

CASE REPORT

Control of *Salmonella* Dublin in a bovine dairy herd

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Abstract

Salmonella enterica serovar Dublin (*S. Dublin*) was diagnosed in a dairy herd after signs of acute gastroenteritis and sepsis. Two hundred eighty-three Holstein cattle were sampled resulting in 700 observations, and serology for *S. Dublin* was performed. Holstein cattle sampled were divided by origin and arrival date to determine on-farm exposure. Prevalence estimates were calculated and compared with control measures implemented on the dairy during the outbreak. One group of cows, presumed to be the original carrier animals, had the highest overall seroprevalence (76.5%). Seroprevalence decreased throughout the study, coinciding with testing and management changes. This report documents biosecurity measures that identified *S. Dublin* after the purchase of subclinical carrier cattle and the steps taken to successfully control herd transmission.

KEYWORDS

cattle, control, dairy, prevalence, salmonellosis

1 | INTRODUCTION

Salmonella enterica serovar Dublin (*S. Dublin*) increasingly has been reported among cattle. In a previous study of 1800 *Salmonella* isolates identified from both clinical and nonclinical submissions, the most common serotype was *S. Dublin* (18%) compared historically to *Salmonella* Newport and *Salmonella* Typhimurium.¹ Herd management actions and vaccination have been suggested in veterinary literature for controlling infection among dairy herds.²⁻⁵ Efforts to control *S. Dublin* are best exemplified in Denmark, based on implementation of a national surveillance program.^{6,7}

Between-herd national prevalence of *S. Dublin* in the United States has not been reported. Published studies describe only within-herd prevalence using antibody detection to *S. Dublin*.^{8,9} Internationally, prevalence of *S. Dublin* is underestimated because of the

potential for asymptomatic infection and latent carriers of disease.^{9,10} Given host adaptability and severe manifestations of disease in cattle, it is critical that control measures for *S. Dublin* are well defined. Such control methods have yet to be described for dairy operations in the United States.

Our objective was to describe the control of a *S. Dublin* outbreak on a dairy farm in the southeastern United States that successfully controlled transmission, as determined by changes in herd seroprevalence. Documenting a model of success may provide national dairy operations guidance in controlling *S. Dublin*.

2 | MATERIALS AND METHODS

2.1 | Pre-*Salmonella* diagnosis management

The herd evaluated maintains approximately 150 to 200 lactating Holstein-Friesians, milked twice daily. The lactating herd number is

Abbreviations: CI, confidence interval; ODC, optical density; OR, odds ratio; SRP, siderophore receptor protein.

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influenced by research and teaching needs designated by the unit director. The herd is housed in a sand-bedded, free-stall barn. Routine cleaning practice includes removing fecal material and wet sand at each milking and weekly rebedding with fresh or recycled sand. The barn utilizes a flush system allowing excrement to be carried away using nonpotable water. This water is recycled from a manure lagoon located immediately adjacent to the barn. Flushing occurs four times daily. The barn is structured such that flush water moves down slope from a tank reservoir and flows through multiple pens, not crossing a central alleyway. Flush water also is used to clean the common working and parlor holding pens. Water from this area flows into the adjacent free stall alleyway at approximately mid-way from the upper slope of that alleyway. A total mixed ration is provided through the central alleyway, which cattle access using head stanchions.

Vaccination routines are in place depending on the age and reproductive status of cattle. Protocols for dry and lactating herd members include a multivalent Clostridial toxoid, multivalent killed respiratory vaccine, and an *Escherichia coli* mastitis vaccine at dry-off. At 21 days in milk, additional doses of the aforementioned vaccines are readministered.

2.2 | Animals

All animal work was performed under the guidance of the Institutional Animal Care and Use Committee at University of Tennessee, Knoxville. Animals included in this report were Holstein cattle housed at the University of Tennessee's East Tennessee Research and Education Dairy Unit, located in East Tennessee, United States (35°45'51.5"N 83°50'30.4"W). The dairy consists of multiple lactating cattle groups originating from various purchase locations. For purposes of this study, 5 groups were created based on time and region of origin. Group 1 contained cattle from the original herd, which were born and reared on site (n = 179). Groups 2 and 3 represented cattle that arrived in late 2014 from farms located on the east coast of the United States and southeastern United States, respectively (n = 3 and 4, respectively). Group 4 consisted of cattle from the midwestern United States that arrived in mid-2015 (n = 33) and group 5 consisted of cattle from southeastern United States that arrived in early 2017

(n = 65). Upon arrival, cattle were mixed with lactating and dry cattle of the existing herd with no isolation. Clinically ill adult cattle (n = 3) with signs of hemorrhagic enteritis and sepsis were presented for veterinary evaluation in the early months of 2017. *Salmonella* Dublin was confirmed on fecal culture. After confirmation in clinically ill animals, blood samples were collected from all adult cattle >20 months of age at 3 time points in 2017. An intermittent sampling occurred in May and was reserved for those cattle that tested positive in March or had newly calved into the herd. Blood samples (20 mL) were collected from the coccygeal vein. Bulk tank milk samples were collected at 6 time points from the herd between October 2017 and June 2020. Records for each animal were accessed to obtain information on origination, age, calving date, reproductive status, date left, and reason left.

2.3 | *Salmonella* Dublin ELISA

Blood and bulk tank samples were submitted to the Animal Health Diagnostic Center at Cornell University for testing. Briefly, a S. Dublin ELISA was performed using antibodies against lipopolysaccharide O antigens 1, 9, and 12 based on established laboratory protocols. Results were reported in comparison to a positive control, represented as optical density (ODC%). The given threshold for a positive sample was >35%, as recommended by the manufacturer, with a specificity of 99.4% (95% confidence interval [CI], 98.8%-99.8%).¹¹ Based on the laboratory's suggestion, carrier status was defined as animals having high serum ELISA results (>75%).

2.4 | Post-*Salmonella* diagnosis management

Various control measures were implemented in an attempt to control S. Dublin infection among the herd (Figure 1). Upon initial diagnosis on the dairy, hygiene changes were made, including cleaning all water boxes twice weekly with chlorinated disinfectant, using only new sand as bedding for 2 months, and replacing the bedding once weekly. Flushing interval was decreased to twice daily and was done using nonrecycled municipal water. A *Salmonella* siderophore receptor

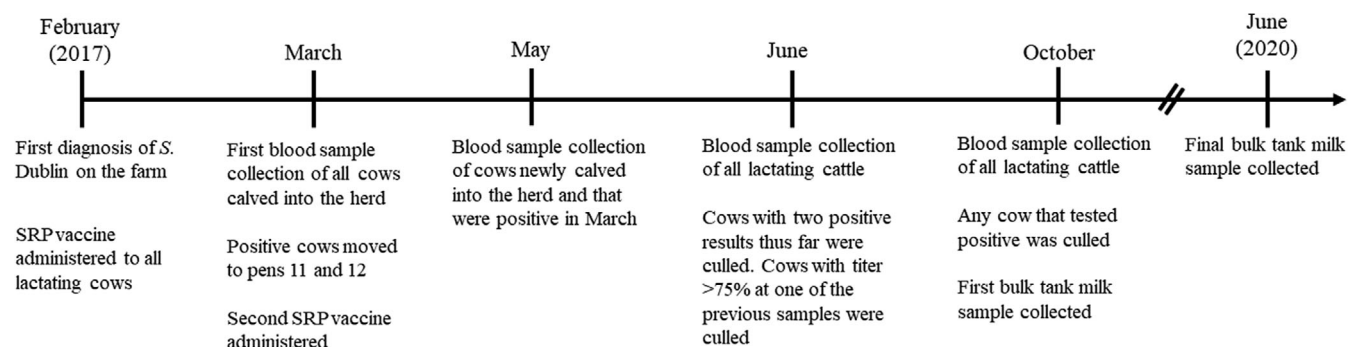


FIGURE 1 Timeline: Linear timeline display critical activities during the study period during the 2017 calendar year, including sample collection for diagnostic testing and culling

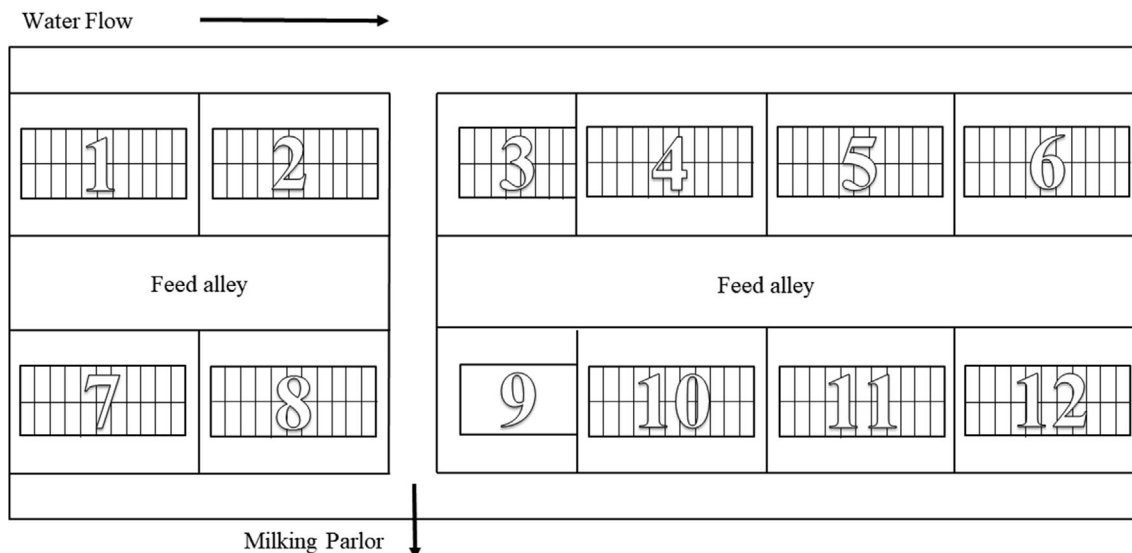


FIGURE 2 Barn schematic: Barn schematic demonstrating pen locations including water, animal, and personnel movement

protein (SRP) vaccine (Zoetis, Parsippany, New Jersey) was administered to all lactating cows a month before the first blood samples were collected and a booster dose of the vaccine was administered a month later. Beginning with the first blood samples, any cow that tested positive was moved to the end of the barn in pens 11 and 12 (Figure 2). These animals were milked first followed by cleaning of the barn before the remainder of the herd was milked. Staff and equipment procedures were implemented to minimize spread among the herd. Machinery used in the bovine housing area was cleaned and disinfected daily by a pressure wash followed by a 10% solution of chlorinated bleach. Employees were required to wear washable protective coveralls, boots, and gloves. A single employee was designated to perform necessary reproductive and health procedures in pens 11 and 12 containing infected cattle. All personal protective equipment was washed and gloves changed after all animal handling. After the second sampling time point, decisions to cull animals were made based on 2 consecutive positive ELISA results, or a positive ELISA result of >75% at the second sample date. Beginning at the third blood sample collection date, decisions to cull were made after only 1 positive ELISA result. The *Salmonella* SRP vaccine was integrated into the prediagnosis vaccination protocol described with administration to heifers 60 days before calving, cows at dry off, and cattle 1 month before calving at close-up.

2.5 | Statistical analysis

Descriptive analysis first was conducted to determine the number of unique animals belonging to each sample group as well as how many times blood samples were collected from each animal throughout the course of the study. Reasons for culling an animal from the herd also were evaluated. Apparent prevalence of *S. Dublin* was calculated in a variety of ways upon controlling for different variables. First, this

value was determined for each sample group having controlled for the several sampling dates. Next, the apparent prevalence for each sampling date was determined having controlled for the different animal groups. Finally, this value was calculated for each of the sample groups at each sampling date after controlling for repeated sampling among the animals. After the estimates of prevalence were determined, univariable analysis of different factors was completed. Effect of date of sampling and sample group independently estimated independently to determine their effects on the odds of a positive *S. Dublin* ELISA result. All factors found significant in the univariable analysis for association with a positive *S. Dublin* ELISA were used in building a model for multivariable logistic regression analysis. Analysis was performed using a generalized estimating equations model to account for the effect of repeated measures among cattle in the study. Effects of interactions were assessed between measured variables. Variables with P values $\leq .05$ were considered significant.

3 | RESULTS

Seven hundred samples were collected from 283 animals over the course of the sample dates. Fifty-eight animals were sampled once, 52 were sampled twice, 136 were sampled 3 times, and 37 were sampled 4 times. In total, 601 samples yielded negative ELISA titers (ie, <35%) for *S. Dublin* whereas 99 were positive. Individual sample sizes and numbers of positive and negative results by group and date are presented in Table 1. Given inadequate sample size, lack of positive diagnosis of *S. Dublin* at any sample date, and temporal resemblance to the original herd, the east coast and southeastern US cows that arrived in late 2014 were merged with the original herd for subsequent analysis. Results of this combination are listed as Group 1a in Table 1.

After controlling for repeated observations and the effects of source and time of sampling, the seroprevalence of *S. Dublin* was

TABLE 1 Distribution of the sample sizes and numbers of positive and negative *Salmonella* Dublin ELISA results for each sample group according to the date of sampling

Group	Source	Sample date	Total positive	Total negative	Total results	Prevalence (%)	95% Confidence interval
1	Original herd	March	31	91	122		
		May	7	21	28		
		June	5	161	166		
		October	3	117	120		
2	East coast US late 2014	March	0	3	3		
		May	0	0	0		
		June	0	4	4		
		October	0	4	4		
3	Southeastern US late 2014	March	0	3	3		
		May	0	0	0		
		June	0	2	2		
		October	0	2	2		
4	Midwestern US mid-2015	March	22	8	30	76.5	54.7-89.7
		May	9	9	18	39.4	17.0-67.3
		June	6	21	27	22.3	8.9-47.1
		October	1	17	18	5.5	0.6-34.4
5	Southeastern US early -2017	March	7	44	51	11.8	5.0-25.3
		May	4	1	5	46.0	7.0-90.5
		June	3	57	60	4.2	1.2-13.4
		October	1	54	55	1.6	0.2-11.2
		May	7	21	28	10.3	4.0-24.1
		June	5	167	172	2.3	0.9-5.8
		October	3	123	126	1.9	0.5-6.1

Note: Apparent prevalence estimates of *S. Dublin* and confidence limits of groups 1a, 4, and 5 are reported after consolidation as according to the date of sampling. Group 1a combines data from the original established herd, East Coast US cattle, and Southeastern US cattle introduced in late 2014; group 4 comprises data from cattle originating from Midwestern United States in 2015; group 5 comprises data from cattle arriving from Southeastern United States in early 2017. All sampling was performed during the 2017 calendar year.

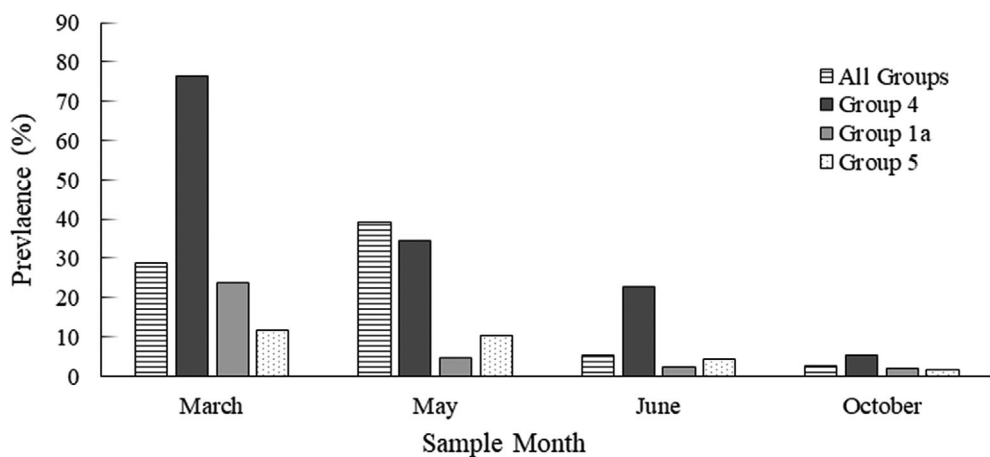


FIGURE 3 Apparent prevalence of *Salmonella* Dublin: Apparent prevalence of *S. Dublin* by source of cattle for each sampling date in 2017. Data from all combined groups are depicted in the vertical lined bars, group 1a data are depicted in the light gray bars; group 4 data are depicted in the dark gray bars; and group 5 data are depicted in the cross-hatched bars

determined for each group at each sample date (see Table 1 and Figure 3). Group 4 cattle, originating from the midwestern United States, had the highest overall prevalence, followed by the combined original herd and then the southeastern US cows arriving in early

2017. An exception was recorded for early 2017 southeastern US cattle at the May sample date, with a large increase in prevalence compared to this group's initial prevalence in March. The magnitude of the effect of animal source and time of sampling on the prevalence of *S.*

TABLE 2 Factors associated with a positive diagnosis of *Salmonella* Dublin

Variable	Category ^a	Odds ratio	95% Confidence interval	P value
Source of cattle	Group 4 vs 5	12.6	4.8-32.9	<.0001
	Group 4 vs 1a	7.6	3.7-16.0	<.0001
	Group 1a vs 5	1.6	0.7-3.7	.23
Sample time	March vs May	3.9	1.9-7.7	.0001
	March vs June	5.7	3.4-9.4	<.0001
	March vs October	8.8	4.3-17.9	<.0001
	May vs June	1.4	0.7-3.0	.30
	May vs October	2.2	0.9-5.4	.07
	June vs October	1.5	0.8-2.9	.19

^aGroup 1a combines data from the original established herd, East Coast US cattle, and Southeastern US cattle introduced in late 2014; Group 4 comprises data from cattle originating from Midwestern United States in 2015; Group 5 comprises data from cattle arriving from Southeastern United States in early 2017.

Dublin was analyzed using odds ratio (OR) calculations, after accounting for repeated observations (Tables 2). Largest ORs were observed when comparing the cows from the midwestern United States to those from southeastern United States arriving in early 2017 and to those from the original herd. These observations indicated that cattle from the midwestern United States were more likely to be positive for *S. Dublin* than cows from the other 2 groups. Comparing the first sample date to the others in relation to a positive diagnosis yielded the highest ORs, meaning cows were more likely to test positive in March than at other dates. Each of the 6 bulk tank milk samples collected biannually from 2017 to 2020 tested negative for *S. Dublin*, with all titers <35%. After negative bulk tank results, management procedures were returned to pre-salmonella diagnosis methods with continued use of the SRP vaccine and serologic testing of new herd additions. Thirty-two animals were culled based on the *S. Dublin* ELISA results alone in 2017 as described above. One hundred seventy cattle were culled throughout the duration of the 3-year study period, with the most common reason being reproductive problems

4 | DISCUSSION

Overall, results indicate that group 4, the midwestern US cows that arrived in mid-2015, had the highest apparent seroprevalence of *S. Dublin* among the herd. Although not enough data are available to conclude that this group was responsible for the introduction and outbreak of *S. Dublin*, it seems that these cows were more susceptible to disease. These results highlight the importance of testing animals for *S. Dublin* before purchase, along with testing for other infectious diseases.

Our report focuses on an individual, medium-sized herd that experienced an outbreak of *S. Dublin* in the early spring of 2017. On the basis of medical records and personal accounts, management changes made throughout the course of the outbreak were described and compared to the apparent seroprevalence estimates, as determined by individual cattle serum samples submitted for *S. Dublin*

ELISA testing. Our study was unique in that control measures implemented could be defined as being successful in controlling *S. Dublin* infection in a North American dairy herd according to the decrease in prevalence. Culling decisions and enhanced biosecurity efforts are best described in terms of controlling and eliminating *S. Dublin* from persistently infected herds.³ This also was the focus of the efforts made on the dairy in our report and the results presented potentially support the validity to the recommendations made.

After diagnosis of *S. Dublin*, the administration of the SRP vaccine was implemented to aid in control of the pathogen. This vaccine exploits the SRP proteins located on many species of bacteria, specifically derived from *S. enterica* serovar Newport.⁴ Because of conservation of the proteins among bacteria species, the SRP vaccine had been incorporated as prophylaxis for salmonellosis. However, the efficacy of this generic vaccine against *S. Dublin* infection has not been quantified. Two *S. Dublin* vaccines are approved for use in cattle in the United States. Studies evaluating serologic responses of dairy calves and pregnant cattle have been reported.⁵ The protective effect of these antibodies, however, has yet to be determined. Given the results of these studies, it is unlikely a generic vaccine, such as the SRP vaccine, would induce *S. Dublin*-specific antibodies that potentially would interfere with *S. Dublin* diagnosis and prevalence data.

Although *S. Dublin* is host-adapted to cattle, it also causes severe disease in humans.¹² Therefore, prevention and control of *S. Dublin* in cattle is critical in order to limit exposure of humans. Estimates of the prevalence in the United States have yet to be determined, making it difficult to understand the distribution of this organism and establish the success of larger control efforts. More research is required to quantify the regional and national prevalence of *S. Dublin* throughout the United States. Only then can measures of control more accurately be assessed.

Because of the retrospective nature of our study, access to data was limited and information bias may exist surrounding recall of management procedure changes occurring during the outbreak. Culling decisions were altered during the study, which may affect prevalence data by the removal of more or fewer positive cows from the herd

corresponding to different sample dates. Previous studies identified more *Salmonella* isolates from periparturient cows but found no significant effect of days in milk of lactating cows on positive diagnosis.^{13,14}

However, because of the relatively small sample size of our study and the low number of positive animals, such risk factors could not be analyzed as related to *S. Dublin* specifically.

Our study indicates the feasibility of markedly decreasing the prevalence *S. Dublin* in a dairy herd by implementation of control methods. Future research is needed to better understand the pathogenesis of *S. Dublin* in adult cattle. This knowledge could be applied to developing more efficient and accurate diagnostic methods in order to prevent outbreaks of disease on farms. Until more is known about *S. Dublin*, veterinarians and producers can find value in the success of control methods described in this report, which can be applied to their operations in the face of an outbreak.

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