.98).

Second part: Comparing *Salmonella* isolation and serology in slaughtered pigs

In total, 123 pigs from 15 herds were sampled. The descriptive results for the bacteriology and serology using the different cut-off values are given in table 3. 59.35% of the blood samples had an OD% larger than 10 and 20.33% larger than 40, while only 30.23% (cut-off OD 40%) to 65.11% (cut-off OD 10%) of the animals from which *Salmonella* had been isolated were seropositive.

	Total	Thessaly	Macedonia	Central Greece	Epirus	Crete
No of Herds	39	7	14	12	5	1
Total No of Animals	688	110	225	217	117	19
No of Sows	314	39	86	124	59	6
No of Finishers	374	71	139	93	58	13
No of Animals OD < 10%	169 - 24.56%	54 - 49.09%	48 - 21.33%	47 - 21.66%	17 - 14.53%	3 - 23.07%
No of Sows OD < 10%	58 - 18.47%	10 - 25.64%	11 - 12.79%	33 - 26.61%	4 - 6.78%	-
No of Finishers OD < 10%	111 - 29.68%	44 - 61.97%	37 - 26.62%	14 - 15.05%	13 - 22.41%	3 - 23.07%
No of Animals OD > 10%	519 - 75.44%	56 - 50.91%	177 - 78.67%	170 - 78.34%	100 - 85.47%	16 - 84.21%
No of Sows OD > 10%	256 - 81.53%	29 - 74.36%	75 - 87.21%	91 - 73.39%	55 - 93.22%	6 - 100%
No of Finishers OD > 10%	263 - 70.32%	27 - 38.03%	102 - 73.38%	79 - 84.95%	45 - 77.59%	10 - 76.92%
No of Animals OD > 20%	329 - 47.82%	31 - 28.18%	107 - 47.56%	184 - 84.79%	56 - 47.86%	6 - 31.58%
No of Sows OD > 20%	173 - 55.1%	22 - 56.41%	50 - 58.14%	69 - 55.65%	30 - 50.85%	2 - 33.33%
No of Finishers OD > 20%	156 - 41.71%	9 - 12.68%	57 - 41%	60 - 64.62%	26 - 44.83%	0
No of Animals OD > 40%	122 - 17.73%	14 - 12.72%	38 - 16.89%	59 - 27.19%	10 - 8.55%	1 - 5.26%
No of Sows OD > 40%	64 - 20.38%	12 - 30.77%	18 - 20.93%	30 - 24.19%	3 - 5.08%	1 - 16.67%
No of Finishers OD > 40%	58 - 15.5%	2 - 2.82%	20 - 14.39%	29 - 31.18%	7 - 12.07%	-
No of Animals 40% < OD < 70%	111 - 16.13%	12 - 10.9%	25 - 11.11%	57 - 26.26%	10 - 8.55%	1 - 5.26%
No of Sows 40% < OD < 70%	58 - 18.47%	10 - 25.64%	11 - 12.79%	30 - 24.19%	3 - 5.08%	1 - 16.67%
No of Finishers 40% < OD < 70%	53 - 14.17%	2 - 2.82%	14 - 10.07%	27 - 29.03%	7 - 12.07%	-
No of Animals OD > 70%	11 - 1.6%	2 - 1.81%	7 - 3.11%	2 - 0.9%	-	-
No of Sows OD > 40%	6 - 1.91%	2 - 5.13%	4 - 4.65%	-	-	-
No of Finishers OD > 40%	5 - 1.34%	-	3 - 2.16%	2 - 2.15%	-	-

Table 1: *Descriptive results for Salmonella spp for sows and finishers based on serology.*

	Thessaly	Macedonia	Central Greece	Epirus	Crete
Thessaly	1				
Northern Greece	0,939072982	1			
Macedonia	0,84314398	0,941822167	1		
Epirus	0,910475885	0,997138042	0,947632736	1	
Crete	0,930553359	0,996114908	0.916101671	0,994229	1

Table 2: *Correlation efficient between five geographical areas.*

Number of positive isolates				
	Total	OD > 10%	OD > 20%	OD > 40%
Serotyping (NRL)	43	28	20	13
Microgen	45	31	23	13
API	50	35	25	15

Table 3: *Descriptive results for Salmonella spp. based on bacteriological isolation and serology with different cut off values.*

Prions are proteinaceous material which causes severe and fatal destruction to the host central nervous system manifesting as bovine spongiform encephalopathy (BSE) in cattle, scrapie in sheep and variant Creutzfeldt-Jakob Disease (vCJD) in humans. Also known as transmissible spongiform encephalopathies (TSEs), these are rare progressive neurodegenerative disorders that cause fatal infection of the host. Prions are transmitted to humans via the consumption of contaminated foods of bovine origin and can spread horizontally through blood transfusions with an extremely low infective dose which is undetectable. The infective agent has a prolonged incubation period making identification and treatment of symptoms difficult.

A total of 123 pigs were included in the study, of which 50 and 45 pigs were found *Salmonella* positive in at least one sample using the API 20E (Biomerieux, France) and the MicrogenTM GnA+B-ID (Microgen Bioproducts Ltd, UK) Systems respectively. However, the NRL identified 47 isolates as *Salmonella* spp. coming from 43 pigs. The isolates were assigned to fourteen serovars, the predominant of which was *S. Typhimurium* (11 isolates), followed by *S. enterica* subsp. *enterica* ser. 4,5,12:i:- (9), *S. enterica* subsp. *enterica*. 6,7:k:- (7), *S. enterica* subsp. *enterica* ser. 4,12:i:- (4), *S. Bredeney* (3) and one each for serovars *S. Agona*, *S. Derby*, *S. Infantis*, *S. Meleagridis*, *S. Cerro, S. enterica* subsp. *enterica* ser. 6,14,25: - : 1,2, *S. enterica* subsp. *diarizonae* 61:k:1,5, *S. enterica* subsp. *salamae* 38:b:1,2 and *S. enterica* subsp. *houtenae* 40:g,t:-. Four isolates were registered as 'Rough'. These are *Salmonella* isolates, where the surface antigens have changed so that they cannot be serotyped. The descriptive results for the bacteriology and serology using the different cut-off values are given in tables 4-8.

	Serologically negative	Serologically positive	Total
NRL negative	35	45	80
NRL positive	15	28	43
Total	50	73	123

Table 4: *Two by two table for the Salmonella isolation results and the serological test using the cut-off value of OD 10%.*

	Serologically negative	Serologically positive	Total
NRL negative	53	27	80
NRL positive	23	20	43
Total	76	47	123

Table 5: *Two by two table for the Salmonella isolation results and the serological test using the cut-off value of OD 20%.*

	Serologically negative	Serologically positive	Total
NRL negative	68	12	80
NRL positive	30	13	43
Total	98	25	123

Table 6: *Two by two table for the Salmonella isolation results and the serological test using the cut-off value of OD 40%.*

	NRL	Microgen	API
OD 10%	0,442	0,128	0,062
OD 20%	0,178	0,034	0,037
OD 40%	0,049	0,103	0,039
Microgen	< 0,01		
API	< 0,01		

Table 7: *Results of chi-squared tests for serology, NRL, Microgen and API.*

OD > 10%		OD > 20%		OD > 40%	
No	%	No	%	No	%
73	59,35%	47	37,21%	25	20,33%

Table 8: *Descriptive results for Salmonella spp for finishers based on serology.*

Based on the analyses of chi-squared tests (Table 7) dependence between ELISA and serotyping (NRL) was observed only when using the cut-off value OD 40% ($p = 0.049$). Furthermore, the OR (95% CI) for an animal being Salmonella positive for the cut-off values of 10%, 20% and 40% were 1.452 (0.674 - 3.127), 1.707 (0.800 - 3.641) and 2.456 (1.004 - 6.007) respectively. The individual results for the Salmonella isolation and the serological results for the cut-off values of OD 10%, 20% and 40% are shown in tables 4-6 respectively. Moreover, dependence between ELISA and monophasic variants was also observed when using the cut-off values of OD 20% and OD 40% ($p = 0.029$, $p < 0.000$ respectively).

Furthermore both API 20E (Biomerieux, France) and the MicrogenTM GnA+B-ID (Microgen Bioproducts Ltd, UK) showed strong correlation with the results of the NRL ($p < 0.01$). With regards to serology the Microgen showed correlation only at a cut-off of 20% ($p = 0.034$), while the API correlated well to the cut-offs of OD 20% and OD 40% ($p = 0.037$ and $p = 0.039$ respectively) (Table 7). As for the monophasic variants chi-squared tests showed correlation at the cut-offs of 20% and 40% ($p = 0.029$ and $p < 0.000$ respectively).

Discussion

Since pigs can carry *Salmonella* without any symptoms of disease [20], subclinically infected animals may enter a herd without raising suspicion and spread the infection to other pigs. In this study, seropositive sows were found in herds with seropositive slaughter pigs. This may indicate the occurrence of vertical transmission of infection or merely reflect a generally established *Salmonella* contamination of these farms. Either way, it is suggesting exposure to *Salmonella* and thus constituting a potential risk of introducing infection to a new herd. The findings of the present investigation indicate that seroprevalence is relatively high in Greece. The statistical analyses of seroprevalence between sows and slaughter pigs showed the potential important epidemiological role of sows to the infection with Salmonella spp. Perhaps, the status of sows in a herd could be used as an indication of *Salmonella* prevalence among slaughter pigs. Sows, being carriers of the pathogen for extended periods, are an important risk factor of the pathogen's dissemination among finishers and of environmental contamination [21-23], making imperative the adaptation of effective control measures, as the implementation of all-in/all-out practices, cleaning and disinfection, firstly at the sow level [24].

It should be noted that by varying the cut-off value, the sensitivity and specificity of the test will change [15]. Thus, by increasing the test cut off value the sensitivity decreased. The scientific cut-off value OD% > 10 firstly applied by Nielsen., et al. [9], showed here particularly high rates of prevalence, as 81.53% and 70.32% of the sows and finishers were seropositive respectively. Increasing the cut-off of OD% > 10 to OD% > 40, the sensitivity dropped dramatically to 20.38% for sows and 15.5% for finishers (Table 1).

However, till today, in pork supply chains for most European countries, as in Greece, serological diagnosis of *Salmonella* infections has not yet been implemented into pig herds, thus they have neither a formal control system nor payment differentials based on *Salmonella* contamination, so there is no direct incentive for producers to reduce the *Salmonella* prevalence. The majority of food safety problems, however, have their origin in the primary production stages prior to slaughter and processing [25]. Since the European Union makes it mandatory for all member states to test pork and pork products for all *Salmonella* serovars with public health significance [5-7], the implementation of national screening and surveillance programmes is required. Such programmes are based on bacteriological or, mainly, serological testing or a combination of both with their advantages and disadvantages.

Serological testing aims in the detection of *Salmonella* antibodies against the somatic O-antigens. All available *Salmonella* ELISAs, known as 'mix-ELISAs' are based on Lipo-Poly-Saccharide (LPS) antigens, specific for each serogroup, including a combination of O-antigens and theoretically detecting most occurring serovars in Western-Europe and the United States [26]. Though *Salmonella* is present in almost all parts of the world, the serovars that are most common vary both locally and over time. Thus different countries have different spectra of serovars or cross-reacting bacteria. The serological test used in the present study was able to detect all serovars belonging to serogroups B, C1 and D. However, positive serological results were also found in pigs from which serovars belonging to other serogroups were isolated. These pigs could have been infected with other serovars than the one isolated, at the same time or earlier in their life. On the other hand, *Salmonella* serovars were isolated harbouring O-antigens which the mix- ELISA was not able to detect, as serovars Meleagridis, Cerro, *S. enterica* subsp. *enterica* ser. 6,14,25: - : 1,2, *S. enterica* subsp. *diarizonae* 61:k:1,5, *S. enterica* subsp. *salamae* 38:b:1,2 and *S. enterica* subsp. *houtenae* 40:g,t:- (Table 9). Although some infected pigs might have been misclassified, uninfected pigs would have been very unlikely to be misclassified. However, for a surveillance programme, it is important to know how much of the prevalent *Salmonella* spectrum is covered by the test in the country where it is used and the epidemiological importance of the serovars which are missed. Unfortunately, in Greece little data is available on the prevalence and the distribution of serovars of salmonellae in pigs, and thus it was not possible to estimate the detectable proportion [27,28]. In the present study, *S. Typhimurium* and its monophasic variants, a high risk for consumers [29], showed the highest seroprevalence, most probably because of the sensitivity of the used ELISA kit towards this serovar [9] and of its ability to maintain chronic infections [30]. Thus, a matter of issue among scientists and policy-makers is whether a surveillance and control programme should encompass all *Salmonella* or only those serovars which are of public or animal health concern. Therefore, it can be argued that, despite the fact that a limited number of serovars is responsible for most cases of human and animal salmonellosis, thus of minimal epidemiologic importance, all *Salmonella* serovars should be regarded as potential zoonotic pathogens [31,32]. Ideally, ELISAs being a tool for *Salmonella* surveillance should encompass all *Salmonella*, since it is impossible to predict which serovars will increase or decline in importance with regards to public health [33]. In practice, epidemiological surveys for the endemic serovars should be undertaken periodically, so as the test to be adjusted to the country-specific distribution of *Salmonella* serovars, incorporating O-antigens relevant for a particular region.

EFSA [34] stated that the ELISA serological test is a valuable tool for on-farm *Salmonella* surveillance purposes, suggesting that a pig, seropositive for *Salmonella* is twice as likely to *yield a Salmonella* infected carcass. As the intention of these programmes is to detect *Salmonella* infected herds, it is important to know to what extent serological screening methods and the bacteriological isolation of *Salmonella* correlate. Many authors have reported correlations between both diagnostic techniques and agreed that serological testing is a good method for screening at the herd level, but individual correlation is low [11,35]. Serological techniques have proven to be convenient and cost effective methods for screening for antibodies against *Salmonella* [10]. Though serological results cannot distinguish between current and past infections, they are a measure of historical exposure to the pathogen, as they indicate exposure to *Salmonella* at one stage of production prior to sampling. They will not detect infections that occurred shortly (1 to 2 weeks) before sampling, since no measurable specific antibodies would have been developed [11,36]. Perhaps, due to this reason, in the present study isolated serovars detectable by the used kit (*S. Typhimurium, S. enterica* subsp. *enterica* ser. 4,5,12:i:-, *S. enterica* subsp. *enterica* ser. 4,12:i:-, *S. Agona, S. Bredeney, S. Derby, S. Infantis* and *S. enterica* subsp. *enterica*. 6,7:k:-) (Table 9), came from seronegative animals. Thus, serological methods identify previous exposure by detecting the presence of specific antibodies against *Salmonella* using ELISA. This method can identify carriers or animals that are already clear of infection. Serology indirectly reflects exposure to *Salmonella* and therefore indicates different serologic stages including pigs that are no longer harbouring the bacteria, carriers and shedders (Koppen., *et al.* 2001).

			No of negative serum samples (%)	No of positive serum samples at different cut-off values		
Isolate	**Serogroup**	**No of isolates**	**OD < 10%**	**OD > 10%**	**OD > 20%**	**OD > 40%**
S. Typhimurium	B	11	4 (36.36%)	6 (54.54%)	6 (54.54%)	4 (36.36%)
S. enterica subsp. *enterica* ser. 4,5,12:i:-	B	9	0	9 (100%)	9 (100%)	7 (77.78%)
S. enterica subsp. *enterica* ser. 4,12:i:-	B	4	2 (50%)	2 (50%)	2 (50%)	2 (50%)
S. Bredeney	B	3	1 (33.33%)	2 (66.67%)	0	0
S. Agona	B	1	1 (100%)	0	0	0
S. Derby	B	1	0	1 (100%)	1 (100%)	0
S.enterica subsp. *enterica*. 6,7:k:-	C_1	7	2 (28.57%)	5 (71.43%)	4 (57.14%)	3 (42.85%)
S. Infantis	C_1	1	0	1 (100%)	1 (100%)	0
S. Meleagridis	E_1	1	1 (100%)	0	0	0
S. Cerro	K	1	1 (100%)	0	0	0
S.enterica subsp. *enterica* ser. 6,14,25: - : 1,2	H	1	0	1 (100%)	1 (100%)	0
S. enterica subsp. *diarizonae* 61:k:1,5	61	1	1 (100%)	0	0	0
S. enterica subsp. *salamae* 38:b:1,2	P	1	0	1 (100%)	1 (100%)	0
S. enterica subsp. *houtenae* 40:g,t:-	R	1	1 (100%)	0	0	0
Rough[a]		4	1 (25%)	3 (75%)	0	0
Total		47	15 (31.91%)	31 (65.96%)	25 (53.19%)	16 (34.04%)

Table 9: *Serological results and serovars isolated from pigs belonging to variant serogroups.*

[a]: Isolates, where the surface antigens have changed so that they cannot be serotyped.

On the other hand, bacteriological methods, being the gold standard as they will leave little doubt to the presence of the bacteria, express the actual infection status of the animal, including recent transmission or contamination and detect all serovars. The ELISA test has been established as being useful in detecting the prevalence of *Salmonella* on-farm, especially well-suited for identifying high prevalence herds [37], but it is not able to identify individual pigs which are shedding *Salmonella* at the point of slaughter [38]. Moreover slaughter pigs can still harbour *Salmonella* spp. in tissues, as the mesenteric lymph nodes, without being detected in serological tests [11]. A bacteriological test on feces from individual pigs leaving the farm would be the definitive measurement of the on-farm status of individual pigs. Currently testing of individual pigs is impractical due to the time and labour required to sample and the prohibitive cost of analysis. Detection of *Salmonella* in subclinically infected herds, which are much more frequently encountered than herds with clinical signs, can be difficult. Moreover, present culture methods are time-consuming and laborious, requiring pre-enrichment, selective enrichment, indicative plating and bio/serotyping and it may therefore not be practically and economically feasible for most countries to apply these methods routinely for large scale monitoring at the pre-harvest level of pork production. Furthermore, due to the low sensitivity of culture methods caused by the intermittent shedding and low counts of bacteria [1], positive pigs can be missed and apparent 'false-positive' serological results may well represent real infections at one point during production not detected by bacteriological testing [39]. Moreover, in reference *Salmonella* laboratories, the final identification of *Salmonella* is usually done using the Kauffman-White serotyping scheme. However, a minor proportion of *Salmonella* suspect isolates may lack the O-antigens (rough isolates), or may not be serotypeable at all or misclassified [27,40]. Therefore, isolation and identification of *Salmonella* continues to be a challenge, met by incessant development of new media and rapid diagnostic tests [8]. However, in cases where it is important to verify infection with a specific serovar (i.e. trace-back procedures or outbreak investigations) culture methods cannot be replaced by serological tests which react to infections with a variety of serovars.

A central question is how to describe the association between serology and bacteriology. One factor affecting this estimation may be the selected cut-off, the appropriateness of which requires careful consideration. In this study 30.23% (cut-off OD 40%) to 65.11% (cut-off OD 10%) of the animals from which *Salmonella* had been isolated were seropositive. The OR for being *Salmonella* culture positive for OD% above versus below the cut-off value was higher for the cut-off value of OD 40% than for OD 20% and 10%. The higher cut-off (OD 40%) for serological testing had thus more discriminatory power in detecting the *Salmonella* harbouring pigs in comparison with lower cut-off values (OR (95% CI) 2.456 (1.004 - 6.007), a result similar to former studies concluding that there is an increase in the probability of finding *Salmonella* with increasing seropositivity [27,28]. Thus, a weak agreement was found between bacteriology and serology for *Salmonella* diagnosis only at a cut off of OD 40%. However, with increasing the cut-off value, the chance of false negative results will increase, since serovars other than *S. Typhimurium* will often only give a moderate serological response [41]. The risk of failing to detect these infections serologically increases at higher cut-off values. On the other hand, a high cut-off value produces fewer positive samples, making it acceptable for all players in the pork chain to initiate a surveillance programme in a country. Later, when the national *Salmonella* prevalence will be reduced to a certain level, the cut-off value can be reduced [35].

It could be stated that, discrepancy between serology and the microbiological assessment does not indicate that the ELISA test is better or worse than bacteriological isolation. Using the strengths of both methods and compensating for their weaknesses, serological testing can be used as a monitoring tool, indicating exposure to *Salmonella* at one point during production, and bacteriological testing as a means to confirm and locate a current infection in herds. Thus, serology helps to identify the infected population, but it is not useful for determining the infection status of individual animals [42,43]. It should be kept in mind, given the dynamic nature of pig production, that a *Salmonella* herd status cannot be based on results of a single sampling round, since it can change over time [44,45]. Therefore, regular testing is necessary to enable producers, advisors and authorities to react to sudden increases in *Salmonella* prevalence in single herds or at a national level [46].

Conclusion

Food safety assurance strategies against *Salmonella* spp. can be implemented at all levels of food production (i.e. pre-harvest, post-harvest, processing and retail). However, surveillance and control at the pre-harvest level are important aspects of food safety assurance strategies to prevent or reduce the transmission of microbiological contamination at the harvest level of pork production. For this purpose serology has been used to determine the prevalence of salmonellae on pig farms and has been adopted by several countries as Denmark [14] and Germany [13] into national control programs designed to reduce occurrence of salmonellae on the farm and in pork.

Although both bacteriological methods and serology are valuable for food safety assurance, as a measure of the presence of *Salmonella* on a herd-level, serological testing can be regarded as both a sensitive and practical method, which forms the basis for targeted sampling, intervention and logistic slaughter procedures. Whether *Salmonella* ELISA testing should be adopted in national surveillance programs as a method of reducing foodborne disease caused by *Salmonella* serovars depends upon the ability of the test to detect indigenous serovars, availability and ease of testing and cost per sample. Additional considerations include correlation of the serologic response with true subclinical infection, as well as the determination of the cut-off value used to evaluate a sample or a herd as serologically positive.

Identification of infection, rather than disease, is the challenge at farm level for control of Salmonella in pork. Elimination of salmonellae in low prevalence situations or reduction in higher prevalence situations has been the focus for control of zoonotic salmonellae in swine. The ELISA test is a 'predictor of risk, not a statement of absolute microbiological negativity or positivity' [47].

Conflict of Interest

The authors declare no financial or any other conflict of interest.

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