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Milk yield and *Salmonella* in dairy herds. Nielsen et al.

The effect of *Salmonella* introduction on milk yield in 28 Danish dairy cattle herds was evaluated. All but second parity cows had reduced milk yield seven to 15 months after the estimated date of introduction of *Salmonella* into the herd, compared with same parity cows from the same herds in the 12 months before introduction. These results can be used by farmers and the dairy industry to quantify production and economic losses from reduced milk yield following introduction of *Salmonella* into dairy herds.

MILK YIELD AND *SALMONELLA* IN DAIRY HERDS

Evaluation of Milk Yield Losses Associated with *Salmonella* Antibodies in Bulk-Tank Milk in Bovine Dairy Herds

T. D. Nielsen[*1], **L. E. Green**[†] **A. B. Kudahl**[‡], **S. Østergaard**[‡], **L. R. Nielsen**[*]

[*]Department of Large Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Grønnegårdsvej 8, DK-1870 Frederiksberg C, Denmark

[†]School of Life Sciences, University of Warwick, Coventry CV4 7AL England

[‡]Faculty of Agricultural Sciences, Institute of Animal Health and Bioscience, University of Aarhus, Denmark

[1]Corresponding author:

Phone: +45 35 33 30 18, Fax: +45 35 33 30 22, E-mail: tdni@life.ku.dk

ABSTRACT

The effect of *Salmonella* on milk production is not well established in cattle. The objective of this study was to investigate whether introduction of *Salmonella* into dairy cattle herds was associated with reduced milk yield and the duration of any effect. Longitudinal data from 2005 through 2009 were used, with data from 12 months before until 18 months after the estimated date of infection. Twenty-eight case herds were selected based on an increase in the level of *Salmonella* specific antibodies in bulk-tank milk from < 10 corrected optic density percentage (**ODC%**) to ≥ 70 ODC% between two consecutive 3-monthly measurements in the Danish *Salmonella* surveillance program. All selected case herds were conventional Danish Holstein herds. Control herds (n = 40) were selected randomly from Danish Holstein herds with *Salmonella* antibody levels consistently < 10 ODC%. A date of herd infection was randomly allocated to the control herds. Hierarchical mixed effect models with the outcome test day energy corrected milk yield (**ECM**)/cow were used to investigate the daily milk yield before and after the estimated herd infection date for cows in parity 1, 2 and 3+. Control herds were used to evaluate whether the effects in the case herds could be reproduced in herds without *Salmonella* infection. Herd size, days in milk, somatic cell count, season, and year were included in the models. The key results were that first parity cow yield was reduced by a mean of 1.4 kg (95% CI: 0.5 to 2.3) ECM/cow per day from seven to 15 months after the estimated herd infection date, compared with first parity cows in the same herds in the 12 months before the estimated herd infection date. Yield for parity 3+ was reduced by a mean of 3.0 kg (95% CI: 1.3 to 4.8) ECM/cow per day from seven to 15 months after herd infection compared with parity 3+ cows in the 12 months before the estimated herd infection. There were minor differences in yield in second parity cows before and after herd infection, and no difference between cows in control herds before and after the simulated infection date. There was a significant drop in milk yield in affected herds and the reduction was detectable several months after the increase in

bulk-tank milk *Salmonella* antibodies. It took more than a year for milk yield to return to pre-infection levels.

Keywords: *Salmonella*, bulk-tank milk antibody, dairy cattle, milk yield

INTRODUCTION

Salmonella is a common cause of food poisoning with more than 130,000 confirmed cases in the EU in 2008 (Anonymous, 2010b). Although chicken and pork are the major animal sources of *Salmonella,* milk and beef cannot be excluded as a cause of human salmonellosis. In Denmark, *Salmonella (S.)* Dublin is the most frequently isolated serotype from beef with more than 60% of isolates from domestic beef (Anonymous, 2010a). *S.* Dublin was the fourth most common serotype isolated from diseased humans in Denmark in 2009 (Anonymous, 2010a), and this serotype has been reported to lead to higher case mortality rates in humans than other serotypes (Helms et al., 2003). *S.* Dublin is also the most frequently isolated serotype of *Salmonella* in cattle with clinical salmonellosis in Denmark (Anonymous, 2009a). It is host adapted to cattle and can create carrier animals as well as causing endemic infection in cattle herds (House et al., 1993; Veling, 2004). Since 2002, there has been a surveillance program monitoring cattle herds in Denmark, where all dairy herds are tested at three month intervals. In this program, an in-house ELISA test (Eurofins Denmark) is used to detect antibodies against lipopolysaccharide antigens from *S.* Dublin in bulk-tank milk (**BTM**). The ELISA test might cross-react with other *Salmonella* serotypes - in Danish cattle herds mainly *S.* Typhimurium. Herds are classified either “most likely free of *S.* Dublin” (level 1) or “most likely infected with *S.* Dublin” (level 2) (Warnick et al., 2006; Anonymous, 2009a). A shift from test-negative (level 1) to test-positive (level 2) is indicative of *Salmonella*-infection spreading among lactating cows (Nielsen and Ersbøll, 2005).

Decreased milk yield has been reported in cows from herds with *Salmonella* infection. One herd investigated by Anderson et al. (2001) experienced a *S.* Agona outbreak with decreased milk yield. Hermesch et al. (2008) reported that cows vaccinated against *S.* Newport during their dry period, produced on average 1.2 kg per day more milk for the first 90 days in the subsequent lactation than non-vaccinated cows in one dairy herd, but that the expected 305-day yield did not differ significantly. This herd had no clinical signs, although *S.* Newport was isolated from fecal samples of cows. A *S.* Dublin outbreak in one 100 cow dairy herd in England caused a severe drop in milk yield (Bazeley, 2006): a milk-loss of 19,430L over approximately two months was estimated. John (1946) reported severe drop in milk yield and that some cows even stopped producing altogether when infected with *S.* Dublin. In addition, according to Vandegraaff and Malmo (1977) a severe drop in milk production was seen in cows clinically affected by *S.* Dublin, but most were back to normal production within ten days of beginning treatment. In contrast to this, other authors have reported cows shedding *Salmonella* without any signs and overall milk yield similar to that of herds without reports of *Salmonella* infection (Gay and Hunsaker, 1993; Huston et al., 2002). However, overall yield varies from herd to herd, so it might be difficult to show effects of *Salmonella* on milk yield by comparing herds. House et al.(2001) found no effect on 305 day yield in a herd where they compared yield in unvaccinated cows to yield in cows that were vaccinated with an autogenous *S.* Montevideo vaccine or cows that were vaccinated with a modified live *S.* Cholerasuis vaccine. However, in testing the herd for *Salmonella* before the study, nine serotypes of *Salmonella* were isolated from fecal culture of cows, so it is not known which, if any, of the 9 serotypes were affecting milk yield.

Very few studies have included a larger number of herds and, to our knowledge, no studies have quantified the changes in milk yield within herd for an extended period of time before and after herds became infected with *Salmonella*. Furthermore, no studies have estimated how long it takes before the herd milk yield is back to pre-infection levels. This is important information for the farmer and the industry in order to quantify production and economic losses from reduced milk yield. Such information will be useful for the Danish Cattle Federation to motivate farmers to prevent and control *Salmonella*. The estimates are also useful for further research such as simulation modeling of long-term effects of *Salmonella* infection in dairy herds. The objective of the current study was to investigate long-term changes in milk yield in Danish dairy herds that experienced large increases in BTM antibodies directed against *S.* Dublin between 2005 and 2009. A large increase in the concentration of BTM antibodies was assumed to be a sign of spread of *Salmonella* in the herd.

MATERIALS AND METHODS

Salmonella Status of Herds

All Danish dairy herds are tested quarterly in the Danish *Salmonella* surveillance program and a herd is classified as level 2 if the average of the last four BTM ELISA test results is ≥ 25 optical density corrected (**ODC%**), when compared to a negative control test (Nielsen et al., 2007b). The positive predictive value of the herd testing scheme has been estimated to be between 0.47 and 0.88 depending on the prevalence of infected herds and the negative predictive value to above 0.96 when between-herd prevalence is below 30% (Warnick et al., 2006). Thus, level 2-herds are not always infected, whereas level 1-herds are most likely uninfected. It was therefore decided to improve the positive predictive value for detection of newly infected herds in this study by restricting the case herd group to herds with large increases in BTM-antibody levels as described in the section "Selection of herds" below.

Selection of Herds

The study was based on registry data from the Danish Cattle Database (Knowledge Centre for Agriculture, Cattle) from January 2005 to December 2009. Selection of herds was based on their BTM *Salmonella* ODC%-measurements from the Danish surveillance program. A herd was included as a case herd, if it had an antibody response < 10 ODC% in at least three samples over a minimum of one year followed by an increase to ≥ 70 ODC% and the test following the initial high test was ≥ 25 ODC% to exclude potentially false positive. Out of approximately 3300 dairy herds, 44 herds fulfilled these criteria. Two herds had an antibody response < 25 ODC% in the test following the initial test, but antibody response ≥ 25 ODC% in subsequent tests. This indicated that they were infected with *Salmonella* and they were also included as case herds. The 46 herds were stratified on main breed, farming type (conventional or organic), and herd size and were analyzed descriptively. The largest group consisted of conventional Danish Holstein dairy herds and 28 herds with a minimum of 40 cows in the study period were selected as case-herds. The following herds were excluded from the model: five herds with no milk yield recordings around the estimated time of infection, four herds not consisting of Danish Holsteins (one Jersey, two Danish Reds and one Crossbreed), one herd consisting of < 40 cows in the study period and eight organic herds. Forty control herds were randomly selected from conventional Danish Holstein herds with > 40 cows in the study period and antibody response < 10 ODC% throughout the study period.

Test day energy corrected milk yield (Test day ECM)

The outcome variable was test day energy corrected milk yield **(test day ECM)** in kg. It was measured as part of the milk recording scheme, a voluntary system in which information of individual cow milk yield is routinely recorded up to 11 times per year. Milk yield in kg, somatic cell count (SCC), fat and protein percentages are recorded in this program and reported back to the farmer. Test day ECM is calculated as in Equation (1):

Test day ECM = (milk in kg*(383*percent fat + 242*percent protein + 780.8))/3140 Eq. (1)

This is a common way to calculate test day ECM in Denmark and is a slight modification of the calculation proposed by Sjaunja et al. (1990).

From the test day ECM recordings, a basic lactation curve was modeled as a function of days in milk (**DIM**) truncated at 305 days and Wilmink's function: exp(ECM)$^{(-0.05*\mathrm{DIM})}$ (Wilmink, 1987). Wilmink's function is an exponential function that models the natural shape of lactation curves by adjusting for DIM with increasing milk yield until around day 60 and then decreasing milk yield throughout the rest of the lactation.

Time Period (T)

An estimated infection date of 61 days prior to the registered increase in BTM-*Salmonella* ODC% was set for each case herd. This was chosen to allow for spread of *Salmonella* from the animal initially infected to other animals in the herd and it accounted for the fact that it takes two weeks from infection to seroconversion (Robertsson, 1984). Furthermore, we were unlikely to identify the first day of high ODC%, because herds were only tested every three months. A variable for 3-month time periods (**T**) was included in the model, to represent time to and from infection, where T_0 was one to three months after the estimated infection date, T_1 was four to six months after infection, T_{-1} was one to three months before estimated infection date and so forth. T-values ranged from T_{-4} to T_5. A simulated infection date, weighted by year and month of infection in the case herds, was set for each control herd to ensure that T_i were comparable for control and case herds. Three control herds had estimated infection dates late in 2008 so there were no test day ECM observations in T_5.

Season

Test day ECM displayed a marked seasonality with highest yield in spring and lowest in fall. A sine curve was created for each parity with amplitude depending on the difference between year-quarter with highest and lowest yield for the control herds, where year-quarters were January to March, April to June, July to September and October to December. This difference in yield between spring and fall was 1.5, 1.5 and 1.9 kg test day ECM for parity 1, 2 and 3+ respectively. The sine curve was given by:

$$\text{Sine} = \text{difference in milk yield}*\text{sine}\ (2*\pi*\text{year-quarter}/4) \qquad \text{Eq. (2)}$$

The sine value was hence constant throughout each quarter of a year and had only 4 values for each parity. Model fit for parity 3+ cows was better when seasonality was included as season (March to May, June to August, September to November and December to February) rather than the sine-curve. Hence, season was included in the model for this parity instead of year-quarter.

Other Confounding Variables

Other variables known to affect milk yield were included in the study: year, log somatic cell count **(LogSCC),** parity (1, 2 and 3+). All data were extracted from the milk recording scheme. Herd size was calculated as the mean number of cows per test date and was included at herd-level. One control herd increased in size from approximately 80 to 200 cows. Data from this herd were excluded after the herd size increased (meaning that data from part of T_4 and all of T_5 were deleted).

Data Analysis

Descriptive statistics were performed in SAS® v. 9.2. Effects on test day ECM were analyzed using a multilevel model in MLwiN 2.21 (Rasbash et al., 2009). The outcome variable had a normal distribution. The hierarchical structure of the data was test day ECM within cow within herd, and we used an iterative generalized least square means procedure for estimations. There were 1.6 parities per cow on average, so each parity was modeled separately. All relevant 2-way interactions were included in the model by forward selection, if they were significant at 5% and if they improved model fit. The final model for parity 1 and 2 was:

$$\text{Test day ECM}_{\text{ijk}} = \beta 0_{ijk} + \text{DIM}(X_{ijk}) + \exp(\text{ECM})^{(-0.05*\text{DIM})}(X_{ijk}) + \text{Log(SCC)}(X_{ijk}) + \text{Sine}(X_{ijk}) + \text{Year} + \text{T} + \text{T*DIM}(X_{ijk}) + \text{T*Sine}(X_{ijk}) + \text{T*Year} + \text{Year*Sine}(X_{ijk}) + v_k + u_{jk} + e_i \qquad \text{Eq. (3)}$$

For parity 3+ the final model was:

$$\text{Test day ECM}_{\text{ijk}} = \beta 0_{ijk} + \text{DIM}(X_{ijk}) + \exp(\text{ECM})^{(-0.05*\text{DIM})}(X_{ijk}) + \text{Log(SCC)}(X_{ijk}) + \text{Season} + \text{Year} + \text{T} + \text{T*DIM}(X_{ijk}) + \text{T*Season} + \text{T*Year} + v_k + u_{jk} + e_i \qquad \text{Eq. (4)}$$

For all models, test day ECM_{ijk} is milk yield on test day *i* for cow *j* in herd *k*, β0 is the intercept on test day *i* for cow *j* in herd *k*, X_{ijk} are the fixed effects varying by cow observation, v_k random effect of herd, u_{jk} random effect of cow and e_i residual error at the outcome level for test day ECM.

Test day ECM was modeled from 12 months (T_{-4}) before to 18 months (T_5) after the estimated infection date for the herd. Control and case herds were modeled separately. The final models for control herds were applied to the respective parity case herd data to assess associations between test day ECM and *Salmonella.* Year 2005 was used as baseline in the model, and data were centered on mean of logSCC (4) (corresponding to a cell count of approximately 55,000 per ml). Fall was used as baseline for parity 3+. Standard residuals for each level in the model and predicted vs. observed test day ECM were plotted to asses model fit.

RESULTS

The 68 herds in the dataset included 119,814 test day ECM observations from 11,959 cows, with 5,436 cows in the case herds and 6,523 cows in the control herds. Comparison of case and control herds is presented in Table 1. Each cow contributed between one and 26 observations (mean = 10). The case herds were on average larger than the control herds, with more cow observations and cows per herd as well as more cows per test date. Descriptions of logSCC and milk yield for the different parities can be seen in Table 2. Case herds had a lower proportion of parity 3+ observations than control herds. The distribution of observations in T_i can be seen in Table 3. Generally, there were fewer observations in T_5 due to the fact that some herds had an estimated time of infection late in 2008.

Results from the model for case herds for parities 1 and 2 are given in Table 4 and for parity 3+ in Table 5. Interactions between T and DIM, Year and Season / Sine were significant in all parities. An interaction between Sine and Year for parity 1 and 2 was also significant (data shown in Appendix 1). Parity 1 cows had reduced yield in T_3 and T_4 (10 to 15 months after the estimated herd infection date), as well as borderline significantly reduced yield in T_2 (seven to nine months after the estimated herd infection date). Parity 3+ cows had the largest reduction in yield for the period (T_2 to T_4). The mean daily milk loss in the period seven to 15 months after the estimated herd infection was 1.4 kg ECM/cow per day (95% CI: 0.5 to 2.3 kg) for parity 1 cows and 3.0 kg ECM/cow per day (95% CI: 1.3 to 4.8 kg) for parity 3+ cows (Figure 1). Parity 2 cows had decreased yield in T_4. For a herd with 100 -cow years and 36, 32 and 32 % of the cows in parity 1, 2 and 3+ respectively, the mean loss in milk production would be more than 40,000 kg ECM (95% CI: 8,000-153,000) in the first year after infection.

Milk yield from cows in control herds was lower in T_{-2} for parity 2 (mean = -2.7 kg ECM/cow per day, 95% CI: -3.7 to -0.8 kg) and borderline significantly reduced in parity 1 in T_{-1} (mean = -1.0 kg ECM/cow per day, 95% CI: -2.0 to 0.1 kg) (Figure 1).

Average herd size was not significant in either control or case herds and did not act as a confounder on other variables so it was omitted from the models. Likewise, the interaction between T and Wilmink's function was tested in the models, but did not change the model estimates or significance of other variables and was therefore left out. Plots of standard residuals and predicted vs. observed test day ECM showed acceptable model fit for all parities (data not shown). There were only minor correlations between T and calendar month, although estimated infection date was strongly seasonal (data not shown).

DISCUSSION

Results

In our study there was a significant reduction in milk yield seven to 15 months after the estimated herd infection date (T_2 to T_4) for cows in parity 1 and 3+. These findings are similar to those reported by others where newly infected cows or herds had a decrease in milk yield (Vandegraaff and Malmo, 1977; Anderson et al., 2001; Bazeley, 2006) but we have quantified the milk loss. Other authors reported that there was no association between *Salmonella* infection and milk yield , however, in these studies the time of introduction of *Salmonella* was not known, so these authors were merely reporting associations between seropositivity and milk yield (McClure et al., 1989; Huston et al., 2002; Van Kessel et al., 2007).

The biggest overall reduction in yield was seen in parity 3+ cows. Other authors report greater reductions in milk yield in higher parity cows with mastitis (Bennedsgaard et al., 2003) and greater susceptibility to mastitis (Breen et al., 2009), and a similar pattern with lameness (Amory et al., 2008; Sanders et al., 2009). It is therefore possible that parity 3+ cows' milk yield was more affected when they were infected with *Salmonella*. The smaller reduction in milk yield in parity 2 cows compared to the other parities was also observed in a smaller study, where milk yield from cows with high antibody levels was compared to milk yield for herd mates with low antibody levels in endemically infected herds (data not published). A possible explanation for this pattern could be different management strategies (e.g. culling patterns) in case herds compared with control herds as a result of herd infection. The ratio between parity 1 and 2 observations decreased over time in case herds, whilst it remained constant in control herds. Consequently it is possible that farmers in case herds culled a larger proportion of parity 2 cows due to poor milk production and that this might explain why there appear to be a different pattern in this parity compared to parity 1 and 3+.

It took 15 months (until T_5) before milk yield was back to pre-infection levels, suggesting that either infected cows were affected for a long time or that infection spread slowly through the herd and different cattle were affected over a prolonged period. It was not possible to discern which of these occurred in our study because *Salmonella* status was a herd variable. Even though the BTM antibody levels generally decreased after the initial sudden increases, 19 of the 28 infected herds still had BTM antibody levels > 25 ODC% at T_5 (data not shown). Previous studies have shown that *Salmonella* can be present in herds without necessarily affecting the milk yield and it is possible that herd immunity develops with repeated exposure and re-infection of the cows (Steinbach et al., 1996). Some herds had a second increase in BTM antibody level 1 to 2 years after the initial increase, and this could indicate a re-infection of the cows in these herds which may have led to repeated periods of decreased milk yield. However, there were insufficient data to analyze the differences in milk yield losses in the case herds with persistently high antibodies and herds where antibodies returned to lower levels within the study period.

The variance of milk yield was greater before than after the estimated infection date in case herds, and greater in case herds than in control herds. Descriptive analyses of the data confirmed this pattern. It is probably due to factors that were not adjusted for in the model, such as presence of other diseases, management routines and purchase patterns. Such diseases might not affect all cows leading to higher variance in milk yield in case herds than control herds. Unfortunately, we did not have information available about other diseases in the herds.

Herd classification

We used an increase in BTM antibody level as sign of introduction of *Salmonella* to the herd. The cut-off level for a herd classified as level 2 in the Danish surveillance program is ≥ 25 ODC%. The negative predictive value of this has been estimated to be 0.98-0.99 when the overall herd prevalence is 0.15-0.30, meaning 1-2% false negative herds (Warnick et al., 2006). We used cut-off < 10 ODC% for the control herds to increase the probability that cows in the control herds had had no antibodies and hence had no exposure to *Salmonella*. Thus, we believe that the control herds were unlikely to have been misclassified. Likewise, we used a cut-off of ≥ 70 ODC% for the case herds to increase our confidence that there was active infection with *Salmonella* in the herds. Furthermore, we only included case herds with antibody levels ≥ 25 ODC% following the initial high test value. This reduced the risk of herds being false positives. The positive predictive value of the surveillance program has been estimated to be 0.68 to 0.88 depending on the underlying true prevalence of between herd infection (Warnick et al., 2006). By using the higher cut-off point for case herds, we believe that the positive predictive value was improved, which increased our confidence that the case herds were truly infected with *Salmonella*.

There is no way of knowing which cows in the case herds had clinical signs of salmonellosis, which were subclinical infected and which were non-diseased or non-infected, because it was not possible to obtain animal level data on infection status. This would have required frequent repeated measurements at animal level over a long period of time and even then it would still be complicated to correctly classify the cows to determine infection dates for each animal (Nielsen et al., 2004; Nielsen et al., 2007a). Therefore, the estimates of milk yield changes were estimated as averages and variations across all cows in the respective parities in the selected case herds. However, Hoorfar et al. (1995) reported that herds with outbreaks of salmonellosis caused by *S.* Dublin within the last six months all had BTM antibody levels OD > 0.5, a cut-off equivalent to approximately 30 - 40 ODC% in the ELISA used in the surveillance program. In this study, we have used a higher cut-off for inclusion of case herds, so it is likely that some cows had clinical signs of salmonellosis during the spread of the infection. Nielsen and Ersbøll (2005) found that although not all cows need to be infected to cause a large increase in BTM-antibodies, the prevalence of antibody-positive cows (ODC% > 25) was usually above 50% at BTM ELISA values of 70 ODC%, and herds with such high BTM ELISA values were frequently found bacteriological test-positive. This suggests that a large proportion of the cows were exposed to *Salmonella* bacteria in the case herds selected for our study, but it is likely that at all time points after the estimated time of infection, there were both uninfected and infected cows present in each case herd. The infection could then continue to spread over the following six to 12 months. Because increase in BTM antibodies happened prior to reduction in milk yield, it is likely that introduction of *Salmonella* to the herd caused the reduction in yield.

In the Danish surveillance program antibodies towards group D antigens are measured, which in cattle is very often *S.* Dublin. There might be a difference in how much infection with different *Salmonella* serotypes affects milk yield. Since *S.* Dublin is host adapted to cattle it might affect yield, whereas non host adapted serotypes such as *S.* Menhaden might not. There is a risk of other serotypes cross-reacting with the test used in the Danish surveillance program. In Denmark, this would mainly be *S.* Typhimurium. However, the most frequently isolated serotype from cattle is *S.* Dublin (Anonymous, 2009a), and we therefore consider the majority of the case herds to have been infected with *S.* Dublin.

Infection date

BTM detection of *Salmonella* had a seasonal trend, with most herds being infected from August through December. This is similar to the patterns observed in the national surveillance program, where there is an increase in herds with high BTM antibody levels in the fall. Consequently, simulated infection dates for control herds were weighted by year and month of infection as in the case herds. Hence, we believe that the pattern seen after T_0, was due to *Salmonella*.

Strength and limitation of study

Our study included 68 dairy herds and is, to our knowledge, the largest study modeling associations between *Salmonella* and milk yield. Furthermore it describes the yield from 12 months before to 18 months after estimated herd infection. The next largest study of *Salmonella* and milk yield was 24 herds (Anderson et al., 1997) with *S*. Menhaden infection. Clinical signs were mainly diarrhea which affected 0 to 40% (mean 7%) of production groups. The eight case herds had similar production levels to the 16 control herds.

Other confounding variables than those included in this study could lead to decreased milk yield (e.g. management). We used registry data for this study, so it was not possible to include management practices but including the random effect of farm accounted for between herd unexplained variance in yield. There were fewer parity 3+ observations in the case herds than in the control herds, but similar numbers of observations for parity 1. This could be an indication that there were different management practices in the case and control herds. However, the ratio between parity 1 and parity 3+ for the case herds was constant throughout the T-periods, which indicates that the management practices (e.g. culling decisions) did not change for the case herds after estimated herd infection. One peculiarity in the results was the significantly reduced milk yield for parity 2 cows in T_{-2} in control herds (four to six months before the artificially selected infection date for the herd). This is difficult to explain but could be due to other confounding variables not included in the model.

Control herds were selected randomly from all conventional Danish Holstein dairy herds with consistently low BTM antibody levels. Case herds in the period 2005-2009 with conventional farming practice and Danish Holstein cows were included in the study, and on average these herds were larger than the control herds. However, there was no significant difference in herd size between case and control herds and herd size did not affect test day ECM when included in the model , so the difference in herd size between case and control herds appeared not to affect the results. It is not known whether other breeds of cattle or organic herds would be affected in a similar way to the study herds if *Salmonella* was introduced into the herd, but approximately 73% of Danish dairy cows are Holsteins (Anonymous, 2009b) and 90% are on conventional farms (Knowledge Centre for Agriculture, Cattle), so this study is likely to represent the majority of Danish farms.

CONCLUSIONS

There is a significant drop in milk yield in *Salmonella* infected herd, mean estimated milk yield loss for a herd with 85 cows was 29,000 kg ECM in the 18 months following estimated time of introduction of infection to the herd. The reduction is detectable several months after the increase in bulk-tank milk *Salmonella* antibodies. It took more than a year for milk yield to return to pre-infection levels.

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Table 1. Attributes of 40 control study herds and 28 case study herds with large, sudden increases in bulk tank milk *Salmonella* antibody levels indicative of recent herd infection

	Case herds (n = 28)					Control herds (n = 40)				
	Mean	Median	Q1[1] Q3	Min Max	Total	Mean	Median	Q1 Q3	Min Max	total
Observations	1,961	1,871	1015 2495	520 3,792	54,911	1,623	1,505	825 2,318	265 3,505	64,903
Observations/ cow	10.1	9	5 15	1 26	54,911	10.0	9	5 15	1 25	64,903
Cows	194	203	107 266	62 433	5,436	163	161	99 221	44 336	6,523
Cows/ test date	79	79	46 106	21 236	693	68	67	47 88	10 155	956

[1] Q1=25% quartile and Q3= 75% quartile

Table 2. Descriptive statistics for energy test day corrected milk yield (test day ECM) and log to somatic cell count (LogSCC) for 40 control herds and 28 case herds with large, sudden increases in bulk tank milk *Salmonella* antibody levels indicative of recent herd infection

	Case herds (n=28)					Control herds (n=40)				
	Mean	SD[1]	5% quartile	95% quartile	n	Mean	SD	5% quartile	95% quartile	n
Test day ECM										
Parity 1	26.9	5.7	17.3	35.6	21,723	26.7	5.5	17.5	35.4	22,669
Parity 2	30.8	7.9	17.6	43.5	16,282	30.6	7.7	17.8	43.0	18,104
Parity 3+	31.3	8.7	16.6	45.7	16,906	31.9	8.7	17.8	46.1	24,130
LogSCC	4.79	1.2	3.2	7.1	54,403	4.77	1.2	3.2	7.1	64,384

[1] Standard deviation

Table 3. Distribution of observations in 3-months time periods T_i for 40 control herds and 28 case herds with large, sudden increases in bulk tank milk *Salmonella* antibody levels indicative of recent herd infection

T[1]		T_{-4}	T_{-3}	T_{-2}	T_{-1}	T_0	T_1	T_2	T_3	T_4	T_5	Total
Month[2]	Start	-12	-9	-6	-3	1	4	7	10	13	16	
	End	-10	-7	-4	-1	3	6	9	12	15	18	
	Parity											
Case												
	1	2,332	2,426	2,259	1,802	2,558	2,197	2,145	2,095	2,159	1,750	21,723
	2	1,693	1,829	1,713	1,296	1,757	1,602	1,619	1,675	1,653	1,445	16,282
	3	1,779	1,820	1,683	1,412	1,956	1,675	1,573	1,640	1,871	1,497	16,906
Control												
	1	2,488	2,160	2,449	2,162	2,558	2,330	2,322	2,006	2,190	2,004	22,669
	2	1,975	1,797	1,933	1,607	2,029	1,877	1,979	1,711	1,761	1,435	18,104
	3	2,497	2,118	2,490	2,180	2,768	2,460	2,668	2,229	2,608	2,112	24,130

[1] Time period in 3-month intervals

[2] Start and end month of time period relative to estimated herd infection date

Table 4. Multilevel analysis for fixed effects on test day energy corrected milk yield (test day ECM) for parity 1 and 2 for 28 Danish Holstein herds with large, sudden increases in bulk tank milk *Salmonella* antibody levels indicative of recent herd infection

		Parity 1				Parity 2			
Variable		Mean	s.e.[1]	LCLM[2]	UCLM[3]	Mean	s.e.	LCLM	UCLM
Intercept		26.55	1.06	24.46	28.63	34.70	2.27	30.24	39.16
DIM[4]		-0.02	0.00	-0.02	-0.02	-0.05	0.00	-0.05	-0.04
$\text{Exp(ECM)}^{(-0.05*\text{DIM})}$		-5.77	0.20	-6.16	-5.37	-5.03	0.29	-5.59	-4.47
LogSCC[5]		-0.25	0.03	-0.31	-0.18	-0.51	0.04	-0.59	-0.43
Year	2005	0	-	-	-	0	-	-	-
	2006	1.43	0.88	-0.29	3.15	0.91	2.15	-3.31	5.12
	2007	3.31	0.90	1.54	5.08	3.05	2.17	-1.20	7.30
	2008	3.65	0.95	1.79	5.51	3.30	2.21	-1.03	7.63
	2009	5.19	1.06	3.11	7.26	5.28	2.31	0.76	9.81
Sine season		-0.29	0.27	-0.82	0.23	-0.44	0.39	-1.21	0.33
Standardized test day ECM/time period (months relative to estimated herd infection)									
-4	(-12 through -10)	1.29	0.92	-0.51	3.09	2.84	2.20	-1.47	7.14
-3	(-9 through -7)	1.46	0.92	-0.34	3.26	2.81	2.19	-1.49	7.11
-2	(-6 through -4)	0.15	0.95	-1.71	2.00	1.47	2.21	-2.85	5.80
-1	(-3 through -1)	0.14	0.92	-1.67	1.95	-0.72	2.17	-4.97	3.53
0	(1 through 3)	0	-	-	-	0	-	-	-
1	(4 through 6)	0.85	0.46	-0.05	1.75	1.89	0.67	0.57	3.20
2	(7 through 9)	-0.82	0.45	-1.71	0.06	1.24	0.67	-0.07	2.55
3	(10 through 12)	-1.30	0.47	-2.23	-0.37	-0.94	0.70	-2.30	0.43
4	(13 through 15)	-1.99	0.48	-2.93	-1.04	-1.73	0.70	-3.10	-0.37
5	(16 through 18)	0.36	0.45	-0.52	1.25	0.48	0.65	-0.79	1.75
Random effects									
Herd level variance		8.95	2.45			11.96	3.31		
Cow level variance		15.53	0.43			24.93	0.80		
Test day ECM level variance		11.30	0.12			18.34	0.22		

[1]Standard error of the mean [2]Lower confidence limit [3]Upper confidence limit [4]Days in milk [5]Log somatic cell count

Table 5. Multilevel analysis for main fixed effects on test day energy corrected milk yield (test day ECM) for parity 3 or higher for 28 Danish Holstein herds with large, sudden increases in bulk tank milk *Salmonella* antibody levels indicative of recent herd infection

Variable			Mean	s.e.[1]	LCLM[2]	UCLM[3]
Intercept			39.24	1.97	35.39	43.10
DIM[4]			-0.05	0.00	-0.06	-0.05
$\text{Exp(ECM)}^{(-0.05*\text{DIM})}$			-6.28	0.32	-6.90	-5.65
LogSCC[5]			-0.81	0.04	-0.89	-0.72
Year	2005		0	-	-	-
	2006		-0.06	1.87	-3.72	3.60
	2007		0.32	1.88	-3.37	4.01
	2008		-0.69	1.92	-4.46	3.07
	2009		1.33	2.03	-2.65	5.30
Season	Fall		0	-	-	-
	Winter		-0.50	0.41	-1.30	0.30
	Spring		3.01	0.93	1.18	4.83
	Summer		0.66	0.46	-0.24	1.57
Standardized test day ECM/time period (months relative to estimated herd infection)						
	-4	(-12 through -10)	1.42	1.91	-2.33	5.18
	-3	(-9 through -7)	2.12	1.93	-1.66	5.90
	-2	(-6 through -4)	0.75	2.18	-3.52	5.01
	-1	(-3 through -1)	-1.24	1.95	-5.07	2.59
	0	(1 through 3)	0	-	-	-
	1	(4 through 6)	-0.49	0.83	-2.12	1.14
	2	(7 through 9)	-4.27	1.27	-6.75	-1.79
	3	(10 through 12)	-3.62	0.76	-5.12	-2.12
	4	(13 through 15)	-1.22	0.62	-2.43	-0.01
	5	(16 through 18)	1.33	0.64	0.08	2.57
Random effects						
Herd level variance			7.98	2.92		
Cow level variance			27.75	1.02		
Test day ECM level variance			26.02	0.30		

[1] Standard error of the mean [2] Lower confidence limit [3] Upper confidence limit [4] Days in milk [5] Log somatic cell count

APPENDIX 1

Results for interactions in multilevel analysis for fixed effects on energy corrected milk yield for parity 1 and 2 for 28 Danish Holstein herds with large, sudden increases in bulk tank milk *Salmonella* antibody levels indicative of recent herd infection

	Parity 1				Parity 2			
Variable	Mean	s.e.[1]	LCLM[2]	UCLM[3]	Mean	s.e.	LCLM	UCLM
DIM[4]*T[5]								
DIM*T$_{-4}$	0.005	0.002	0.001	0.009	-0.007	0.003	-0.013	-0.001
DIM*T$_{-3}$	0.001	0.002	-0.003	0.005	-0.010	0.003	-0.016	-0.004
DIM*T$_{-2}$	0.003	0.002	-0.001	0.007	-0.006	0.002	-0.010	-0.002
DIM*T$_{-1}$	0.003	0.001	0.001	0.005	0.003	0.002	-0.001	0.007
DIM*T$_0$	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DIM*T$_1$	0.001	0.001	-0.001	0.003	-0.007	0.002	-0.011	-0.003
DIM*T$_2$	0.005	0.002	0.001	0.009	-0.003	0.002	-0.007	0.001
DIM*T$_3$	0.006	0.002	0.002	0.010	0.003	0.002	-0.001	0.007
DIM*T$_4$	0.003	0.002	-0.001	0.007	-0.001	0.002	-0.005	0.003
DIM*T$_5$	-0.003	0.002	-0.007	0.001	-0.005	0.003	-0.011	0.001
Sine*T								
Sine*T$_{-4}$	0.23	0.22	-0.19	0.66	0.42	0.33	-0.21	1.06
Sine*T$_{-3}$	-0.74	0.26	-1.25	-0.22	-1.63	0.40	-2.41	-0.86
Sine*T$_{-2}$	0.20	0.21	-0.21	0.61	0.03	0.30	-0.56	0.63
Sine*T$_{-1}$	0.60	0.22	0.16	1.03	0.70	0.33	0.06	1.34
Sine*T$_0$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sine*T$_1$	-0.29	0.24	-0.76	0.17	-0.50	0.37	-1.21	0.22
Sine*T$_2$	0.34	0.21	-0.08	0.76	-0.34	0.32	-0.97	0.29
Sine*T$_3$	0.97	0.23	0.52	1.41	1.03	0.34	0.36	1.69
Sine*T$_4$	0.19	0.21	-0.22	0.60	-0.88	0.31	-1.48	-0.28
Sine*T$_5$	-0.07	0.28	-0.61	0.47	-1.01	0.40	-1.79	-0.23
Year*T								
2006*T$_{-4}$	-0.56	0.92	-2.37	1.25	-0.31	2.18	-4.58	3.96
2006*T$_{-3}$	0.48	0.91	-1.30	2.27	1.63	2.18	-2.64	5.89
2006*T$_{-2}$	0.62	0.94	-1.23	2.47	0.92	2.19	-3.36	5.21
2006*T$_{-1}$	-0.56	0.92	-2.37	1.25	0.84	2.16	-3.39	5.07
2006*T$_0$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2006*T$_1$	-0.51	0.46	-1.41	0.39	-1.29	0.68	-2.62	0.04
2006*T$_2$	-0.21	0.52	-1.22	0.81	2.91	0.79	1.37	4.45
2006*T$_3$	-0.02	0.58	-1.16	1.12	-0.38	0.84	-2.03	1.26
2006*T$_4$	2.28	0.70	0.91	3.64	1.80	0.93	-0.03	3.63
2006*T$_5$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2007*T$_{-4}$	-0.47	0.95	-2.33	1.39	-0.53	2.20	-4.85	3.79
2007*T$_{-3}$	0.10	0.95	-1.77	1.96	0.22	2.20	-4.10	4.54

2007*T_{-2}	1.10	-0.96	2.99	-0.79	1.82	2.21	-2.51	6.14
2007*T_{-1}	0.69	0.93	-1.14	2.51	1.70	2.17	-2.55	5.96
2007*T_0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2007*T_1	-1.06	0.43	-1.90	-0.21	-1.30	0.62	-2.52	-0.08
2007*T_2	-1.12	0.40	-1.91	-0.33	-2.31	0.59	-3.46	-1.16
2007*T_3	-1.31	0.42	-2.13	-0.48	1.19	0.62	-0.03	2.40
2007*T_4	-0.01	0.42	-0.83	0.81	-0.02	0.61	-1.21	1.16
2007*T_5	-0.55	-0.39	0.22	-1.32	-1.21	0.57	-2.31	-0.10
2008*T_{-4}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2008*T_{-3}	1.35	0.98	-0.58	3.27	1.77	2.23	-2.60	6.13
2008*T_{-2}	1.24	0.96	-0.64	3.13	-1.33	2.20	-5.63	2.98
2008*T_{-1}	0.00	0.93	-1.83	1.83	1.13	2.16	-3.11	5.36
2008*T_0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2008*T_1	-0.48	0.40	-1.26	0.30	-0.12	0.56	-1.22	0.99
2008*T_2	0.52	0.38	-0.21	1.26	0.14	0.54	-0.91	1.19
2008*T_3	1.37	0.39	0.61	2.12	2.66	0.55	1.59	3.74
2008*T_4	1.18	0.36	0.48	1.88	1.45	0.51	0.45	2.44
2008*T_5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2009*T_{-4}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2009*T_{-3}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2009*T_{-2}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2009*T_{-1}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2009*T_0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2009*T_1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2009*T_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2009*T_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2009*T_4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2009*T_5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sine*Year								
2005	-	-	-	-	-	-	-	-
2006	-0.17	0.24	-0.63	0.30	0.00	0.35	-0.69	0.69
2007	-0.31	0.26	-0.81	0.19	0.06	0.37	-0.65	0.78
2008	0.09	0.28	-0.46	0.64	0.52	0.40	-0.26	1.29
2009	-0.67	0.33	-1.31	-0.03	-0.04	0.47	-0.96	0.89

[1]Standard error of the mean [2]Lower confidence limit [3]Upper confidence limit [4]Days in milk [5]T_{-4} is 12 to 10 months before estimated herd infection, T_{-3} is nine to seven months before, T_{-2} is six to four months before, T_{-1} is three to one months before, T_0 is one to three months after, T_1 is four to six months after, T_2 is seven to nine months after, T_3 is 10 to 12 months after, T_4 is 13 to 15 months after and T_5 is 16 to 18 months after.

Results for interactions in multilevel analysis for fixed effects on energy corrected milk yield for parity 3 or higher in 28 Danish Holstein herds with large, sudden increases in bulk tank milk *Salmonella* antibody levels indicative of recent herd infection

	Parity 3+			
Variable	Mean	s.e.[1]	LCLM[2]	UCLM[3]
DIM[4]*T[5]				
DIM*T_{-4}	-0.003	0.002	-0.007	0.001
DIM*T_{-3}	-0.008	0.002	-0.012	-0.004
DIM*T_{-2}	-0.006	0.002	-0.010	-0.002
DIM*T_{-1}	0.003	0.002	-0.001	0.007
DIM*T_{0}	0.000	0.000	0.000	0.000
DIM*T_{1}	-0.004	0.002	-0.008	0.000
DIM*T_{2}	0.000	0.002	-0.004	0.004
DIM*T_{3}	0.001	0.002	-0.003	0.005
DIM*T_{4}	-0.003	0.002	-0.007	0.001
DIM*T_{5}	-0.008	0.002	-0.012	-0.004
Year*T				
2006*T_{-4}	1.45	1.92	-2.30	5.21
2006*T_{-3}	0.74	1.91	-3.00	4.47
2006*T_{-2}	2.00	1.98	-1.88	5.89
2006*T_{-1}	0.99	1.93	-2.80	4.77
2006*T_{0}	0.00	0.00	0.00	0.00
2006*T_{1}	2.12	0.68	0.78	3.46
2006*T_{2}	1.81	0.94	-0.03	3.65
2006*T_{3}	3.79	0.94	1.95	5.63
2006*T_{4}	2.61	0.97	0.71	4.50
2006*T_{5}	0.00	0.00	0.00	0.00
2007*T_{-4}	0.36	1.93	-3.42	4.13
2007*T_{-3}	0.62	1.93	-3.17	4.40
2007*T_{-2}	4.07	2.01	0.13	8.01
2007*T_{-1}	1.34	1.96	-2.51	5.18
2007*T_{0}	0.00	0.00	0.00	0.00
2007*T_{1}	0.65	0.67	-0.65	1.95
2007*T_{2}	0.66	0.65	-0.61	1.93
2007*T_{3}	0.21	0.64	-1.05	1.47
2007*T_{4}	0.14	0.63	-1.10	1.38
2007*T_{5}	-1.02	0.65	-2.29	0.24

	2008*T_{-4}	0.00	0.00	0.00	0.00
	2008*T_{-3}	1.17	1.94	-2.63	4.96
	2008*T_{-2}	2.38	2.01	-1.56	6.33
	2008*T_{-1}	0.24	1.96	-3.61	4.08
	2008*T_0	0.00	0.00	0.00	0.00
	2008*T_1	1.90	0.57	0.79	3.01
	2008*T_2	2.59	0.60	1.40	3.77
	2008*T_3	3.85	0.59	2.70	5.00
	2008*T_4	1.30	0.52	0.28	2.32
	2008*T_5	0.00	0.00	0.00	0.00
	2009*T_{-4}	0.00	0.00	0.00	0.00
	2009*T_{-3}	0.00	0.00	0.00	0.00
	2009*T_{-2}	0.00	0.00	0.00	0.00
	2009*T_{-1}	0.00	0.00	0.00	0.00
	2009*T_0	0.00	0.00	0.00	0.00
	2009*T_1	0.00	0.00	0.00	0.00
	2009*T_2	0.00	0.00	0.00	0.00
	2009*T_3	0.00	0.00	0.00	0.00
	2009*T_4	0.00	0.00	0.00	0.00
	2009*T_5	0.00	0.00	0.00	0.00
Season*T					
	Spring*T_{-4}	-2.70	1.27	-5.19	-0.21
	Spring*T_{-3}	-2.27	1.03	-4.29	-0.26
	Spring*T_{-2}	-3.93	1.66	-7.19	-0.67
	Spring*T_{-1}	-0.60	1.19	-2.92	1.73
	Spring*T_0	0.00	0.00	0.00	0.00
	Spring*T_1	-3.31	1.08	-5.43	-1.20
	Spring*T_2	0.10	1.53	-2.90	3.10
	Spring*T_3	-0.28	1.11	-2.45	1.90
	Spring*T_4	-2.14	1.20	-4.48	0.21
	Spring*T_5	-1.48	1.02	-3.49	0.52
	Summer*T_{-4}	-1.23	0.60	-2.40	-0.05
	Summer*T_{-3}	-0.68	1.13	-2.89	1.54
	Summer*T_{-2}	0.07	1.23	-2.34	2.48
	Summer*T_{-1}	0.33	0.64	-0.92	1.59
	Summer*T_0	0.00	0.00	0.00	0.00
	Summer*T_1	-0.88	1.22	-3.26	1.50
	Summer*T_2	1.41	1.07	-0.68	3.50
	Summer*T_3	1.34	0.64	0.08	2.59

Summer*T_4	0.83	0.60	-0.35	2.00
Summer*T_5	0.44	1.19	-1.90	2.78
Winter*T_{-4}	0.33	0.55	-0.75	1.41
Winter*T_{-3}	-0.68	1.13	-2.89	1.54
Winter*T_{-2}	-0.15	1.38	-2.85	2.56
Winter*T_{-1}	2.04	1.02	0.04	4.05
Winter*T_0	0.00	0.00	0.00	0.00
Winter*T_1	0.27	0.67	-1.03	1.58
Winter*T_2	3.52	1.22	1.13	5.91
Winter*T_3	0.86	1.29	-1.66	3.39
Winter*T_4	0.49	0.52	-0.52	1.50
Winter*T_5	0.57	0.54	-0.50	1.63

[1] s.e.=standard error of the mean [2] LCLM=lower confidence limit [3] UCLM=upper confidence limit [4] Days in milk [5] T_{-4} is 12 to 10 months before estimated herd infection, T_{-3} is nine to seven months before, T_{-2} is six to four months before, T_{-1} is three to one months before, T_0 is one to three months after, T_1 is four to six months after, T_2 is seven to nine months after, T_3 is 10 to 12 months after, T_4 is 13 to 15 months after and T_5 is 16 to 18 months after.