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A European longitudinal study in *Salmonella* seronegative- and seropositive-classified finishing pig herds

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SUMMARY

Surveillance and control are important aspects of food safety assurance strategies at the pre-harvest level of pork production. Prior to implementation of a *Salmonella* surveillance and control programme, it is important to have knowledge on the dynamics and epidemiology of *Salmonella* infections in pig herds. For this purpose, 17 finishing pig herds initially classified as seropositive and 15 as seronegative, were followed for a 2-year period through serological and bacteriological sampling. The study included 10 herds from Denmark, 13 from The Netherlands, 4 from Germany and 5 from Sweden and was performed between October 1996 and May 1999. The *Salmonella* status of finishing pig herds was determined by an initial blood sampling of approximately 50 finishing pigs close to market weight per herd. The development of the *Salmonella* status of the selected herds was assessed at seven subsequent sampling rounds of 25 blood samples from finishing pigs, 25 blood samples from grower pigs and 10 pen faecal samples each, approximately 3 months apart. The odds for testing finishers seropositive, given that growers were found seropositive previously were 10 times higher than if growers were seronegative (OR 10·0, 95% CI 3·2–32·8). When *Salmonella* was isolated from pen faecal samples, the herd was more likely to be classified seropositive in the same sampling round, compared to no *Salmonella* being detected (OR 4·0, 95% CI 1·1–14·6). The stability of an initially allocated *Salmonella* status was found to vary noticeably with time, apparently irrespective of a seropositive or seronegative classification at onset of the study. Given the measured dynamics in the occurrence of *Salmonella* in pig herds, regular testing is necessary to enable producers, advisors and authorities to react to sudden increases in the *Salmonella* prevalence in single herds or at a national level.

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INTRODUCTION

Food safety assurance strategies can be implemented at all levels of food production (i.e. pre-harvest, post-harvest, processing and retail). Monitoring, prevention and control efforts at the pre-harvest level are important elements of food-safety assurance strategies to prevent or reduce the transmission of microbiological contamination at the harvest level of pork production [1]. In contrast to clinical salmonellosis, subclinical *Salmonella* infections in pigs are common in many pig-producing countries [2–5]. Since pigs can carry *Salmonella* without obvious symptoms of disease, there is a need for tools that can be used to identify herds housing infected animals, which ultimately can lead to contaminated pork products for human consumption.

Prior to implementation of a surveillance and control programme, it is important to have knowledge of the dynamics and epidemiology of *Salmonella* infections in pig herds. Pig production is a dynamic process with intensive traffic of animals and feedstuffs. Each contact with the outside world, be it introduction of new stock, feed, humans, pets, birds, wildlife or other, constitutes a potential risk for the introduction of infection. Questions regarding herd incidence, persistence of infection or contamination and possible seasonal patterns need to be answered. In addition, the tools used to assess the *Salmonella* status of a herd need to be evaluated in a practical setting over a period of time. Therefore, this study sets out to investigate these aspects of *Salmonella* epidemiology, by following finishing pig herds over a period of time through serological and bacteriological sampling.

The study was part of a larger international research project, entitled '*Salmonella* in Pork (SALINPORK)' [6] and was mainly funded by the European Commission. The objective of this study was to investigate the stability of *Salmonella* herd status over time, seasonal variation in the incidence of herd infections, the herd incidence of *Salmonella* infections from the grower to the finisher production stage and the relation between serological and bacteriological herd classification. In order to assess these factors, finishing pig herds classified as seropositive and seronegative were followed over a 2-year period, by serological and bacteriological testing. The study included finishing pig herds from Germany, Denmark, The Netherlands and Sweden and was performed between October 1996 and May 1999.

MATERIALS AND METHODS

Herd selection

A subsample of herds participating in an international risk factor study [7] was selected for this study, based on their serological *Salmonella* herd status. Within-herd seroprevalence was determined by blood sampling of approximately 50 finishing pigs close to market weight. A herd was assigned seropositive *Salmonella* status if more than 4% of the blood samples from finishers tested positive at a cut-off of 10 OD% (optical density percentage), allowing for a maximum of two false-positive results out of 50 blood samples for herd selection purposes. As always, the final inclusion criterion in this study was the farmer's willingness to participate. Due to practical circumstances, some variation in the selection process between countries occurred, as described below.

Germany

In Germany, four farmers (two seronegative and two seropositive herds) were willing to take part in the longitudinal study. After two visits one farmer stopped the project because of an outbreak of Classical Swine Fever close to his herd.

Denmark

Five seronegative and five seropositive herds were selected, based on the results from the preceding 4 months, obtained from the Danish routine serological meat juice survey [1].

The Netherlands

Five seronegative and five seropositive herds were selected. However, due to the Classical Swine Fever outbreak in The Netherlands from February 1997 to April 1998, one participant decided not to cooperate any longer and three farmers were forced out of production before completion of the study. To compensate for the loss of follow-up sampling rounds, two additional seropositive herds were included in the study, as well as a second pig house in one of the original seropositive herds, which was analysed here as a separate herd.

Sweden

Selection of finishing pig herds for the follow-up study was focused on selection of different areas due to

differences in the seroreactions between areas [8]. Two herds from the south of Sweden were selected (an area with high prevalence of seroreactors), two herds from the middle of Sweden (low prevalence of seroreactors) and one herd from the north of Sweden (low prevalence of seroreactors).

Sample collection and analysis

The *Salmonella* status of a finishing pig herd was determined by an initial blood sampling round (see Herd selection above). The development of the *Salmonella* status of the selected herds was assessed by seven subsequent sampling rounds. At each round, 25 blood samples from finishers close to slaughter and 25 blood samples from growers (with a minimum weight of 20 kg) were taken. In addition, 10 pen faecal samples of approximately 25 g, each representing $\geqslant 5$ pigs, were taken per visit. Testing was done approximately 3 months apart, so that each herd was to be followed for 2 years. To study the development of the seroprevalence from one age group to the next, pens with growers which were sampled in one round were to be sampled again 3 months later in the next round when pigs had reached the finishing stage.

Blood samples were analysed for antibodies against *Salmonella* O-antigens 1, 4, 5, 6, 7 and 12 with an indirect mix ELISA [9, 10]. Results were expressed as optical density percentage of a known positive (OD%). Samples with an OD% >10 were considered positive, which is usually referred to as the scientific cut-off [9]. Each participating country performed the microbiological analysis of their own faecal samples in four steps (i.e. pre-enrichment, enrichment, plating out and confirmation), using a method referred to as NMKL71 [11]. Serotyping of *Salmonella* isolates was performed according to the Kaufmann–White scheme [12] using the routine procedure of each participating laboratory. Non-typable strains or autoagglutinable strains (rough) were verified as *Salmonella enterica* by conventional biochemical analysis. All isolates were forwarded to the Danish Institute for Food and Veterinary Research for further typing.

During follow-up, a herd was classified as seropositive if more than 4% of the blood samples from finishing pigs were positive, allowing for one false-positive result out of 25 samples. A herd was considered bacteriologically positive if *Salmonella* was isolated from one or more pen faecal samples.

Statistical analysis

Stability of a Salmonella *status during the follow-up period*

Exploratory analyses were performed to compare the mean duration of an initially allocated seronegative or seropositive *Salmonella* status (i.e. period until first change of status), using a non-parametric one-way ANOVA (PROC NPAR1WAY) [13] on rank scores. The average duration of a steady state, i.e. any single period without status shift, was compared between the two initial *Salmonella* statuses with a *t* test, after testing the assumption of normality by using a procedure that produces univariate statistics and information on the distribution of numeric variables (PROC UNIVARIATE). The association between the probability of a herd being classified as seropositive and the initial *Salmonella* herd status was modelled in a repeated-measures logistic regression analysis, specifying a compound symmetry correlation structure between steady-state periods within the same herds. This correlation structure applies only to observations within the same grouping level, while observations with different grouping levels are assumed to be uncorrelated.

Correlation between the serological status of growers and finishers

The correlation coefficient between the proportion of seropositive growers in one sampling round and seropositive finishers in the following [i.e. cross-autocorrelation (growers$_t$, finishers$_{t+1}$)] was calculated using Spearman's rank correlation test. The probability of finishing pigs testing seropositive, given the status of grower pigs in the previous sampling round was also modelled in a repeated-measures logistic regression. The correlation between subsequent sampling rounds within each herd was taken into account by specifying a first-order autoregressive correlation matrix in the SAS procedure for generalized linear models, PROC GENMOD [repeated subject = herd/type = ar(1);] [13]. Herd factors, collected in the European risk factor survey [7] were screened as covariates together with country of origin and type of feed used (tri-variable screening). Candidate variables with a *P* value of $\leqslant 0{\cdot}25$ were included in a full model (including two-factor interaction terms) which was subsequently reduced by backwards elimination. The final model only included variables with a *P* value of $\leqslant 0{\cdot}05$. Predictive values of the grower status for the

Table 1. *Frequency distribution of the within-herd* Salmonella *seroprevalence of 32 herds at onset of a longitudinal study of seropositive- and seronegative-classified finishing pig herds in Germany, Denmark, The Netherlands and Sweden*

Initial herd status	Within-herd sero-prevalence (%)	No. of herds	% of herds	Cumulative % of herds
Seronegative	0	9	60·0	60·0
	1–2	1	6·7	66·7
	3–4	5	33·3	100
	Total	15	100	
Seropositive	5–25	3	17·6	17·6
	26–50	3	17·6	35·3
	51–75	5	29·4	64·7
	>75	6	35·3	100
	Total	17	100	

Table 2. *Frequency distribution of the number of herds shifting from one* Salmonella *status to the other during the observation period during a longitudinal study of 17 seropositive- and 15 seronegative-classified finishing pig herds in Germany, Denmark, The Netherlands and Sweden. Herds were assigned a seropositive status if more than 4% of sample were >10 OD%*

Initial *Salmonella* status	No. of status shifts	No. of herds	% of herds
Seronegative herds	0	6	40
	1	2	13
	2	6	40
	3	1	7
	Total	15	100
Seropositive herds	0	6	35
	1	8	47
	2	2	12
	3	1	6
	Total	17	100

status of finishers in the subsequent sampling round were calculated from a 2×2 table.

Correlation between serological and bacteriological herd classification

The association between serological and bacteriological classification of *Salmonella* herd status was modelled in a repeated-measures logistic regression analysis, specifying a first-order autoregressive correlation matrix in PROC GENMOD in SAS [13]. Covariates for the multiple linear logistic regression were found with the same procedure as described above (i.e. trivariable screening and backwards elimination). To assess whether the association differed between and within herds, a random effects model was specified using the GLIMMIX macro from SAS [13], where parameter estimates were allowed to vary within and between herds. Possible seasonal variation in the occurrence of subclinical *Salmonella* infections was evaluated in these models in several ways: by including month of observation, by defining four seasons, and by weighting calendar months as 'grades of summer' by assuming a linear association (i.e. July = 7; June and August = 6; May and September = 5; April and October = 4, etc.).

In all models, 'country of origin' is included to adjust for between-country variation and possible differences in test performance within countries. Since the subsample of herds can not be regarded as representative for the entire pig production in the participating countries, the parameter estimates for 'country' are not meaningful and are, therefore, not reported here.

RESULTS

A total of 32 finishing pig herds participated in the longitudinal study, 17 of which were initially classified as seropositive and 15 initially as seronegative. In total, 9844 blood samples and 1506 pen faecal samples were analysed. Table 1 shows the frequency distribution of the within-herd seroprevalence of the selected herds at onset of this study.

Stability of *Salmonella* status during the follow-up period

In order to investigate the stability of an assigned *Salmonella* status, the number of times a herd changed status during the observation period was recorded. Table 2 shows the frequency distribution of the number of status shifts for herds initially designated seronegative and seropositive. The number of status shifts varied from 0 to 3.

Overall, 12 out of 32 herds (38%) continued to have the same *Salmonella* status as recorded at the start of the study during the observation period. Among the six seronegative herds (40%) that remained negative, three herds were sampled over the entire 2-year period, while sampling was stopped

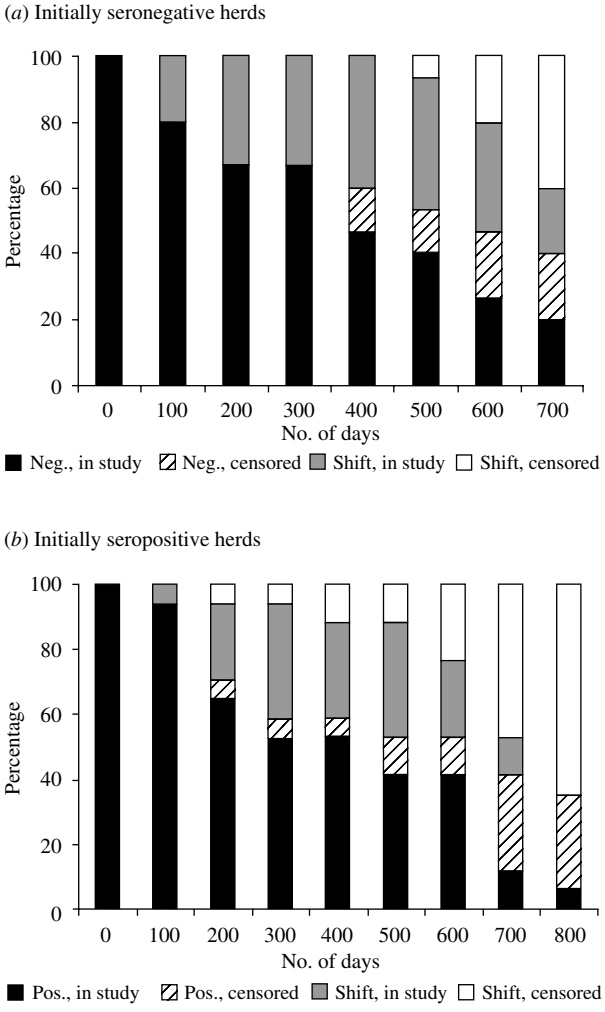

Fig. 1. Survival curves over the duration of an initial seronegative (*a*) and initial seropositive (*b*) *Salmonella* status in periods of 100 days in a longitudinal study of 32 finishing pig herds in Germany, Denmark, The Netherlands and Sweden.

prematurely for the remaining three herds (after 12–18 months). Among the six seropositive herds (35%) that remained seropositive, only one herd was followed over the entire 2-year period. Two of the seropositive herds were sampled after 6 and 15 months respectively, and three herds were missing 5–13 months between sampling rounds. Six out of nine seronegative herds, which became seropositive, returned to their negative status after 3–7 months. Three negative herds had already changed status in the first sampling round, two of which returned to their negative status within two sampling rounds. The period of time it took for eight seropositive herds to become seronegative during the observation period varied from 2 to 22 months.

Table 3. *Comparison of the average duration in days* (S.D.) *between and within steady* Salmonella *status for initial seronegative and seropositive classification of 32 finishing pig herds in a longitudinal study performed in Germany, Denmark, The Netherlands and Sweden, using the t test*

	Initial status		
	Seronegative	Seropositive	
Average duration of steady status			
Seronegative (S.D.)	384 (218)	304 (146)	$P=0{\cdot}3285$
Seropositive (S.D.)	214 (132)	396 (274)	$P=0{\cdot}0287$
	$P=0{\cdot}0424$	$P=0{\cdot}3543$	

Figure 1 shows the percentage of herds maintaining their initial status over the observation period. Herds which withdrew from follow-up without changing status, as well as withdrawal of herds after changing status are indicated as censored. No significant difference in the mean duration of the initial status between seropositive and seronegative herds could be shown in a non-parametric one-way ANOVA ($P=0{\cdot}5457$, Wilcoxon two-sample test). On average, herds that were classified as seronegative in the initial sampling round, remained negative for 381 days, ranging from 49 to the end of the follow-up of seronegative herds at 695 days. Seropositive herds stayed positive for 419 days on average, ranging from 33 to the end of the follow-up of seropositive herds at 861 days. No evidence of seasonal variation in the mean duration of the initial status was found. The average length of a steady-state period for both initially seropositive and seronegative herds is shown in Table 3. There was no difference in the average duration of a seronegative period between initially seronegative and seropositive herds ($P=0{\cdot}3285$, *t* test).

However, initially seronegative herds were classified as seronegative for longer periods than they were classified as seropositive ($P=0{\cdot}0424$, *t* test). The average duration of positive *Salmonella* status was found to be significantly longer for herds initially classified as seropositive compared to herds initially classified as seronegative ($P=0{\cdot}0287$, *t* test). During the observation period, the duration of seropositive *Salmonella* status did not differ significantly from the duration of seronegative status for herds that were initially classified as seropositive ($P=0{\cdot}3543$, *t* test).

Table 4. *The final model describing the association between the probability of a herd being classified as seropositive depending on the initial* Salmonella *herd status, the duration of a steady status and the type of feed used in a herd, in a longitudinal study of 32 finishing pig herds from Germany, Denmark, The Netherlands and Sweden*

Variable	Level	OR	95% CI*	*P* value
Initial herd status	Seropositive	7·54	3·37–16·85	<0·0001
	Seronegative	1		
Steady state	No. of days	0·99	0·99–1·00	0·0451
Feed type	Wet	0·32	0·17–0·60	0·0003
	Dry	1		

* Likelihood ratio based confidence limits.
OR, Odds ratio; CI, confidence interval.

Table 5. *The odds ratio, positive and negative predictive value of the serological* Salmonella *classification of grower pigs in one sampling round* (*n*) *compared to the classification of finishing pigs in the next round* (*n*+1), *based on 115 correlated sampling rounds, in a longitudinal study of 32 seropositive and seronegative classified finishing pig herds in Germany, Denmark, The Netherlands and Sweden*

	Finishers (round *n*+1)		
	Seropositive	Seronegative	Total
Growers (round *n*)			
Seropositive	18	6	24
Seronegative	21	70	91
Total	39	76	115

Odds ratio, 10·0; positive predictive value, 0·75; negative predictive value, 0·77.

The logistic regression model showed that the odds for an initially seropositive herd to be classified as seropositive during follow-up, is approx. 7·5 times the odds for an initially seronegative herd (see Table 4), after multiple status periods within herds, the effect of country, feed type and the length of a steady-state period have been considered. Being fed wet feed appears to have a protective effect against seropositive herd classification. With an increasing length of a steady herd status, the probability of a seropositive herd classification decreases. This effect is small (OR 0·99) because it represents a decreased probability per extra day of steady status. Recalculated for an extra 30-day period yields an odds ratio (OR) of 0·91 [95% confidence interval (CI) 0·833–0·997].

Correlation between the serological status of growers and finishers

Since animals were not individually identified, and it was possible that different pens were sampled at the next sampling round, it was investigated on a herd level whether the status of growers in one sampling round was correlated to the status of finishers in the next sampling round. In total, 115 observations from 29 herds were available where results from growers in one round could be linked to results from finishers in the next sampling round. In 70 correlated sampling rounds (61%), both growers and finishers were seronegative. On 21 occasions (18%), growers were seronegative, while the finishers in the subsequent sampling round were seropositive. Both growers and finishers were seropositive in 18 sampling rounds (16%). In the entire study, only six herds had a single sample round with low proportions of seroreactors (1 or 2 positive out of 25 samples) among growers when no seropositive samples were found among finishers. No *Salmonella* was isolated in these sampling rounds. Table 5 shows these scenarios in a 2×2 table.

The odds for finishers testing seropositive, given that growers were seropositive in the previous sampling round were 10 times higher than if the growers were seronegative (OR 10·0, 95% CI 3·2–32·8). In a repeated-measures logistic regression analysis, the effect of between-country variation ($P=0{\cdot}0495$) and the initial herd status ($OR_{seronegative}$ 0·30, 95% CI 0·12–0·79, $P=0{\cdot}0149$) on this estimate were found to be significant. However, correcting for these factors resulted in almost identical odds (OR 9·5, 95% CI 2·6–34·5, $P=0{\cdot}0006$). The positive predictive value of a seropositive sampling round among growers was 0·75, while the negative predictive value was 0·77. Figure 2 shows a lag-plot of the correlation between growers in one sample round and finishers in the following round in the same herd.

Two distinct correlation patterns could be identified. First, in herds with a high proportion of seroreactors among growers (i.e. >0·25) in one sample round, a high proportion of seroreactors among finishers was found in the subsequent sampling round (28–100%). Across countries and herds, finding more than 4% seropositive samples among growers in one sample round (i.e. allowing for one possible false-positive sample), was positively correlated with

Table 6. *Overview of* Salmonella *serotypes isolated from grower and finishing units in a longitudinal study of 32 seropositive- and seronegative-classified finishing pig herds in Germany, Denmark, The Netherlands and Sweden. Note that the totals in this table do not represent prevalence estimates, since herds were not selected randomly*

	Germany		Denmark		The Netherlands		
Age group	Serotype	*n*	Serotype	*n*	Serotype	*n*	Sweden
Growers	*S*. Typhimurium	5	*S*. Typhimurium	11	*S*. Typhimurium	3	
	S. Derby	1	*S*. Tennessee	3			
Totals	6/115 (5·2%)		14/332 (4·2%)		3/150 (2·0%)		0/35 (0·0%)
Finishers	*S*. Typhimurium	2	*S*. Typhimurium	24	*S*. Typhimurium	4	
	S. Rough	1	*S*. Tennessee	1	*S*. London	3	
					S. Panama	2	
					S. Infantis *S*.	1	
					Bovis morbificans	1	
					S. O21:-:-	1	
Totals	3/115 (2·6%)		25/372 (6·7%)		12/247 (4·9%)		0/140 (0·0%)

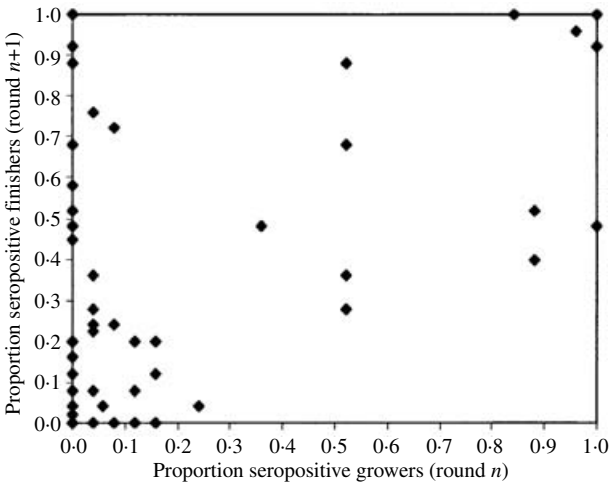

Fig. 2. Lag-plot of the relation between the proportion of growers that test seropositive for *Salmonella* in one sampling round and the proportion seropositive finishers in the next round in the same slaughter pig herds in a longitudinal study of 32 finishing pig herds in Germany, Denmark, The Netherlands and Sweden.

finding seropositive finishers in the next sampling round ($r=0{\cdot}72$, $P<0{\cdot}0001$, Spearman). Secondly, in herds with lower proportions of seroreactors among growers (i.e. $<0{\cdot}16$), both low and high proportions (from 0·0 up to 1·0) of seropositive finishers were observed in the following sampling round.

Correlation between serological and bacteriological herd classification

Table 6 shows an overview of the *Salmonella* serotypes that were isolated from both grower and finisher pens in each country during the longitudinal study. *S*. Typhimurium was the predominant serotype in all participating countries in both age categories. In The Netherlands, the diversity of isolated serotypes was larger among finishing pigs than grower pigs. Figure 3 shows the proportion of culture-positive pen faecal samples in correlation to the proportion seropositive samples in the same sampling round for growers (triangular markers) and finishers (diamond-shaped markers). A total of 84% of all *Salmonella* isolations were accompanied by a serological response.

In 74% of all sampling rounds, serological and bacteriological herd classification were in agreement. In eight sampling rounds taken in five different herds, *Salmonella* was cultured from pen faecal samples when the herd was classified as seronegative. In three cases, *S*. Typhimurium was isolated, which was accompanied by a weak serological response (i.e. 4% of samples were seropositive) at the threshold of a seronegative status as defined in this study. In the remaining sampling rounds, *S*. Typhimurium (1), *S*. Tennessee (2), *S*. Infantis (1) and *S*. Derby (1) were isolated.

A positive correlation between bacteriological results and a positive serological status in the same sampling round was found ($r=0{\cdot}32$, $P=0{\cdot}0001$, Spearman). In the repeated-measures logistic regression analysis it was found that when *Salmonella* was isolated from pen faecal samples in the same sampling round the herd was approximately four times more likely to be classified seropositive, compared to no *Salmonella* being detected (see Table 7). Herds which were initially classified as seropositive

Table 7. *The final model describing the association between the probability of a herd being classified as seropositive for* Salmonella *depending on the bacteriological herd classification, the initial herd status, the age category of the pigs, feed type and whether the caretaker washes hands consistently before taking care of the animals. The study included 32 finishing pig herds, which were classified as either seropositive or seronegative for* Salmonella *at onset of a longitudinal study performed in Germany, Denmark, The Netherlands and Sweden*

Variable	Level	OR	95% CI	*P* value
Bacteriological classification	Positive	3·98	1·09–14·55	0·0368
	Negative	1		
Initial herd status	Seropositive	4·90	2·29–10·48	<0·0001
	Seronegative	1		
Age category	Finishers	4·20	2·14–8·27	<0·0001
	Growers	1		
Feed type	Wet	0·07	0·02–0·33	0·0007
	Dry	1		
Washing hands	No	4·44	1·63–11·63	0·0024
	Yes	1		

OR, Odds ratio; CI, confidence interval.

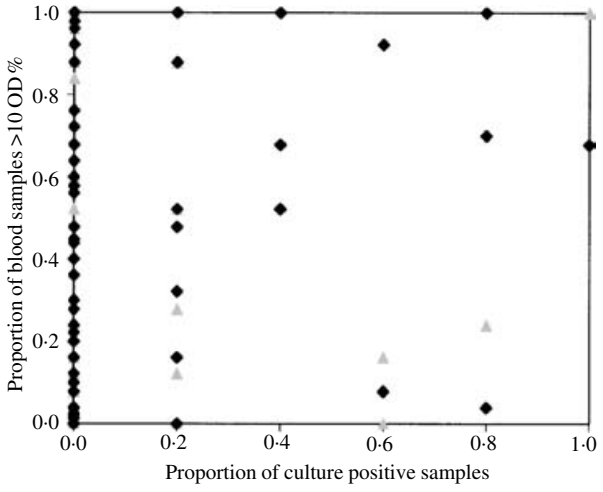

Fig. 3. Scatterplot illustrating the correlation between the proportion of pen faecal samples that are culture-positive for *Salmonella* and the proportion of *Salmonella* seropositive samples (cut-off 10 OD%) in sampling rounds for growers and finishers in a longitudinal study of 32 finishing pig herds in Germany, Denmark, The Netherlands and Sweden. ▲, Growers; ◆, finishers.

were approximately five times more likely to be classified seropositive during follow-up. Herds were also four times more likely to be classified seropositive based on the serological profile of finishers compared to growers. Herds fed with wet feed were approximately 13 times less likely to be classified as seropositive compared to herds fed with dry feed. Herds of which the caretaker(s) did not consistently wash their hands before caring for the animals were more than four times more likely to test seropositive compared to herds were the caretaker did. In the random effects model, the covariance parameter estimates showed no significant differences of effect between ($P=0{\cdot}1312$) and within ($P=0{\cdot}3107$) herds.

DISCUSSION

Stability of a *Salmonella* status during the follow-up period

The stability of an initially allocated *Salmonella* status, was found to vary noticeably. In total, 62% of herds in this study shifted from their initial status at least once during observation, this may have been even more had all herds been followed for the entire 2-year period. Since herds were re-evaluated every 3 months, their status could depend on the status of newly introduced animals, the status of the feed, the presence of residual contamination, seasonal variation, a momentary breach in the hygiene barrier, or even sampling error. There was no difference between seropositive and seronegative herds in the average time an initial herd status was maintained for herds that changed status during the follow-up period. A possible explanation could be that the herds, which changed *Salmonella* herd status during this study,

either became newly infected during the study period or had been seropositive at one or more occasions before the onset of this study, rendering the allocation of their initial herd status more or less arbitrary. Also, this simple analysis does not take into account differences in follow-up time and censoring of observations. There may also be a problem in the comparison itself. A shift in status does not mean the same for seropositive and seronegative herds. For a herd initially classified as seronegative, the case definition is a shift to seropositive status, while for an initially seropositive herd, the case definition is a shift to seronegative status. Although *Salmonella* is a common subclinical infection in pigs, the ability to measure a change in status (i.e. sensitivity of the method) may not be the same for initially seropositive and initially seronegative herds (i.e. differential).

Steady status periods varied from 1 month to more than 2 years, when the study was terminated. The fact that seropositive periods were found to last longer for initially seropositive herds compared to initially seronegative herds may be explained by the fact that most of these herds in this study had a relatively high within-herd seroprevalence. There may also be (risk) factors present in those herds, which increase the probability of positive *Salmonella* status, or a lack of (protective) factors which decrease the probability of positive *Salmonella* status. Therefore, herds that may have had problems with *Salmonella* over longer periods had a greater probability of being selected as initially seropositive in this study. Herds fed with wet feed were found to be three times less likely to be classified as seropositive at a sampling round compared to herds fed with dry feed. The protective effect of wet feed against *Salmonella* infections, or its detection, compared to dry feed has been described previously [14–19].

There are many factors that may lead to a change of *Salmonella* status, both single events or exposures as well as more structural or management-related factors. Among these is the introduction of contaminated feed and/or infected animals [20–23], a change in feed or management strategy and contact with the surrounding environment [24]. This means that *Salmonella* herd status can change from one production cycle to another and that the status measured at a particular point in time may not be valid 6 months later. However, herds that are consistently negative over a longer period of time than in this study do exist in most pig- producing countries [25]. It is difficult to find common factors for these herds, which could explain why they remain negative. In the absence of *Salmonella*, farmers might practice management strategies, commonly accepted as risky with respect to *Salmonella* infection or contamination, without negative consequences.

Correlation between the serological status of growers and finishers

When comparing the serological *Salmonella* status of growers in one sampling round with the serological status of finishers in the subsequent sampling round, two distinct infection scenarios were found: the first showing a positive correlation between the proportion of seropositive growers and finishers, the second where the serological status of finishers appears to be independent from a negative or low serological status of growers. In herds with a high proportion of seroreactors among growers (i.e. $>0{\cdot}25$), only high proportions of seroreactors among finishers were found (i.e. $>0{\cdot}28$). Therefore, a high proportion of seropositive growers in a herd can be regarded as indicative for a high proportion of seropositive finishing pigs. At least in these herds, antibodies from an infection contracted at the grower stage or at the beginning of the finishing period (at 25 kg body weight) may still be measurable in the finishing unit. This is supported by observations by Kranker et al. [26], who were able to measure antibodies against *Salmonella* infections that were contracted at the nursery stage of production. However, no distinction can be made between residual antibody levels from an earlier infection or a new infection obtained in the finishing unit in this study. Kranker et al. [26] observed a variety of transmission patterns during all stages of pre-harvest pig production, including early infection, late infection, clearing or low level of infection and re-infection of pigs. Nonetheless, the presence of high proportions of seropositive growers and finishers probably reflects an infection that is well spread throughout the various production stages in the herd. In herds with lower proportions of seroreactors among growers (i.e. $<0{\cdot}16$), both low and high proportions of seropositive finishers were observed (from 0 to 1). This suggests that in those cases where low proportions of seroreactors among growers result in high proportions of seroreactors among finishers, infection first occurs in the finishing unit, indicating a source of *Salmonella* related to the finishing unit (e.g. contamination of the environment, different feed or older pigs). In these cases, the probability that

finishers still harbour and shed bacteria at the time of slaughter is much higher in this situation than if infection occurs earlier during production [26]. When low proportions of seroreactors among growers remain as low proportions of seroreactors among finishers two possible explanations can be offered, both which may occur simultaneously. It may be that (low levels of) antibodies of previously acquired infections are still measurable in the finishing stage of production, or that new infections occurred with *Salmonella* serotypes that only evoke minor immune responses. On a few occasions, a moderate proportion of seropositive growers was less apparent or even absent in the subsequent sampling round among finishers. This could suggest the possibility of an early infection in these pigs, which was not followed by a re-infection during the later stages of production, either because exposure did not occur or because pigs develop some level of immunity after infection. However, Kranker et al. [26] observed re-infection of pigs with measurable levels of antibodies against *Salmonella*. In our study, animals were not individually identified. Since only 3 months separate growers from ready-to-ship finishers, and antibodies can usually be measured over a longer period, this observation could also be explained by other animals being sampled in the subsequent round. Although the predictive information of a seronegative and seropositive batch of growers is almost equal in this case, the positive predictive value can easily be improved by increasing the herd level cut-off for growers. In this study, if 25% or more of the growers tested seropositive, the next sampling round would yield seropositive finishers. The negative predictive value could be improved by increasing the herd level cut-off for finishers, but this seems of little practical value and should, therefore, be discouraged.

Correlation between serological and bacteriological herd classification

Traditionally, researchers have relied on bacteriological methods to detect *Salmonella* contamination or infection. Bacteriological testing methods may not be practically and economically feasible in a situation where regular testing of a large number of samples is involved, particularly at the pre-harvest level. Therefore, serological testing may be a practical alternative, especially since latent carriers or intermittent shedders may be detected by this method. This study showed a generally good correlation between bacteriological and serological classification of finishing pig herds. This correlation was also found in other studies [17, 27, 28]. However, there were still a number of sampling rounds in which the results from both methods would not lead to the same conclusion. A few possible explanations can be offered. First, a number of serotypes were found in this study, which either could not be detected by the serological test because of the specific mixture of O-antigens or towards which the test showed poor sensitivity [6]. Secondly, when comparing serological results to bacteriological results, it should be borne in mind that the results may reflect different stages of infection. After infection, a bacteriological peak can be measured first, followed by a serological response, if any. Experimental inoculation studies have shown that the interval between the peak of the bacteriological and serological response ranges between 7 and 30 days [6, 9]. Kranker et al. [26] found that under natural (or practical) conditions, the average time period between the two peaks is larger (i.e. approximately 60 days). This means that in the early stages of an infection, no serological response can be measured, while positive bacteriological results can be found. In the late stage of an infection, where pigs may have cleared themselves from infection, it is often still possible to measure antibodies against *Salmonella*, classifying them as seropositive. Therefore, serological testing provides a measure of historical exposure that may not correlate closely to the microbiological burden at the time of sampling. Latent carriers or intermittent shedders can also be found serologically positive, without being able to detect *Salmonella* bacteriologically. These animals are still a potential risk for carcass contamination and as such of public health concern [29]. Although it could be argued that when a pig has cleared itself from infection it no longer constitutes a public health risk, the antibody response indicates previous exposure to *Salmonella* somewhere in the production chain. The problem is that neither bacteriological nor serological sampling results will be able to indicate with 100% certainty that an animal has cleared itself from infection, thus leaving the possibility that the animal is still a carrier of *Salmonella*. When comparing serological and bacteriological results under practical circumstances, the most likely reason for obtaining a 'false-positive' serological result is generally believed to be poor sensitivity of bacteriological sampling methods [28].

Apart from a positive correlation to bacteriological sampling results, the probability of testing a herd

seropositive was found to depend on seropositive *Salmonella* herd status at the onset of the study, sampling finishers as opposed to growers, feeding on dry feed as opposed to wet feed and not washing hands before taking care of the animals. The association with an initially seropositive herd status is not surprising since these herds were selected based on the ability of the serological test to detect antibodies in these herds. The probability of testing seropositive for *Salmonella* at some point during production increases with age, simply because of increasing exposure time (i.e. time at risk) and opportunity. As shown before, being fed wet feed decreases the probability of pigs testing seropositive [14–19]. The mechanism behind this protective effect is probably a combination of poor growing conditions for *Salmonella* on the feed (i.e. low pH, competitive flora) and increased pig resistance (i.e. positive influence on gut flora). The increased odds for testing seropositive if the caretaker did not wash his hands before taking care of the pigs was also found in the risk factor study [7], of which the herds in this study are a subsample of. Although this may very well be a real risk factor, it could also reflect the herd owner's general approach to hygiene. Even though the factor COUNTRY contributed significantly to the final model (i.e. had taken country-related factors, including herd selection method, into account), there was no interaction between the factors in the final model and the country of origin.

Even though sampling took place over more than 2 years in total, there was no obvious indication of seasonal variation in this study. While the number of herds in this study is probably too small to be able to demonstrate a possible influence of season on the occurrence of *Salmonella* in pig herds, Edel et al. [30] and van Schie [25] were also unable to demonstrate an indication of seasonality in the incidence of *Salmonella* isolations from Dutch pigs.

CONCLUSIONS

The *Salmonella* status of a herd can change over time. 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. A high proportion of seropositive growers in a herd was found to be indicative for a high proportion of seropositive finishing pigs. A serologically derived herd status was found to have a moderate correlation to a bacteriologically derived one. 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 should this be desired.

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