Evaluation of milk yield losses associated with *Salmonella* antibodies in bulk tank milk in bovine dairy herds

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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 determine the duration of any such effect. Longitudinal data from 2005 through 2009 were used, with data from 12 mo before until 18 mo 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 optical density percentage (ODC%) to ≥70 ODC% between 2 consecutive three-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 yield of energy-corrected milk (ECM)/cow were used to investigate daily milk yield before and after the estimated herd infection date for cows in parities 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. Yield in first-parity cows was reduced by a mean of 1.4 kg (95% confidence interval: 0.5 to 2.3) of ECM/cow per day from 7 to 15 mo after the estimated herd infection date, compared with that of first-parity cows in the same herds in the 12 mo before the estimated herd infection date. Yield for parity 3+ cows was reduced by a mean of 3.0 kg (95% confidence interval: 1.3 to 4.8) of ECM/cow per day from 7 to 15 mo after herd infection compared

with that of parity 3+ cows in the 12 mo before the estimated herd infection. We observed minor differences in yield in second-parity cows before and after herd infection and observed no difference between cows in control herds before and after the simulated infection date. Milk yield decreased significantly in affected herds and the reduction was detectable several months after the increase in bulk tank milk *Salmonella* antibodies. It took more than 1 yr for milk yield to return to preinfection levels.

Key words: *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 European Union in 2008 (European Food Safety Authority, 2010). 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* Dublin is the most frequently isolated serotype from beef with more than 60% of isolates from domestic beef (National Food Institute, 2010). *Salmonella* Dublin was the fourth most common serotype isolated from diseased humans in Denmark in 2009 (National Food Institute, 2010), and this serotype has been reported to lead to higher case mortality rates in humans than other serotypes (Helms et al., 2003). *Salmonella* Dublin is also the most frequently isolated serotype of *Salmonella* in cattle with clinical salmonellosis in Denmark (National Food Institute, 2009). It is host-adapted to cattle and can create carrier animals and cause endemic infection in cattle herds (House et al., 1993; Veling, 2004). Since 2002, a surveillance program has monitored cattle herds in Denmark, where all dairy herds are tested at 3-mo intervals. In this program, an in-house ELISA test (Eurofins Denmark, Glostrup, Denmark) is used to detect antibodies against LPS antigens from *Salmonella* Dublin in bulk-tank milk (BTM). The ELISA test might cross-react with other *Salmonella*

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serotypes, mainly *Salmonella* Typhimurium, in Danish cattle herds. Herds are classified either “most likely free of *Salmonella* Dublin” (level 1) or “most likely infected with *Salmonella* Dublin” (level 2; Warnick et al., 2006; National Food Institute, 2009). 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 *Salmonella* Agona outbreak with decreased milk yield. Hermes et al. (2008) reported that cows vaccinated against *Salmonella* Newport during their dry period, produced on average 1.2 kg/d more milk for the first 90 d in the subsequent lactation than nonvaccinated cows in one dairy herd, but that the expected 305-d yield did not differ significantly. That herd had no clinical signs, although *Salmonella* Newport was isolated from fecal samples of cows. A *Salmonella* Dublin outbreak in a 100-cow dairy herd in England caused a severe decrease in milk yield (Bazeley, 2006): a milk-loss of 19,430 L over approximately 2 mo was estimated. John (1946) reported a severe decrease in milk yield and that some cows even stopped producing altogether when infected with *Salmonella* Dublin. In addition, according to Vandegraaff and Malmo (1977), a severe decrease in milk production was seen in cows clinically affected by *Salmonella* Dublin, but most cows returned to normal production within 10 d 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-d yield in a herd where they compared yield in unvaccinated cows to that in cows vaccinated with an autogenous *Salmonella* Montevideo vaccine or in cows vaccinated with a modified live *Salmonella* Cholerasuis vaccine. However, in testing the herd for *Salmonella* before the study, 9 serotypes of *Salmonella* were isolated from fecal cultures of cows, so it is not known which, if any, of the 9 serotypes were affecting milk yield.

Compared with our study, very few studies have included a larger number of herds and, to our knowledge, none have quantified the within-herd changes in milk yield for an extended period before and after herd infection with *Salmonella*. Furthermore, no studies have estimated how long it takes before the herd milk yield returns to preinfection levels. This is important information for the farmer and the industry to quantify production and economic losses from reduced milk yield.

Such information will be useful for the Danish Cattle Federation (Aarhus, Denmark) to motivate farmers to prevent and control *Salmonella*. The estimates of milk yield effects 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 *Salmonella* 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 4 BTM ELISA test results is ≥ 25 corrected optical density percentage (ODC%) compared with 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 has been estimated to be above 0.96 when between-herd prevalence is $< 30\%$ (Warnick et al., 2006). Thus, level 2 herds are not always infected, whereas level 1 herds are most likely uninfected. We decided, therefore, 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 Selection of Herds section below.

Selection of Herds

The study was based on registry data from the Danish Cattle Database (Knowledge Centre for Agriculture, Cattle, Århus, Denmark) 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 (1) it had an antibody response < 10 ODC% in at least 3 samples over a minimum of 1 yr followed by an increase to ≥ 70 ODC% and (2) the test following the initial high test was ≥ 25 ODC% (to exclude potential false positives). Out of approximately 3,300 dairy herds, 44 herds fulfilled these criteria. Two additional herds had an antibody response < 25 ODC% in the test following the initial test but an antibody response ≥ 25 ODC% in subsequent tests. This indicated that they were infected with *Salmonella* and they were 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: 5 herds with no milk yield recordings around the estimated time of infection, 4 herds not consisting of Danish Holsteins (1 Jersey, 2 Danish Reds, and 1 crossbreed), 1 herd consisting of <40 cows in the study period, and 8 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 ECM Yield

The outcome variable was test-day ECM yield (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 (kg), SCC, and fat and protein percentages are recorded in this program and reported to the farmer. Test-day ECM is calculated as follows:

$$\text{Test-day ECM} = [\text{milk in kg} \times (383 \times \% \text{ fat} + 242 \times \% \text{ protein} + 780.8)] / 3,140. \quad [1]$$

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 DIM truncated at 305 d and the Wilmlink function: $\exp(\text{ECM})^{(-0.05 \times \text{DIM})}$ (Wilmlink, 1987). The Wilmlink function is an exponential function that models the natural shape of lactation curves by adjusting for DIM with increasing milk yield until around d 60 and then decreasing milk yield throughout the rest of the lactation.

Time Periods

An estimated infection date of 61 d before 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 2 wk from infection to seroconversion (Robertson, 1984). Furthermore, we were unlikely to identify the first day of high ODC%, because herds were only tested every 3 mo. A variable for 3-mo time periods (**T**) was included in the model to represent time before and

after infection, where T_0 was 1 to 3 mo after the estimated infection date, T_1 was 4 to 6 mo after infection, T_{-1} was 1 to 3 mo before estimated infection date and so on; 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 T_5 had no test-day ECM observations.

Season

Test-day ECM displayed a marked seasonality with the highest yield in spring and the lowest in fall. A sine curve was created for each parity, with amplitude depending on the difference between year-quarter with highest and lowest yields 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 of test-day ECM for parities 1, 2, and 3+, respectively. The sine curve was given by:

$$\text{Sine} = \text{difference in milk yield} \times \sin(2 \times \pi \times \text{year-quarter}/4). \quad [2]$$

The sine value was thus 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 SCC, 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 the herd level. One control herd increased from approximately 80 to 200 cows, and data from this herd were excluded after 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 (version 9.2, SAS Institute, Cary, NC). Effects on test-day ECM were analyzed using a multilevel model in MLwiN 2.21 (Rasbash et al., 2009). The outcome variable had a

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

Item	Case herds (n = 28)					Control herds (n = 40)				
	Mean	Median	Q1–Q31	Range	Total	Mean	Median	Q1–Q3	Range	Total
Observations	1,961	1,871	1,015–2,495	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

¹Q1 = 25% quartile; Q3 = 75% quartile.

normal distribution. The hierarchical structure of the data was test-day ECM within cow within herd, and we used an iterative generalized least squares 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 as follows:

$$\begin{aligned} \text{Test-day ECM}_{ijk} = & \beta 0_{ijk} + \text{DIM}(X_{ijk}) \\ & + \exp(\text{ECM})^{(-0.05 \times \text{DIM})}(X_{ijk}) + \text{Log}(\text{SCC})(X_{ijk}) \\ & + \text{Sine}(X_{ijk}) + \text{Year} + \text{T} + \text{T} \times \text{DIM}(X_{ijk}) \\ & + \text{T} \times \text{Sine}(X_{ijk}) + \text{T} \times \text{Year} + \text{Year} \times \text{Sine}(X_{ijk}) \\ & + v_k + u_{jk} + e_i. \end{aligned} \tag{3}$$

For parity 3+ the final model was

$$\begin{aligned} \text{Test-day ECM}_{ijk} = & \beta 0_{ijk} + \text{DIM}(X_{ijk}) \\ & + \exp(\text{ECM})^{(-0.05 \times \text{DIM})}(X_{ijk}) + \text{Log}(\text{SCC})(X_{ijk}) \\ & + \text{Season} + \text{Year} + \text{T} + \text{T} \times \text{DIM}(X_{ijk}) \\ & + \text{T} \times \text{Season} + \text{T} \times \text{Year} + v_k + u_{jk} + e_i. \end{aligned} \tag{4}$$

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 mo before (T_{−4}) to 18 mo after (T₅) 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 an SCC of approximately 55,000 cells/mL. Fall was used as baseline for parity 3+. Standard residuals for each level in the model and predicted versus observed test-day ECM were plotted to assess model fit.

RESULTS

The 68 herds in the data set 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 1 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 are given in Table 2. Case herds had a lower proportion of parity 3+ observations than did control herds. The distribution of observations in

Table 2. Descriptive statistics for test-day ECM yield and log to SCC (LogSCC) for 40 control herds and 28 case herds with large, sudden increases in bulk tank milk *Salmonella* antibody levels indicative of recent herd infection¹

Item	Case herds (n = 28)					Control herds (n = 40)				
	Mean	SD	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

¹n represents number of test-day ECM samples.

Table 3. Distribution of observations in 3-mo 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

Item	Time period (start and end months ¹)										Total
	T ₋₄ (-12 to -10)	T ₋₃ (-9 to -7)	T ₋₂ (-6 to -4)	T ₋₁ (-3 to -1)	T ₀ (1 to 3)	T ₁ (4 to 6)	T ₂ (7 to 9)	T ₃ (10 to 12)	T ₄ (13 to 15)	T ₅ (16 to 18)	
Case											
Parity 1	2,332	2,426	2,259	1,802	2,558	2,197	2,145	2,095	2,159	1,750	21,723
Parity 2	1,693	1,829	1,713	1,296	1,757	1,602	1,619	1,675	1,653	1,445	16,282
Parity 3	1,779	1,820	1,683	1,412	1,956	1,675	1,573	1,640	1,871	1,497	16,906
Control											
Parity 1	2,488	2,160	2,449	2,162	2,558	2,330	2,322	2,006	2,190	2,004	22,669
Parity 2	1,975	1,797	1,933	1,607	2,029	1,877	1,979	1,711	1,761	1,435	18,104
Parity 3	2,497	2,118	2,490	2,180	2,768	2,460	2,668	2,229	2,608	2,112	24,130

¹Start and end months of period relative to estimated herd infection date are given in parentheses.

T₁ can be seen in Table 3. Generally, T₅ included fewer observations because 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 or sine were significant in all parities. An interaction between sine and year for parity 1 and 2 was also significant (data shown in Appendix Tables A1 and A2). Parity 1 cows had reduced yield in T₃ and T₄ (10 to 15 mo after the estimated herd infection date), as well as borderline significantly reduced yield in T₂ (7 to 9 mo after the estimated herd infection date). Parity 3+ cows had the largest reduction in yield for the period T₂ to T₄. The mean daily milk loss in the period 7 to 15 mo after the estimated herd infection was 1.4 kg of ECM/cow per day (95% CI: 0.5 to 2.3 kg) for parity 1 cows and 3.0 kg of 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 T₄. 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 of 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 of ECM/cow per day, 95% CI: -3.7 to -0.8 kg) and borderline significantly reduced in T₋₁ for parity 1 (mean = -1.0 kg of ECM/cow per day, 95% CI: -2.0 to 0.1 kg; Figure 1).

Average herd size was not significant in 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 the Wilmink function was tested in the models, but did not change the model estimates or significance of other variables and was therefore excluded. Plots of standard residuals and predicted versus observed test-day ECM showed acceptable model fit for all parities (data not shown). Only minor correlations were observed between T and calendar month, although the estimated infection date was strongly seasonal (data not shown).

DISCUSSION

In our study, we observed a significant reduction in milk yield 7 to 15 mo after the estimated herd infection date (T₂ to T₄) for cows in parities 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 (McClure et al., 1989; Huston et al., 2002; Van Kessel et al., 2007) reported no association between *Salmonella* infection and milk yield; however,

Table 4. Multilevel analysis for fixed effects on 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

Variable	Parity 1				Parity 2			
	Mean	SEM	LCL ¹	UCL ¹	Mean	SEM	LCL	UCL
Intercept	26.55	1.06	24.46	28.63	34.70	2.27	30.24	39.16
DIM	−0.02	0.00	−0.02	−0.02	−0.05	0.00	−0.05	−0.04
Exp(ECM) ^(−0.05×DIM)	−5.77	0.20	−6.16	−5.37	−5.03	0.29	−5.59	−4.47
Log SCC	−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 ²								
−4 (−12 to −10)	1.29	0.92	−0.51	3.09	2.84	2.20	−1.47	7.14
−3 (−9 to −7)	1.46	0.92	−0.34	3.26	2.81	2.19	−1.49	7.11
−2 (−6 to −4)	0.15	0.95	−1.71	2.00	1.47	2.21	−2.85	5.80
−1 (−3 to −1)	0.14	0.92	−1.67	1.95	−0.72	2.17	−4.97	3.53
0 (1 to 3)	0	—	—	—	0	—	—	—
1 (4 to 6)	0.85	0.46	−0.05	1.75	1.89	0.67	0.57	3.20
2 (7 to 9)	−0.82	0.45	−1.71	0.06	1.24	0.67	−0.07	2.55
3 (10 to 12)	−1.30	0.47	−2.23	−0.37	−0.94	0.70	−2.30	0.43
4 (13 to 15)	−1.99	0.48	−2.93	−1.04	−1.73	0.70	−3.10	−0.37
5 (16 to 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		

¹LCL and UCL = lower and upper confidence limits.²Time period in months relative to estimated herd infection is shown in parentheses.

in those studies, the time of introduction of *Salmonella* was not known, so the authors merely reported associations between seropositivity and milk yield.

The greatest 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 milk yield of parity 3+ cows was more affected when they were infected with *Salmonella*. The smaller reduction in milk yield in parity 2 cows compared with the other parities was also observed in a smaller study, where milk yield from cows with high antibody levels was compared with that of herd mates with low antibody levels in endemically infected herds (T. D. Nielsen and L. R. Nielsen, unpublished data). 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, whereas it remained constant in control herds. Consequently, it is possible that farmers in case herds culled a

larger proportion of parity 2 cows because of poor milk production, which might explain the different pattern in this parity compared with parity 1 and 3+.

It took 15 mo (until T₅) for milk yield to reach pre-infection levels, suggesting either that 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 T₅ (data not shown). Previous studies have shown that *Salmonella* can be present in herds without necessarily affecting milk yield, and it is possible that herd immunity develops with repeated exposure and reinfection of the cows (Steinbach et al., 1996). Some herds had a second increase in BTM antibody level 1 to 2 yr after the initial increase, and this could indicate a reinfection of the cows in these herds that may have led to repeated periods of decreased milk yield. However, because of insufficient data, we were unable to analyze the differences in milk yield losses in the case herds with persistently

Table 5. Multilevel analysis for main fixed effects on 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	SEM	LCL ¹	UCL ¹
Intercept	39.24	1.97	35.39	43.10
DIM ⁴	-0.05	0.00	-0.06	-0.05
Exp(ECM) ^(-0.05×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 ²				
-4 (-12 to -10)	1.42	1.91	-2.33	5.18
-3 (-9 to -7)	2.12	1.93	-1.66	5.90
-2 (-6 to -4)	0.75	2.18	-3.52	5.01
-1 (-3 to -1)	-1.24	1.95	-5.07	2.59
0 (1 to 3)	0	—	—	—
1 (4 to 6)	-0.49	0.83	-2.12	1.14
2 (7 to 9)	-4.27	1.27	-6.75	-1.79
3 (10 to 12)	-3.62	0.76	-5.12	-2.12
4 (13 to 15)	-1.22	0.62	-2.43	-0.01
5 (16 to 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		

¹LCL and UCL = lower and upper confidence limits.²Time period in months relative to estimated herd infection is shown in parentheses.

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 was greater in case herds than in control herds. Descriptive analyses of the data confirmed this pattern, and 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 to 0.99 when the overall herd prevalence is 0.15 to 0.30, indicating 1 to 2% false-negative herds (Warnick et al., 2006). We used

a <10 ODC% cut-off for the control herds to increase the probability that cows in the control herds had no antibodies and hence 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 confidence that active infection with *Salmonella* existed in the herds. Furthermore, we only included case herds with antibody levels ≥ 25 ODC% following the initial high test value, which 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*.

It is impossible to know which cows in the case herds had clinical signs of salmonellosis, which were subclinically infected, and which were nondiseased or noninfected, because it was not possible to obtain animal-level data on infection status. This would have required

Figure 1. Predicted daily milk yield mean (kg of ECM) relative to that 1 to 3 mo after *Salmonella* infection (T0) for case ($n = 28$) and control ($n = 40$) herds. T₋₄ is 12 to 10 mo before estimated herd infection, T₋₃ is 9 to 7 mo before, T₋₂ is 6 to 4 mo before, T₋₁ is 3 to 1 mo before, T₀ is 1 to 3 mo after, T₁ is 4 to 6 mo after, T₂ is 7 to 9 mo after, T₃ is 10 to 12 mo after, T₄ is 13 to 15 mo after, and T₅ is 16 to 18 mo after herd infection. Bars represent 95% CI of mean milk yield.

frequent repeated measurements at the animal level over a long period; even then, it would be complicated to classify the cows correctly to determine infection dates for each animal (Nielsen et al., 2004, 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 *Salmonella* Dublin within the last 6 mo all had BTM antibody levels optical density >0.5 , a cut-off equivalent to approximately 30 to 40 ODC% in the ELISA used in the surveillance program. In this study, we 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 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 to be bacteriologically positive. This suggests that a large proportion of the cows in the case herds selected for our study were exposed to *Salmonella* bacteria, but it is likely that at all time points after the estimated time of infection, both uninfected and infected cows were present in each case herd. The infection could then continue to spread over the following 6 to 12 mo. Because the increase

in BTM antibodies occurred before reduction in milk yield, it is likely that introduction of *Salmonella* to the herd caused the decrease in yield.

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

Infection Date

Detection of *Salmonella* in BTM showed 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 an increase in herds with high BTM antibody levels occurs in the fall. Consequently, simulated infection dates for control herds were weighted by year and month of in-

fection as in the case herds. Hence, we believe that the pattern seen after T_0 was due to *Salmonella*.

Strengths and Limitations of the Study

Our study included 68 dairy herds and is, to our knowledge, the largest study to model associations between *Salmonella* and milk yield. Furthermore, it describes the yield from 12 mo before to 18 mo after estimated herd infection. The next largest study of *Salmonella* and milk yield followed 24 herds (Anderson et al., 1997) with *Salmonella* Menhaden infection. In that study, clinical signs were mainly diarrhea, which affected 0 to 40% (mean 7%) of production groups. The 8 case herds had similar production levels to the 16 control herds.

Confounding variables other 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; however, including the random effect of farm accounted for unexplained between-herd variance in yield. The case herds had fewer parity 3+ observations than the control herds but case and control herds had similar numbers of observations for parity 1. This could be an indication of 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 time 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 (4 to 6 mo before the artificially selected infection date for the herd). This is difficult to explain but could be due to 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 to 2009 with conventional farming practice and Danish Holstein cows were included in the study, and these herds were larger on average than the control herds. However, we observed 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 did not appear 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* were introduced into the herd, but approximately 73% of Danish dairy cows are Holsteins and 90% are on conventional farms (Knowledge Centre

for Agriculture, 2009), so the results of this study are likely to be representative of the majority of Danish farms.

CONCLUSIONS

A significant decrease in milk yield occurred in *Salmonella*-infected herds, with mean estimated milk yield loss for a herd with 85 cows being 29,000 kg of ECM in the 18 mo 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, and milk yield did not return to preinfection levels for more than 1 yr following infection.

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APPENDIX

Table A1. Results for interactions in multilevel analysis for fixed effects on ECM yield for parities 1 and 2 for 28 Danish Holstein herds with large, sudden increases in bulk tank milk *Salmonella* antibody levels indicative of recent herd infection

Variable ¹	Parity 1				Parity 2			
	Mean	SEM	LCL ²	UCL ²	Mean	SEM	LCL	UCL
DIM × T								
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

Continued

Table A1 (Continued). Results for interactions in multilevel analysis for fixed effects on ECM yield for parities 1 and 2 for 28 Danish Holstein herds with large, sudden increases in bulk tank milk *Salmonella* antibody levels indicative of recent herd infection

Variable ¹	Parity 1				Parity 2			
	Mean	SEM	LCL ²	UCL ²	Mean	SEM	LCL	UCL
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

¹T₋₄ is 12 to 10 mo before estimated herd infection, T₋₃ is 9 to 7 mo before, T₋₂ is 6 to 4 mo before, T₋₁ is 3 to 1 mo before, T₀ is 1 to 3 mo after, T₁ is 4 to 6 mo after, T₂ is 7 to 9 mo after, T₃ is 10 to 12 mo after, T₄ is 13 to 15 mo after, and T₅ is 16 to 18 mo after herd infection.

²LCL and UCL = lower and upper confidence limits.

Table A2. Results for interactions in multilevel analysis for fixed effects on ECM 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

Variable ¹	Parity 3+			
	Mean	SEM	LCL ²	UCL ²
DIM × T				
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

Continued

Table A2 (Continued). Results for interactions in multilevel analysis for fixed effects on ECM 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

Variable ¹	Parity 3+			
	Mean	SEM	LCL ²	UCL ²
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

¹T₋₄ is 12 to 10 mo before estimated herd infection, T₋₃ is 9 to 7 mo before, T₋₂ is 6 to 4 mo before, T₋₁ is 3 to 1 mo before, T₀ is 1 to 3 mo after, T₁ is 4 to 6 mo after, T₂ is 7 to 9 mo after, T₃ is 10 to 12 mo after, T₄ is 13 to 15 mo after, and T₅ is 16 to 18 mo after herd infection.

²LCL and UCL = lower and upper confidence limits.