

Prevalence and risk factors for *Salmonella* in veal calves at Danish cattle abattoirs

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SUMMARY

The study's objectives were to determine herd- and animal-level prevalence and herd-level risk factors for *Salmonella* in dairy-bred veal calves at slaughter in Denmark. In total, 1296 faecal samples were collected at five cattle abattoirs in Denmark during 2007–2008. The animals came from 71 randomly selected specialized veal-calf producers that delivered more than 100 animals to slaughter per year. *Salmonella* Dublin bacteria were isolated from 19 samples from 12 herds and *Salmonella* Typhimurium was isolated from one sample. The apparent prevalence of herds delivering *Salmonella*-shedding animals to slaughter was 18% (95% CI 9–27). The overall estimated true prevalence of shedding calves at slaughter was 1·3%. Veal-calf herds that purchased animals from herds not classified as low risk in the Danish *Salmonella* surveillance programme had significantly ($P=0\cdot03$) higher risk of delivering *Salmonella*-shedding calves to slaughter. The results emphasize the importance of efforts in the dairy industry to ensure food safety for consumers.

Key words: Abattoir, bacteriology, risk factor, *Salmonella*, veal.

INTRODUCTION

Salmonellosis is one of the most common zoonoses in the world. It was the second most commonly reported human zoonosis in the European Union (EU) in 2008 [1] with a total of 131 468 confirmed cases. The most common sources of human *Salmonella* infections were eggs, pork and poultry, and *Salmonella* (*S.*) Enteritidis and *S.* Typhimurium were the most frequently

reported serovars. However, beef cannot be completely ignored as a source of *Salmonella* infection in humans even though most member states of EU, including Denmark, reported very low (<1·0%) proportions of beef carcass swab samples testing positive, although some member states reported higher prevalences (up to 7·5%). In Denmark, about 16–36 human cases of salmonellosis were attributed to beef produced in Denmark and about half that number (3–25) to imported beef in 2008. Furthermore, for a large proportion (~40–55%) of salmonellosis cases in humans the source was unknown [2]. Some of these could be from beef or through direct contact with cattle. In 2008, 28 people were recorded as being

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hospitalized in Denmark with *S. Dublin*. This serotype is known to be more invasive, difficult to treat and leads to higher mortality in humans than other *Salmonella* serotypes [3]. This particular serotype is host-adapted to cattle. Thus the source of human cases of *S. Dublin* is likely to be either beef produced in Denmark, imported beef or direct contact with infected animals and their surroundings.

Specialized veal-producers purchase bull calves from dairy herds around the age of 2 weeks and rear these animals until ready for slaughter. In Denmark, most veal calves are slaughtered before the age of 12 months. They may be infected with *Salmonella* if bacteria are present in the rearing herd or they may become infected or contaminated during transportation and lairage at the abattoir. In a study from 93 abattoirs in the UK in 2003, seven different *Salmonella* spp. were found in 36/2553 (1.4%) faecal samples collected from cattle slaughtered at age <30 months. *S. Mbandaka* was found in 10 (27.8%), *S. Typhimurium* in 10 (27.8%), *S. Dublin* in eight (22.2%) and *S. Derby* in four (11.1%) of the 36 positive samples. The median age of the tested cattle was 24 months [4]. An older study of apparently healthy veal calves in the UK found 31/720 (4.3%) animals tested infected with *Salmonella*. Twenty-three of these were *S. Dublin*. However, only eight (1.1%) of the tested animals had *Salmonella* in the intestinal contents. The rest were found infected by culture of internal organs, lymph nodes or carcass surfaces [5]. In a review of the importance of *S. Dublin* in humans in Denmark, Lester *et al.* [6] concluded that reduction in human *S. Dublin* infections could be obtained through stricter regulations for the slaughtering of animals from *S. Dublin*-infected herds, optimal hygiene at abattoirs and increased cooperation between the veterinary and medical professions concerning investigation of routes of infection. It is therefore relevant for the veterinary authorities and the Danish beef industry to learn more about prevalence, serotype distribution and risk factors for *Salmonella* infection in cattle at slaughter.

There is a lack of studies of risk factors for *Salmonella* in veal calves. However, suggestions of factors of importance for *Salmonella* occurrence in cattle generally include hygienic factors in the herds, e.g. flies in pens [7], contact with poultry manure or wild bird manure, outdoor calving, herd size and herd expansions [8]. Hygiene and contacts at markets and in vehicles are also likely to be important risk factors before slaughter [9]. In Danish dairy herds, risk

factors for becoming infected in 2003 included herd size, number of purchased cattle from test-positive herds and number of test-positive neighbour herds. Organic herds were less likely to recover than conventional herds indicating that different types of management can influence the occurrence of *Salmonella* in cattle herds [10]. Off-farm rearing of heifers has been acknowledged as an important risk of infection with multi-drug-resistant *Salmonella* in US dairy herds [11]. One study also reported that for heifers and cows, recent antimicrobial treatment increased the probability of isolating *Salmonella* from faecal samples [12]. Moreover, *Salmonella* has been associated with high calf mortality in dairy herds. This may be due to both direct effects of the infection and underlying management factors [13].

The Danish Cattle Federation and the Danish Veterinary and Food Administration initiated an active surveillance programme for *S. Dublin* in 2002. In short, the programme consists of testing based on regular bulk-tank milk testing of dairy herds and blood sampling mainly at slaughter of non-dairy herds [14]. All collected samples are tested for antibodies directed against serogroup-D *Salmonella* antigens. *S. Dublin* is by far the most important serogroup-D *Salmonella* type for cattle. Whereas herd classification in the programme is not perfect, it is aimed at classifying herds into groups with low (level 1) and high (level 2) risk of becoming infected with *S. Dublin* infection, plus a third group (level 3) of herds diagnosed with clinical salmonellosis from *S. Dublin*. Level 1 herds that purchase animals from level 2 herds become classified as level 2 herds for a period of at least 3 weeks and until new tests from the herd allow it to be promoted to level 1 [15]. This has markedly reduced the movement of animals from level 2 herds to level 1 herds and has contributed to a marked reduction from 26% to 9% national dairy herd-level prevalence from 2002 to 2010. The incidence of human cases of *S. Dublin* has not decreased proportionally over the same time period. Trade and hygienic slaughter restrictions apply to level 3 herds. However, it is not clear to what extent cattle from levels 1 and 2 pose a risk of introducing *Salmonella* to the abattoir and whether trade and hygienic slaughter restrictions may be relevant for other herds than those with clinical salmonellosis in order to further improve food safety.

The objectives of this study were (1) to estimate the prevalence of specialized veal production herds that deliver *Salmonella* to abattoirs in Denmark via

shedding animals, (2) to estimate the prevalence of veal bull calves that carry *Salmonella* bacteria in the colorectal contents at slaughter and determine the concentration of bacteria in *Salmonella*-positive faecal samples and (3) to determine herd risk factors for *Salmonella* in veal calves at slaughter. Such risk factors can potentially be used to classify high-risk herds so that special hygienic measures can be taken at transportation, lairage and slaughter to avoid contamination of carcasses.

MATERIALS AND METHODS

Selection of herds

In the study design the aim was to sample 20 bull calves from each of about 80 specialized veal-calf herds from 1 October 2007 until sufficient samples had been collected by sampling a maximum of five animals per delivery from each of these herds. Four cattle abattoirs from three different companies were selected by convenience, and they slaughtered calves from all over the country. Herds were selected based on number of slaughtered bull calves at these four abattoirs in the period 1 September 2006 to 1 September 2007. In total, 200 herds slaughtered more than 100 bull calves aged between 6 and 14 months at these four abattoirs in that period. Out of these 200 herds, 81 herds were randomly selected to participate in the project and sampling was initiated in November 2007. When sampling was stopped in April 2008, 70 herds had calves sampled at the four selected abattoirs and one herd was sampled at a fifth small private abattoir. The last 10 selected herds had either ceased production or changed to delivering calves to an abattoir not included in the study.

Collection of faecal samples

When bull calves between the age of 6 and 14 months from any of the selected herds entered the abattoirs on Mondays to Thursdays, the first five calves in the delivery were marked for sampling. If there were fewer than five animals delivered for slaughter in one day they were all sampled. After removal of the gastrointestinal tract at the slaughter line, faecal samples were collected by cutting into the rectum or colon with a hot-water sterilized knife. About 70 g faecal material was collected aseptically and placed in a container which was marked and stored at 4 °C. Samples were sent to the analysing laboratory on the same day or the following morning.

Bacteriological culture method

Faecal samples were all examined at the Regional Northern Laboratory of the Veterinary and Food Administration (Aalborg, Denmark) according to ISO 6579:2002/Amd 1 2007 [16]. Faecal material (25 g) was mixed in 225 ml buffered peptone water (BPW) and left for pre-enrichment at 37 °C for 18 ± 2 h. Inoculation of 0.1 ml test material onto modified semi-solid Rappaport–Vassiliadis medium base (MSRV agar) plates was followed by incubation for 48 ± 6 h at 41.5 °C. MSRV plates were read after 24 ± 3 h and after 48 ± 6 h. Material from MSRV plates suspected to be positive was inoculated on xylose lysine deoxycholate agar (XLD) (Oxoid CM0469), *Salmonella* chromogenic agar (SCA) (Oxoid CM1007) and modified Brilliant-Green Phenol-Red lactose sucrose agar (BPLS agar) (Oxoid CM0329) plates and incubated at 37 °C for 18–24 h. Isolates suspected to be *Salmonella* positive at XLD, SCA and BPLS were identified using *Salmonella* antiserum Poly A-I + Vi and API: ID 32 E. Serotyping and confirmation of positive isolates were conducted at the National Food Institute, Technical University of Denmark (Copenhagen).

All *Salmonella*-positive faecal samples were cultured by a semi-quantitative method, where the samples prior to pre-enrichment were diluted tenfold with BPW. Five dilutions of each sample were examined separately as described above.

Data for risk factor analyses

The following explanatory factors were assessed in the statistical analyses: purchase patterns, herd size, and calf mortality. Data for the risk factor assessments were collected from the Danish Cattle Database. All data were compiled at herd level. Purchase patterns were evaluated as the total number of animals purchased, number of animals purchased from herds not classified as low-risk herds and number of purchase events from herds not classified as low risk during the period 1 September 2006 to 1 September 2007 (the year before selection of herds for the study). Herd size was defined as the average number of male cattle in the herd during the same period. This number was adjusted for the number of days each animal spent in the herd.

The number of calves that died or were euthanized between ages 1 and 180 days from January 2007 to January 2008, adjusted for the average number of calves in the herds, was used as an estimate of the calf

mortality at herd level. The calculation method has been described in detail elsewhere [13]. The mortality percentage was transformed for the statistical analyses using the natural logarithm because the distribution was very right-skewed.

Statistical analysis

Apparent herd-level and animal-level prevalence of faecal shedding was calculated directly from the laboratory results and an estimated true animal-level prevalence was calculated by adjusting the apparent prevalence estimates by the estimated sensitivity and specificity of the bacteriological culture test from a previous study [17].

The association between the herd probability of delivering animals shedding *Salmonella* to the abattoir (yes/no) and the explanatory factors described above was tested by logistic analysis using PROC GENMOD in SAS[®] v. 9.2 (SAS Institute, USA). All explanatory factors were included in a full model and removed by stepwise backwards elimination requiring a significance level of 5% to remain in the final model. After reducing the model to only significant effects, all explanatory factors were then re-introduced one by one in the model to evaluate possible confounding and test for interactions with the main effects. However, total number of animals purchased, number of animals purchased, and number of purchase events from herds not classified as low risk were highly correlated and could therefore not be included in the model simultaneously.

RESULTS

Bacteriological culture results and estimated prevalences

In total, 1296 faecal samples were collected from 71 herds. Due to practical constraints at the abattoirs the number of samples collected from each herd was not easy to control and thus varied from 5 to 40 (mean = 18.3, s.d. = 6.5) in the final dataset. In total, 20 faecal samples were culture positive for *Salmonella* spp. The animals testing positive came from 13/71 herds. One herd had four positive samples, one herd had three, two herds had two each and nine herds had one culture-positive sample. All isolates were *S. Dublin* except one which was *S. Typhimurium* DT40. No herd had more than a single serotype isolated. It was possible to perform semi-quantitative estimation

of *Salmonella* concentrations in 18 of the *S. Dublin*-positive samples. Sixteen samples had <1 colony-forming unit (c.f.u.)/g faeces, one had between 1 and 10 c.f.u./g and one had between 100 and 1000 c.f.u./g.

The apparent prevalence of veal herds delivering animals carrying *Salmonella* infections in the colon or rectum to the abattoirs was 18.3% (95% CI 9.3–27.3). The overall apparent prevalence of culture-positive individual animals across all 71 herds was 1.5% (95% CI 0.9–2.2). In the 12 *S. Dublin* culture-positive herds the apparent prevalence of *Salmonella*-shedding animals varied from 4.3% to 20%.

The estimated true prevalence of shedding animals at slaughter was calculated assuming an average test sensitivity of 80% based on a previous study of spiked samples with low concentrations of *S. Dublin* bacteria [17], and a test specificity of 99.5% allowing for a small risk of cross-contamination of the samples at the abattoir or laboratory. The true prevalence estimate of shedding animals at slaughter was 1.3% across all herds, and it varied between 4.8% and 24.5% in the 12 culture-positive herds.

Descriptive analysis of risk factors

Table 1 provides the distribution of risk factors for each *Salmonella*-positive and -negative herd.

Risk factor analyses

One explanatory factor was significantly associated with faecal culture positivity in the logistic analysis, namely the number of times the veal herds purchased animals from herds not classified as low risk in the surveillance programme (intercept = -2.14, parameter estimate = 0.068, $P = 0.03$). The predicted association is depicted in Figure 1. None of the other confounders or interactions were significant. However, number of animals purchased from herds not classified as low risk was borderline significant ($P = 0.06$) in a univariable logistic regression model. Herd size was also borderline significant when included in a univariable logistic regression ($P = 0.06$).

DISCUSSION

In this study we estimated the prevalence and concentrations of *Salmonella* in faecal samples at slaughter in Danish dairy calves reared in specialized beef production herds that delivered more than 100 calves

Table 1. Distribution of risk factors with continuous outcomes [described by 25% (Q1) and 75% (Q3) quartiles] relative to bacteriological *Salmonella* status of 71 specialized veal herds

Risk factor	Positive	Negative
Calf mortality		
Q1	0.034	0.025
Median	0.046	0.044
Q3	0.060	0.092
Herd size		
Q1	332	238
Median	521	357
Q3	878	579
Number of purchased animals		
Q1	301	309
Median	595	483
Q3	1227	844
Number of purchase events from herds not classified as low risk		
Q1	4	1
Median	13	4
Q3	18	10
Number of calves purchased from herds not classified as low risk		
Q1	73	12
Median	259	105
Q3	399	226

to slaughter per year. These herds are interesting because they contribute the highest load of *Salmonella* to the abattoirs due to the highest herd prevalence and the high number of animals delivered to slaughter at an early age. We found that at least 18% of the veal herds delivered *Salmonella*-shedding calves to slaughter in the study period from November 2007 to April 2008. Some herds were probably misclassified as negative due to poor sample size and lack of diagnostic sensitivity of the bacteriological faecal culture test. The concentrations of bacteria in the culture-positive samples were very low which further adds to lack of sensitivity in the culture test. We used a sensitivity of 80% based on a previous study on spiked samples [17], but the concentrations may in fact have been lower in this study and other strains may have been involved, so we may have overestimated the sensitivity and thus underestimated the true prevalence of infected animals.

The herds were randomly selected from veal herds delivering more than 100 animals to slaughter per year, and sampling was aimed to be spread out over several deliveries, in order to estimate the true prevalence of animals carrying *Salmonella* bacteria in

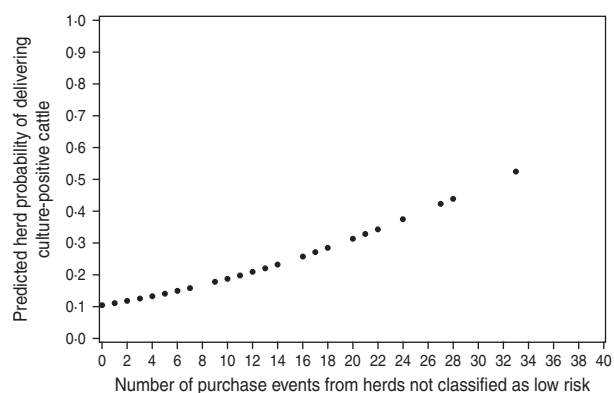


Fig. 1. Predicted probability of veal herds delivering *Salmonella*-shedding animals to slaughter vs. number of purchase events from high-risk herds during a 1-year period prior to sampling.

their intestines thereby acting as important reservoirs of contamination of carcasses at the abattoirs. The estimate of 1.3% shedding animals corresponds well to the 1.4% found in a similar study from the UK [4]. However, the majority of the isolates were *S. Dublin* in our study, whereas in the study by Milnes *et al.* [4] only 22% of the isolates were *S. Dublin*. The overall prevalence might have been higher if we had collected the samples in late summer and early autumn instead of winter and early spring [18, 19].

Calf mortality was not associated with *Salmonella* shedding at slaughter in our study. This may be because there are other more common reasons for calf mortality in veal calves than *Salmonella*, including viral diseases such as BRSV and enzootic pneumonia.

Purchase of animals from herds that could not be classified as low risk (level 1) in the *Salmonella* surveillance programme was found to be associated with isolation of *Salmonella* from the herds. This is most likely because such herds are often infected with *Salmonella* and infectious animals are purchased into the veal herd. Similar associations have been found in dairy herds [10, 20]. This result is important because it provides an option for the control of *Salmonella* in the cattle industry. If purchase from other than low-risk herds can be limited or stopped – for instance by reducing the number of infected dairy herds or by legislation against such trade, the number of infected veal herds can be reduced. This would be expected to lead to reduced input of infectious animals to the abattoirs.

Although excretion of *S. Dublin* bacteria at the time of slaughter was generally low with an anticipated low impact on food safety, high shedders will occur at the slaughter line from time to time, but cannot easily be predicted based on register data and

controlled *per se*. The results of this study strongly suggest that *Salmonella* infection can be controlled in veal herds by avoiding purchase of calves from infected herds. This should probably be supported by management aimed at controlling the spread of *Salmonella* between calves within the herds.

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DECLARATION OF INTEREST

None.

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