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Feed-borne Outbreak of *Salmonella* Cubana in Swedish Pig Farms: Risk Factors and Factors Affecting the Restriction Period in Infected Farms

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Feed-borne Outbreak of Salmonella Cubana in Swedish Pig Farms: Risk factors and Factors affecting the restriction period in infected farms. Acta vet. scand. 2006, 47, 13-22. – In 2003, a feed-borne outbreak of Salmonella Cubana occurred in Sweden as a result of contamination in a feed plant. Salmonella Cubana was detected in 49 out of 77 pig farms having received possibly contaminated feed. In this study, potential risk factors for farms being salmonella positive were examined, and a survival analysis was performed to investigate risk factors affecting the restriction period for salmonella positive farms.

The median restriction time for all 49 farms was 17 weeks. An increased risk for farms being salmonella infected (positive in feed and/or faeces) was seen for fattening farms and farms feeding soy. The survival analysis showed that herds with a low level of infection and farms with a high hygiene level had shorter restriction times.

This study is unique as it investigates a real outbreak of feed-borne salmonella, where the source of infection was reliably identified, the period of exposure could be defined and data were collected from all exposed farms.

Feed-borne, pigs, swine, riskfactor, outbreak, soy meal, restriction period, salmonella, Salmonella Cubana

Introduction

In Sweden, only a few cases of salmonella in food-producing animals are reported each year (Thorberg and Engvall, 2001; National Veterinary Institute, 2001; *Boqvist et al*., 2003; National Veterinary Institute, 2004). This situation can be attributed to the organised Swedish salmonella control that started in 1961 to ensure safe animal products for human consumption. All findings of Salmonella spp. in feed, animals or food of animal origin are notifiable by law and action is always taken to eliminate the infection. Whenever *salmonella* is isolated, a prompt investigation and trace-back of the infection is performed and infected herds are put under restrictions, regardless of serotype. A clean-up plan is instituted and approved by the Swedish Board of Agriculture. Thorough cleaning and disinfection of the premises and all possibly contaminated areas are performed. Animal movements and spreading of manure are restricted. The herd is not declared free from infection until all animals in the herd are negative in two consecutive faecal samplings one month apart, and adequate cleaning and disinfection have been completed.

In May 2003, *Salmonella* Cubana was detected in faecal samples from a routine sampling in a fattening pig herd. Through trace-back from the pig herd, contamination with S. Cubana was discovered in the swine feed production line of a Swedish feed plant. Investigations revealed that 77 pig farms had received feed during the critical period, when the contamination of the feed line was still undetected (from the end of April until the 10th of June 2003). Restrictions were put on the farms until sampling of the animals and the farms' feeding system had been carried out. On farms where salmonella was isolated from either the animals or the feeding system, extensive cleaning and disinfection was carried out and the restrictions were not lifted until two whole-herd samplings one month apart showed negative results.

In Sweden, control of feed is integrated in the national salmonella control programme. *Salmonella* Cubana has been detected in raw feed materials several times during the last decades (*Malmqvist et al.,* 1995; *Boqvist et al.,* 2003; National Veterinary Institute, 2004). In animal production S. Cubana has been found only twice, in 1995 in layers and in 1997 in a gilt producing herd (Boqvist et al., 2003). No reports of S. Cubana in association with clinical disease in animals have been found. However, S. Cubana has been described as a significant pathogen for humans (Curbelo Hernandez and Martinez Cruz, 1950), and in the 60s and 70s, human cases were associated with carmine dye used in foods (Bridger, 1973). In an American study of travellers' diarrhoea, S. Cubana was associated with mild enteric symptoms only (*Sack et al.,* 1977). Two food-borne outbreaks of S. Cubana in humans have been reported from the USA in 1998, affecting 34 and 14 persons respectively (*Taormina et al.,* 1999, Centers for Disease Control and Prevention, 2005). In Sweden, three cases of human infection with *S.* Cubana have been reported since 1997 (up to summer 2005), two of which were believed to have been infected abroad.

The objectives of this study were to identify risk factors for finding *S.* Cubana in pig herds that received contaminated feed, and to identify factors affecting the length of the time period that infected herds were kept under restrictions.

Materials and methods

Study design

There were 77 epidemiologically separate pig farms that had received possibly contaminated feed. Sampling of the herds was done in late June and included pooled samples with 5-10 pigs per sample, representative of the entire animal population in the herd, as well as different parts of the entire feeding system. An average of 185 samples per herd were taken. In 49 of these farms, *S.* Cubana was detected in the samples from feed and/or faeces that were taken during the outbreak investigations. In the remaining 28 farms all samples were salmonella negative. In 31 of the 49 farms where *S.* Cubana was detected, the bacteria was detected in faecal samples from the animals, as well as in the feeding system in most farms. In 18 farms, positive samples were only found in the feeding system, while all faecal samples were negative.

Data according to a protocol were collected by field veterinarians during the investigations in farms that had received contaminated feed. This information, together with the eradication plans for infected farms, were the main sources of information. For the survival analysis, supplementary data was collected by a questionnaire sent out in December 2003 to the veterinarian responsible for each farm.

The variables included as potential risk factors for becoming salmonella infected were herd size (measured as number of pigs and stable units), type of production (integrated, piglet producing or fattening herd), type of feeding system (dry feed, and wet feed mixed with water or with whey), amount and type of feed delivered from the plant (from the contaminated production line) and the week for, and number of, feed deliveries (Table 1).

At first, farms were classified as positive if any sample was salmonella positive, either from the feeding system or the animals. A separate analysis was also performed classifying only farms

Table 1. Odds ratios for possible risk factors for detecting Salmonella Cubana on farms in the outbreak in pig farms in Sweden 2003.

	Odds ratio (90 % confidence interval)	
	Farm positive in feed and/or faeces (n=49)	Farm positive in faeces (n=31)
Type of production:		
Integrated *vs piglet*	3.14 (1.09 -–9.02)	1.78 (0.61 – 5.15)
Fattening *vs piglet*	3.38 (1.11 – 10.28)	1.25 (0.41 – 3.83)
Herd size:		
No. of pigs (OR per extra 100 pigs)	1.05 (1.00 – 1.10)	1.03 (1.00 – 1.06)
No. of stable units:		
1-5 stable units *vs* ≥11	0.21 (0.05 – 0.85)	0.26 (0.08 – 0.83)
6-10 stable units *vs* ≥11	0.28 (0.06 – 1.29)	0.39 (0.11 – 1.42)
Herd size (divided on type of production):		
Piglet producing		
big *vs small (< 500 pigs)*	17.50 (1.88 – 163.22)	9.33 (1.08 – 81.0)
Fattening		
big *vs small (< 950 pigs)*	0.33 (0.07 – 1.57)	0.30 (0.08 –1.21)
Integrated		
big *vs small (< 1250 pigs)*	1.56 (0.46 – 5.34)	1.76 (0.54 – 5.74)
Type of feed:		
soy *vs other types*	18.32 (3.20 – 104.90)	1.62 (0.68 – 3.85)
Feeding system:		
dry vs wet (H2O)	0.92 (0.34 – 2.51)	0.60 (0.21 – 1.69)
wet (whey) *vs wet* (H2O)	2.50 (0.94 – 6.62)	0.93 (0.37 – 2.33)
wet (whey) *vs dry*	2.73 (1.02 – 7.29)	1.56 (0.60 – 4.07)
Feed delivery week:		
(farms with only one delivery, n=46)		
18 *vs* 23	0.08 (0.01 – 1.22)	N.a.
19 *vs* 23	0.50 (0.06 – 4.03)	0.06 (0.01 – 0.45)
20 *vs* 23	0.21 (0.03 – 1.69)	0.08 (0.01 – 0.70)
21 *vs* 23	0.28 (0.03 – 2.37)	0.06 (0.01 – 0.52)
22 *vs* 23	0.22 (0.03 – 1.96)	0.13 (0.01 – 1.10)
No. of feed deliveries:		
one vs *three or more*	1.13 (0.31 – 4.15)	0.35 (0.10 – 1.29)
two vs *three or more*	0.80 (0.20 – 3.25)	0.45 (0.11 – 1.83)
Amount of feed: (OR per extra ton)	1.04 (1.00 – 1.08)	1.02 (0.99 – 1.04)

Reference variables (baseline) in italics. Odds ratios with $p < 0.10$ in bold. N.a. = not applicable, due to too few observations in one group.

with salmonella positive faecal samples as positive and the rest as negative (including farms with positive samples from the feeding system only).

To identify factors possibly affecting the length of the period under restrictions (i. e. until two salmonella negative, whole herd samplings one month apart and cleaning and disinfection of the premises had been achieved), a total of 13 variables were analysed. The type of production, herd size and type of feeding system (categorised as above) were included in the analyses. In this part of the study, the number of positive samples detected was also included as

Table 2. Odds ratios (OR) from multivariate analysis of potential risk factors for farms being infected with Salmonella Cubana in the outbreak in pig farms in Sweden 2003.

		OR (90% CI	
		Farm positive in feed and/or faeces (n=49)	Farm positive in faeces (n=31)
Type of production:			
Integrated *vs piglet*		3.19 (0.29 - 35.0)	N.i.
Fattening *vs piglet*		38.2 (1.57 - 927.8)	N.i.
Herd size:			
No. of pigs (OR per extra 100 pigs)			
	piglet producing	1.17 (0.90 –1.52)	N.i.
	fattening	0.84 (0.57 – 1.52)	N.i.
	integrated	1.05 (0.80-1.79)	N.i.
1-5 stable units *vs* ≥ 11		N.i.	0.22 (0.06 - 0.75)
6-10 stable units *vs* ≥ 11		N.i.	0.31 (0.08 - 1.23)
Type of feed:			
Other feed types *vs soy*		0.08 (0.01 – 0.75)	N.i.
First feed delivery week:			
18 *vs 22-23*		N.i.	0.27 (0.04 - 1.98)
19 *vs 22-23*		N.i.	0.22 (0.05 - 0.89)
20 *vs 22-23*		N.i.	0.33 (0.07 - 1.50)
21 *vs 22-23*		N.i.	0.15 (0.02 - 1.15)

Reference variables (baseline) are written in italics. N.i. = not included, (not significant in the univariate analysis).

well as the level of infection (measured as number of positive samples detected and number of separate days of sampling with positive samples during the outbreak period (categorised as A: ≤3 or B: >3 positive samples on one day or C: any number on >1 day), see Table 3). Moreover, data regarding the situation on the farm during the eradication work (the possibility to empty units and thus facilitate the movement of pigs during cleaning and disinfection, and external factors that delayed the eradication work, such as harvest) were analysed. The veterinarians' opinion on whether the feeding system and the stable units in general were easy or complicated to clean and disinfect were also included, as well as their view of the hygiene on the farm and the motivation of the farmer (low, middle or high) to commit to the restrictions and the eradication plan. Finally, the veterinarians' opinion of the cleaning and disinfection work performed by the sanitation firm, if such a firm was engaged, was included in the analyses.

The study was concluded while some farms (n= 10) were still under restrictions (at 30 weeks).

Statistical analyses

Statistical analyses were performed using SAS for Windows v8.2 (SAS Institute, Cary, North Carolina, USA). Initially, in the risk factor analysis, two different dependent variables were investigated in a univariate model using logistic regression: 1) positive samples in feed and/or faeces and 2) positive samples in faeces. All independent variables were investigated separately. Odds ratios (OR) with a 90% confidence interval (CI) were calculated. Two multivariate logistic regression models were built using Proc Genmod. Correlation was tested for variables that appeared to be similar using Spearman rank correlation (rsp). There was a correlation between the number of pigs and the amount of feed delivered (rsp=0.8) and the number of pigs and the number of stable units (rsp=0.8). In Model 1, the variable "number of pigs" was the herd size parameter kept in the model while in model 2 it was the "number of stable units", based on the results in the univariate analysis.

Variables that had a p-value of ≤ 0.10 in the univariate analyses were included in the multivariate models. In Model 1 these variables were: number of pigs, type of production and type of feed. An interaction variable between, "number of pigs" and "type of production", was also included. In Model 2 the variables included were: feed delivery week and number of stable units. As the unit of interest in both models was the farm, and because several animals were sampled on each farm, clustering resulting in overdispersion could not be excluded. However, the deviance/degree of freedom were close to 1 in both models indicating that overdispersion was not a major concern.

$P \leq 0.10$ was considered statistically significant.

In the survival analysis of the restriction periods, the dependent variable was the number of weeks under restriction. Of the 49 salmonella infected farms in the study, 10 were still under restriction at the end of the study period and were right censored in the analyses. Univariate and multivariate analyses were performed using Cox proportional-hazards model (Cox, 1972). In the multivariate model, all variables from the univariate analysis were included in the initial model. A backward selection procedure was run until all remaining variables in the model showed a p-value of £ 0.10. The proportional-hazards assumptions was checked by inspecting the plot of the log of the negative log of the survival function versus log of time and by testing for homogeneity of the survival curves by the log rank test (Cantor, 1997).

Results

The results from the univariate analysis of risk factors for farms becoming salmonella infected appear in Table 1. In the multivariate model for farms that were salmonella positive in the feeding system and/or in faecal samples (see Table 2), an increased risk was associated with fattening herds and herds feeding soy. The herd size variable "stable units" indicated a lower risk for

Table 3. Hazard ratios (HR) from univariate analysis of risk factors affecting the restriction period for pig farms infected with Salmonella Cubana. Only significant variables ($p < 0.10$) included. (HR>1 = shorter restriction period, HR<1 = longer restriction period).

	HR	P-value
No. of pos. samples	0.3	0.00
≰3 pos. samples at one occasion	3.9	0.00
Empty stable unit	0.5	0.07
Uncomplicated C & D*; feed systems	1.8	0.08
Uncomplicated C & D*; stables	3.7	0.07
Hygiene level: high	3.8	0.07
Farmers´ motivation: middle	3.9	0.04
Farmers´ motivation: high	2.6	0.07
External factors	0.52	0.06

* C & D = cleaning and disinfection

Table 4. Median restriction time (in weeks) for salmonella infected farms, in the Salmonella Cubana outbreak, grouped by the veterinarians´ opinion of the cleaning and disinfection (C & D).

The veterinarians´ opinion on the C & D		No. of herds	Median restriction time
Feed system:	Uncomplicated C & D	28	14
	Complicated C & D	17	24
Stable:	Uncomplicated C & D	37	16
	Complicated C & D	6	27

smaller farms, when only farms with positive faecal samples were classified as positive. As regards the week of feed delivery, the increased risk for positive faecal samples in herds receiving feed in the later weeks (week 22-23), as compared to the earlier weeks (before week 22) that was demonstrated in the univariate analysis (including farms with only one feed delivery) could only be detected for one week (week 19) in the multivariate analysis (including all farms, regardless of number of feed deliveries).

The median restriction time for all 49 farms was 17 weeks. For farms with positive samples from the feeding system only, the median restriction period was 14 weeks, and for farms with positive faecal samples the median restriction period was 23 weeks. Results that were significant at $p \leq 0.10$ in the univariate analysis of factors affecting the restriction period are shown in Table 3. Those farms that had a feeding system regarded by the veterinarian as difficult to clean and disinfect had a longer median eradication time while those with stables easily cleaned and disinfected had a shorter median eradication time, as can be seen in Table 4.

Those herds with a restriction period exceeding 30 weeks (n=10) received a lower average score by the veterinarian than the other farms as regards the initial hygiene level and the farmer's attitude towards the work that needed to be done. The herds with >30 weeks' restriction period were also getting a lower average score by the veterinarians indicating feeding systems and stables that were difficult to clean and disinfect, in comparison with herds with shorter restriction periods. Moreover, these herds usually had external factors, i.e. factors not caused by the pig production itself, which delayed the eradication work. Some external factors mentioned were harvest work, reorganising of the entire production, plans to stop the production, and social factors making the farmer unable to cope with the situation.

No differences were seen in restriction time between different types of herds, neither type of production or herd size was associated with the restriction period. Farms with a dry feed system were more often mentioned by the veterinarians as having a feed system problematic to clean and disinfect. However, the proportion of farms with a dry feed system was small (18%).

The multivariate survival analysis found a higher hazard for release of salmonella restrictions (i.e. a significantly shorter restriction time) if the number of positive samples were £ 3 and detected on the same day of sampling, in comparison with farms that had positive samples on more than one occasion (hazard ratio=4.1). Farms which the veterinarian regarded as having a high hygienic level had a significantly shorter restriction time than farms with a low hygienic level (hazard ratio=1.9).

Discussion

This is the largest feed-borne salmonella outbreak ever recorded in Sweden. It was regarded as an opportunity to document and draw conclusions about salmonella epidemiology and control in Swedish swine production.

Fattening herds were positive to a greater extent than piglet producing herds (Table 2). This may be explained by the fact that different herd types use different types of feed. In the farms included in this study, soy was over-represented in integrated and fattening herds.

The analyses showed that, in this outbreak, soy was associated with a higher risk of farmcontamination than the other feed types produced on the same production line. Farms feeding soy also fed whey to a high extent. In previous studies, a lower prevalence of salmonella in pigs fed whey has been shown (*van der Wolf et al.,* 1999; *van der Wolf et al.,* 2001; *Lo Fo Wong et al.,* 2004). Several studies also indicate dry or pelleted feed as a significant risk factor, compared to wet feed (*Beloeil et al.,* 2004; *Cook and Miller,* 2005; *O'Connor et al.,* 2005; *Quessy et al.,* 2005). This effect could not be demonstrated in this study. However, possibly due to the difference in risk between soy and the other feed types, such an effect might have been undetectable in this study.

As regards the week of feed delivery, the difference between the univariate and the multivariate risk factor analysis was probably due to the fact that farms receiving feed on more than one occasion were included in the multivariate analysis. Repeated feed deliveries could be expected to obscure any risk associated with a particular feed delivery week. The first herd samplings in each farm were performed within a couple of weeks from the last week of feed delivery from the contaminated plant. A possible explanation for the association seen in the univariate analysis, between positive faecal samples and feed delivered in weeks 22 and 23, is that the pigs receiving contaminated feed during these weeks were still excreting salmonella at the time of sampling.

Lower grade of infection was associated with a shorter restriction time, which appears logical as a low level of infection would be easier to get rid of than a higher level. Higher level of farm hygiene was also associated with a shorter restriction time, which is again what would be expected. Other studies have shown low hygiene level to be a significant risk factor for salmonellosis in swine (*Berends et al.,* 1996; *Funk et al.,* 2001; *Beloeil et al.,* 2004). The variables concerning farm hygiene, farmer's attitude, quality of performance by sanitation firm and difficulty of cleaning were subjective and might be expected to depend on the perception of the individual veterinarian responding to the questions.

Several studies on risk factors for salmonella infection in pigs have been published (*Berends et al.,* 1996; *Funk et al.,* 2001; *van der Wolf et al.,* 2001; *Beloeil et al.,* 2004; *Lo Fo Wong et al.,* 2004; *Cook and Miller,* 2005; *Quessy et al.,* 2005, among others). Many of these use a cross-sectional approach, but sometimes a cohort design has been employed. Apart from the purchase of salmonella positive animals and the risk factors related to feed and hygiene discussed above, not many factors associated with salmonella infection have been consistently identified.

This study is unique, as it investigates a real outbreak where the source of infection was reliably identified, the period of exposure could be defined and data were collected from all exposed farms.

The number of farms affected by the outbreak was rather small, limiting the power of the study and consequently the risk factors could not be expected to be easily detected. Moreover, a study based on data collected in the middle of an outbreak entails some difficulties, as the focus at the time was on eradication and not on data collection and some data that might have been used in the analyses could not be obtained.

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Sammanfattning

Sommaren 2003 påvisades en kontamination med Salmonella Cubana i en svensk foderfabrik. Vidare utredning visade att potentiellt smittat foder hade levererats till 77 svinbesättningar.

I 49 av de 77 besättningarna kunde S. Cubana isoleras vid provtagningar av svin och/eller fodersystem. Dessa besättningar spärrförklarades och sanerades i enlighet med det svenska salmonellakontrollprogrammet. Denna studie omfattar en riskfaktoranalys och en överlevnadsanalys. Riskfaktorer för att en besättning som mottagit foder från den kontaminerade fabriken infekterats med salmonella undersöktes, respektive faktorer som kan ha påverkat spärrtidens längd på de gårdar som befanns salmonellapositiva. För de 49 drabbade besättningar var medianvärdet för spärrtidens längd

17 veckor. En ökad risk för att ha blivit infekterad med salmonella sågs hos gårdar med slaktsvinsproduktion och hos gårdar som köpt soja från den kontaminerade foderfabriken. Det visades även att gårdar med ett lågt smittryck, samt de med en hög hygiennivå, hade kortare spärrtid. Denna studie är unik då den undersöker ett verkligt utbrott av foderburen salmonella där smittkällan identifierats, exponeringsperioden definierats och data samlats in från samtliga gårdar som fått det potentiellt smittade fodret.

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