A structured approach to control of *Salmonella* Dublin in 10 Danish dairy herds based on risk scoring and test-and-manage procedures

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LRN: Study design, data collection and herd visits, data analysis, and preparation of manuscript.

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Both authors read and approved the final manuscript.
Abstract

The aim of this study was to describe a structured approach to effectively reduce *Salmonella* Dublin prevalence in infected dairy herds based on a step-wise procedure. Furthermore, the aim was to describe tools for management and monitoring, and to report on development in prevalence among young stock and adult cattle in 10 case herds that were followed for more than three years.

The five steps in the structured approach were: 1) risk scoring to determine transmission routes within the herd and into the herd; 2) determining a plan of action; 3) performing management changes to close important routes of infection; 4) interpretation of repeated testing of individual animals to detect high-risk animals for special hygienic management or culling; and 5) diagnostic testing of different age groups and bulk tank milk to evaluate progress of control over time.

Serology, true prevalence estimates and changes in herd classification in the Danish surveillance programme for *Salmonella* Dublin were used to assess the progress in the herds during and after the control period. Effective control of *Salmonella* Dublin was achieved in all participating herds through management that focused on closing infection routes mainly in the calving areas and the young calf areas of the herds. It took on average three years to control the infection in the case herds. Bulk tank milk recordings from the four following years indicated that most of the herds might have eradicated the infection.
1. Introduction

*Salmonella enterica* subsp. *enterica* serovar Dublin (*Salmonella Dublin*) is a bacterium that receives much attention in the cattle industry of several countries around the world due to a relatively high prevalence in cattle dense areas and potentially severe zoonotic implications for humans and animal health, welfare and production (Helms, Vastrup, Gerner-Smidt, & Mølbak, 2003; Peters, 1985; Nielsen, Nielsen, Toft, & Houe, 2010). Therefore a national surveillance programme was initiated in Denmark in 2002. In this programme, all cattle herds are tested on a regular basis and placed into one of the three classification levels (Warnick, Nielsen, Nielsen, & Greiner, 2006). Furthermore, farmers in infected herds are encouraged through trade restrictions to control the infection. Herd-specific approaches are required to control the infection, which is often latent in a number of animals that shed the bacteria intermittently. Previous attempts to control *Salmonella Dublin* in dairy herds have proven difficult for farmers. Approximately half of the dairy herds that experience an outbreak of *Salmonella Dublin* become persistently infected (Veling, 2004). A number of infected animals have a latent infection with intermittent bacterial shedding in faeces (Wray, Wadsworth, Richards, & Morgan, 1989; Richardson, 1973) and some animals might become active carriers, i.e. animals that shed bacteria more or less continuously for extended periods of time (House, Smith, Dilling, & Roden, 1993). There are indications in field studies that persistent infections at herd level cannot be stopped solely by culling active carriers detected by bacteriological isolation of *Salmonella*-bacteria in faecal samples (Veling, 2004). Furthermore, test-and-cull strategies might not be cost-effective, especially if within-herd transmission can be prevented without the culling of potential carriers. Vaccination is used as a tool to control *Salmonella Dublin* in several countries. However, vaccination is mainly useful for reducing clinical signs and shedding of bacteria from affected animals. It does not
entirely stop bacteria from spreading to the environment and between animals (Mizuno, McLennan, & Trott, 2008; Segall & Lindberg, 1993).

Calves below the age of 3 months are more susceptible to Salmonella Dublin than older animals leading to more severe clinical signs and more shedding of bacteria in this age group (Nazer & Osborne, 1977; Segall & Lindberg, 1991). Usually, the incidence rate of acute infections is highest in this age-group, but infection (including persistent infection) can occur at any age if the infection dose is sufficiently high. Therefore, control of Salmonella Dublin needs to be focused on stopping transmission to and between young calves, but also to lower the infection pressure in general in the herd environment. Furthermore, an important part of a control programme is to avoid purchase of infected animals from other herds (Nielsen, Warnick, & Greiner, 2007). In the Danish surveillance programme for Salmonella in cattle this is done by providing farmers with access to information about the status of all other cattle herds via the internet.

In a previous study, six persistently infected dairy herds started a voluntary control effort based on changes in management at calving and of the pre-weaned calves. In addition, all adult cattle (>1 year) were monitored serologically and cows with persistently high Salmonella Dublin antibody titres were recommended culled (Jensen, Kjeldsen, & Alban, 2004). Five of the herds managed to control the infection and reduce the proportion of seropositive animals markedly. One herd had a recurrence of salmonellosis four years after the control effort was initiated. The study showed that several of the herds had difficulties carrying through the recommended management changes, and they also purchased new animals from herds with unknown infection status during and after the study period (Jensen et al., 2004). Thus, there appeared to a need for tools to assist farmers wanting to control Salmonella Dublin in their herds.

The aim of this study was to describe a structured approach to effectively control Salmonella Dublin in infected dairy herds based on a step-wise procedure provided in a manual for farmers and
advisors. The approach incorporated didactic principles to assure the necessary commitment and follow-up during the control period required to obtain daily and long-term control efforts. Furthermore, the aim was to describe tools for management and monitoring, and to report on development in seroprevalence among young stock and adult cattle in 10 case herds. Monitoring was done by repeated serological testing of all animals in the participating herds during a three-year period starting in 2003, and through observation of herd classifications in the Danish surveillance programme between 2001 and 2010. The study was designed for illustrative purposes. Thus, it is a descriptive case-study that does not allow for comparison of the effect of different management strategies between the herds.

2. Materials and methods

2.1 Herds

The study was initiated in June 2003 and the participating herds were followed intensively until the end of 2006. After that the herds were monitored via the Danish national surveillance programme for *Salmonella* Dublin in cattle. A convenience sample of 10 dairy herds was found through veterinary practitioners. The herds were located in several regions of the main Danish peninsula, Jutland. The herds were initially all placed in surveillance Level 2 indicating too high antibody levels in bulk tank milk ELISA measurements, and thus likely infected with *Salmonella* Dublin. The surveillance programme classifications are described in more detail in section 2.6 and have also been described and evaluated elsewhere (Warnick et al., 2006). Table 1 gives an overview of the participating herds.
2.2 Didactic risk scoring tool

A tool was developed to assist farmers and cattle health consultants identify transmission routes in the herds and plan control strategies accordingly. This manual was based on ideas from an American manual concerning paratuberculosis control (Rossiter, Hutchinson, Hansen & Whitlock, 1999). It was adjusted to match the infection dynamics of *Salmonella* Dublin and Danish farming practises and legislation. The idea was to use risk scoring to go through the herd systematically from one end to the other and assign risk scores to different relevant barn sections and management practises in the current system on a scale from 0 to a maximum score. The maximum scores were decided by the authors of the manual according to existing knowledge about risk factors for spread of *Salmonella* Dublin in cattle herds, and they were weighted so that the most critical areas for control counted most in the total sum of risk scores (e.g. hygiene and management in the calving area and the pre-weaned calf barn). The scoring system is not very rigid, so the absolute numbers can probably not be used for statistical purposes, but discussions on what each score should be and why stimulate communication between the farmer and his advisor(s). Thus, the manual with both background information and the scoring system was developed as a didactic tool to focus attention of decision makers in the farm on the most critical control points in each specific herd. An English version of the *Salmonella* Dublin risk scoring form is available in the online supplementary material (Risk_scores_Salmonella.xls).

2.3 Plan of action

The next step was to summarise and evaluate the risk scores and to make a plan of action based on the results. Visual presentations of the summary risk scores for each area in the herd were implemented in the risk scoring forms (see supplementary material: Risk_scores_Salmonella.xls). The plan should always contain i) a clear statement of the action, e.g. calf pens to be cleaned,
disinfected and dried out for 2 days before a new calf is allowed into the pen; ii) the name of the
person responsible for the action in the herd; iii) a date for when this action should be implemented,
and iv) an agreement about follow-up on the action. It was recommended that the farmer made this
plan of action in collaboration with a herd health consultant, and that they planned regular follow-
up on the plan, e.g. every 6 months during the control period. It was an important part of the
didactic idea behind the manual that the farmer was actively involved in deciding the plan of action,
so it should not be dictated by the advisor.

2.4 Recording of management changes

The third step was to perform the planned actions. All herds were visited towards the end of
the study period in 2006 and a semi-structured interview was performed by the authors of this
manuscript. In Table 2 is an overview of the main management practises and changes that were
recorded in each herd of relevance for Salmonella-control. Note that the sample size of herds does
not allow for comparison of management practises between herds, so the listed management
practises performed in the herds should mainly be seen as actions that were feasible for the farmers
to perform during the control period.

2.5 Diagnostic tests on animal level

All lactating cows had individual milk samples collected every 3 months. Blood samples were
collected twice per year from all heifers from 3 months of age up to first calving for prevalence
estimation in the young stock. All of these samples were tested using an indirect ELISA detecting
antibodies to Salmonella Dublin lipopolysaccharide O-antigens. The ELISA has been described and
evaluated in other studies (Nielsen & Ersbøll, 2004; Nielsen, Toft, & Ersbøll, 2004). The individual
ELISA results were provided to the farmers in two ways: 1) progress graphs to evaluate if the plan
of action was working and 2) colour-coded decision support lists indicating high-risk animals for special management (i.e. single calving pen with rigorous cleaning) or culling. The classification of the individual animals was done based on repeated ELISA-measurements and modified from House et al. (1993), Smith et al. (1989) and Spier et al. (1990). Animals were classified risk group R=1 (marked in red on the list), if they had at least two samples above 80 ODC% with a minimum of 120 days in between, and the most recent sample was above 80 ODC%, and the average of the last up to four samples was above 80 ODC%. The animals were categorised medium risk indicated by R=2 (marked in yellow on the list), if the most recent ELISA and the average of the last up to four samples were above 50 ODC%, but not high enough to be categorised as R=1. Animals with ELISA values below 50 ODC% in the most recent sample did not have any colour indicators on the decision support lists.

2.6 Diagnostic tests on herd level

In the national surveillance programme for *Salmonella* Dublin all dairy herds automatically had a bulk tank milk sample collected approximately every 3 months from 2001 and onwards. These were tested for antibodies against *Salmonella* Dublin with a bulk tank milk ELISA described elsewhere (Nielsen & Ersbøll, 2005). Herds were classified into three levels based on the average ELISA values of the last four samples, and the value of the latest sample in relation to the average of the previous three. Furthermore, movement of animals and detection of bacteria usually upon clinical suspicion could affect herd classification. The programme validity has been estimated by Warnick et al. (2006). It was found that at a true prevalence of 15%, approximately 1% of Level 1 herds were false negative meaning that they might be infected with *Salmonella* Dublin despite low bulk tank milk antibody levels. It was also found that approximately 20% of the Level 2 herds were
in fact not infected with *Salmonella* (or had too low prevalence for it to be detected), but still had too high antibody levels to be assigned to Level 1.

### 2.7 Statistics

The true prevalence (TP) at each sampling round was estimated from the apparent prevalence (AP, the number of animals with ELISA-values >50 ODC% out of all tested animals), the sensitivity (Se) and specificity (Sp) estimates by formula (1) (Houe, Ersbøll & Toft, 2004):

\[
TP = AP + Sp - 1 / Sp + Se -1
\]  

(1)

The estimates of test validities used in these calculations were Se=0.75 and Sp=0.95 for young stock (serum ELISA) and Se=50% and Sp=90% for adult cows (milk ELISA) (Nielsen, 2003; Nielsen et al., 2004; Nielsen & Ersbøll, 2004).

Paired T-tests were used to compare the within-cows and within-heifer true prevalence estimates at the first and the last sampling rounds across all herds.

### 3. Results and discussion

#### 3.1 Control actions performed in the study herds

Herd characteristics and main performed control actions are summarised in Tables 1 and 2. The date that control actions were initiated was estimated from the farmer interviews. However, it was not possible to determine an exact start date, because some control actions were not started simultaneously within the herds. Farmer behaviour varied markedly between the herds. Herd E had already initiated control of *Salmonella* Dublin 10 months prior to the first sampling, but otherwise control actions were generally started after the time of entry into the study. Herd G did not start control actions in the calf barn until one year into the project. Herds I and J did not consistently stick to the planned control actions. In particular, they were not consistent in the use and
management of calving areas and single pen housing of calves. Herd H, on the other hand, appeared to be doing very well with regard to control actions and the farmer was very motivated to reach Level 1 as quickly as possible in order to be able to sell high quality breeding animals. He was therefore very consistent in removing calves immediately after calving irrespective of calving hour and in cleaning of calf pens between calves. Fast removal of calves from the dam after birth reduces the risk of calves becoming infected from their own mother or from other cows in the calving area (Richardson, 1973). There was, however, a period with indications of new infections among the young stock and cows during winter 2005 in Herd H. Such reoccurrence of new infections might be due to presence of asymptomatic carrier animals in the herd or to surviving infection in the environment (Plym-Forshell & Ekesbo, 1996; Taylor & Burrows, 1971; House et al., 1993; Veling, 2004). None of the herds engaged in major new barn construction projects during the project period, but some rearranged the interior of the barns to obtain a better flow of animals or improved possibilities for cleaning and group sectioning.

Evidence for major risk factors of within-herd transmission to support advices for optimal control strategies is often based on empirical knowledge (Hardman, Wathes, & Wray, 1991; House & Smith, 2004; Richardson, 1973). A major reason for lack of scientific evidence is that comparison of management and housing systems in scientific field studies poses major challenges, not least in longitudinal studies. Field studies of infections that mainly spread through faecal to oral routes require expensive and time-consuming sampling with frequent testing of both animals and environment, and recording of management procedures and changes hereof over long periods of time to lead to significant new knowledge about infection dynamics and within-herd risk factors of Salmonella Dublin (Nielsen, van den Borne, & van Schaik, 2007). Alternatives to observational studies are simulation modelling of within-herd infection dynamics. However, such models also
require reliable input parameters and prior knowledge about risk factors, and often mainly provide hypothetical or theoretical conclusions (Xiao, Bowers, Clancy, & French, 2005).

*Salmonella* Dublin can be transmitted from infectious animals to most age-groups suggesting that strict group-housing is recommendable, and the highly susceptible pre-weaned calves should be housed in clean environment. It should be considered that any manure originating from other animals should not come into contact with susceptible animals (Hardman et al., 1991). Alternative isolated pens for - or culling of - sick animals may be preferable during control, because clinically affected animals shed highest numbers of *Salmonella*-bacteria (Segall & Lindberg, 1991).

### 3.2 Diagnostic tests on animal level

The ELISAs used here have been evaluated for sensitivity and specificity with regard to infection in the individual animal (Nielsen, 2003; Nielsen et al., 2004; Nielsen & Ersbøll, 2004). Estimated sensitivity of approximately 50-88% and specificity of around 88-98% at cut-off 50 ODC% depending on the age of the tested animal suggest that serology has its strength in group or herd diagnostics or if interpreted based on repeated sampling of the same animals.

Bacteriological culture for *Salmonella* has very poor sensitivity (maybe as low as 6-14%) in subclinically infected cattle (House et al., 1993; Nielsen et al., 2004). Consequently, infections in some groups of animals or even in the whole herd would go unnoticed, if bacteriology had been used for evaluation of progress in the control of *Salmonella* Dublin in this study. The sensitivity is poor due to intermittent shedding, low concentrations of bacteria in the samples and other factors related to the origin of the faecal material and the strain of bacteria (Baggesen, Nielsen, Sørensen, Bødker, & Ersbøll, 2007). Thus, use of bacteriology would not provide sound conclusions, and it was decided to use the more cost-effective and sensitive ELISAs in this study.
Farmers were provided with three decision support tools based on individual animal diagnostic testing during the study period:

1) sorted lists of diagnostic test results for all animals tested in the herd (Table 3);
2) high-risk animals identified by fixed criteria as described in the Materials and methods section (Table 4);
3) graphs illustrating the antibody ELISA response in the samples versus the age of the animals on the date of sampling (Figure 1).

These tools were sent out to farmers four to six times per year approximately one month after each sampling round. It was not possible to obtain accurate information about which animals were culled or managed according to these lists in this project. However, in a culling analysis of these herds, it was found that the risk of culling was significantly higher across all herds for heifers and cows that had been classified as R=1 or R=2 during the study (Nielsen & Dohoo, 2010). The types of graphs illustrated in Figure 1 based on sampling of large groups of cattle were highly appreciated by the farmers. However, they require sampling of a large number of animals which is time consuming and expensive. Smaller samples of indicator groups such as calves between 4 and 6 months of age have been suggested by others to be useful for herd diagnostics (Veling, Barkema, van der Schans, van Zijderveld, & Verhoeff, 2002).

### 3.3 Change in seroprevalence and estimated true prevalence

Overall, there was a significant reduction in prevalence from first to last sampling round in both young stock and lactating cows (Figure 2). In lactating cows, the overall estimated true within-herd prevalence across all herds was reduced from 26.9% to 2.7% \((t=5.19, p<0.006)\) from the first to the last sampling rounds. In young stock above the age of three months, the overall within-herd
true prevalence across all herds went from 15.0% to 2.6% ($t=1.48, \ p<0.17$). Only herd H did not reach 0% true prevalence in the study period.

Seroprevalence ≤5% was used as the criteria indicating good control of the infection. Herd H did not reach seroprevalence ≤5% in young stock in the study period. This herd reached Level 1 in the surveillance programme in February 2008. However, the young stock was not tested after December 2006, so it was not possible to say how the young stock seroprevalence developed after the project ended. Among the nine herds that did obtained seroprevalence in young stock ≤5% within the project period, the average time from initiation of control actions to when seroprevalence was ≤5% in young stock, was 13 months (std: 13), ranging from 0 to 30 months. In this project, the farmers were motivated to participate and there was frequent follow-up in the process from project leaders and local advisors. It is possible that successful intervention takes longer in herds with less motivated managers.

### 3.4 Changes in surveillance programme classifications

All herds were classified as Level 2 when the study period was initiated. To reach Level 1 (most likely not infected with *Salmonella Dublin*) in the Danish surveillance classification scheme, the average of the last four bulk tank milk samples had to be below 25 ODC% in antibody ELISA, and the last sample could not have an ODC% value that was 20 ODC% above the average of the previous three samples. Furthermore, herds were not allowed to have purchased animals from herds not in Level 1. The project period officially ended in December 2006. However, because the national surveillance programme was still running, we were able to follow the herds after that. As shown in Table 1, six of the 10 participating herds changed from Level 2 to Level 1 before December 2006. Herd A reached Level 1 in April 2004, but returned to Level 2 because of too high bulk tank milk antibodies in January 2009. In the time period between these two events this farmer
purchased animals from Level 2-herds on several occasions, which lead the herd to be classified as
“Level 2-because of purchase/contact to Level 2”. This might explain why this herd became re-
infected (Nielsen, Warnick, & Greiner, 2007; Vaessen, Veling, Frankena, Graat, & Klunder, 1998).
The last four herds obtained Level 1 before the end of 2008. It was an indication that infection
was not stopped effectively until the end of or after the project period. The time it takes bulk tank
milk antibodies to reduce to levels that will classify the herd in Level 1, after transmission of
*Salmonella* Dublin among all of the young stock has ceased, can be up to around 2 years (Jordan,
Nielsen, & Warnick, 2008).
Nine herds stayed in Level 1 at least until August 2010, which was the last sampling before
submission of this manuscript. The estimated mean time herds claimed to perform control actions
directed against transmission of *Salmonella* Dublin before reaching Level 1 was 24 months (std: 6).
Figure 3 illustrates bulk tank milk *Salmonella* Dublin antibody levels in the participating herds from
before the study period until August 2010. The individual curves show that four of the herds most
likely became infected one to two years before the project started, whereas the rest of the herds had
most likely been infected for at least three years before 2003.

### 3.5 Experiences from interviews

During the herd visits it became evident that it was important that herd-managers had a
thorough understanding of the infection dynamics to manage infections. Control of *Salmonella*
Dublin requires long-term and daily efforts, and the required actions differ from one herd to
another. Consequently, communication is a major challenge in such control programmes. This
includes communication between central decision makers, farmers and local advisors.
Communication can be assisted by tools, which convey background information and infection status
of herds and animals obtained via diagnostic test information over time such as in this study. We suggest that control strategies for *Salmonella* Dublin should contain the following five components:

a) communication;

b) reduction of transmission via changes in management and trade restrictions;

c) detection and management (or culling) of infectious animals;

d) documentation of effect of intervention;

e) continued surveillance.

### 3.6 Control or eradication

There was a clear reduction in the apparent prevalences and estimated true prevalences in all herds, but the apparent prevalences were not 0% in all herds at the end of the study period. The specificity of antibody ELISA is not 100%, primarily because some animals have been exposed to *Salmonella* Dublin, but have cleared the infection, or they have been infected with other *Salmonella* serovars containing cross-reacting antigens. It is therefore possible that the infection was cleared from the herds without it being reflected as seroprevalence at 0%. However, it is also possible that the used diagnostic tests have not been able to identify some infected animals, or that *Salmonella* Dublin-bacteria have remained in the environment. It is therefore not possible to deem the herds “free of *Salmonella* Dublin”, but the infection appears to currently be under control and potentially eradicated from the herd, based on bulk tank milk recordings obtained after the study period ended. Thus, the recommendation to farmers should be to continue management practises to control the infection, but testing can be reduced to a minimum after the low prevalence status has been obtained.

All cattle herds in Denmark have been included in the surveillance programme since 2002 which includes consequences upon trade with herds not in Level 1. Hence, most farmers are likely
to perform some control actions when they are informed about the results and discuss these with colleagues, local advisors etc. It was therefore not possible to include infected control herds to determine, if the structured approach used in the study herds were more effective than leaving it up to self-clearance to occur. However, most of the study herds had been infected for an extended period of time before the project started. Furthermore, previous studies show that persistent infection occurs in half of the dairy herds that become infected with Salmonella Dublin (Veling, 2004), and that restrictive purchase patterns are not enough in itself to stop transmission of infection within the herds (Nielsen et al., 2007). Thus, it is not very likely that self-clearance would have occurred in the study herds without the efforts provided by the farmers in this project.

4. Conclusions

To our knowledge this is the first study to demonstrate that it is feasible to control Salmonella Dublin in endemically infected herds. Effective control of Salmonella Dublin in the study herds was obtained by the use of limited resources through management that focused on closing transmission routes within the herds. We found markedly reduced prevalence of antibody-positive animals in 9 out of 10 herds, and all 10 herds could be classified as most likely free of Salmonella Dublin infection after a follow-up period. It was, however, not possible to compare different management strategies between these herds, so the results of this study should not be interpreted as specific recommendations, but rather as an illustration of a structured approach to controlling Salmonella Dublin in dairy herds that appears to work. It took on average three years from initiation of control actions until monitoring suggested that Salmonella Dublin was no longer spreading and the seroprevalence was low in all age groups of the herd. It cannot be ruled out that more aggressive culling of high-risk animals could speed up the control. However, such a strategy might not be cost-
effective. Such strategies probably have to be studied by simulation modelling, because it is
difficult and resource consuming to study under field conditions.

Supplementary material related to this article can be found online at

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Federation for funding. The farmers and local advisors are thanked for participating with
constructive comments and input in project meetings and interviews and by providing data from
their herds.
References


Table 1 Overview of 10 dairy herds that participated in a Danish *Salmonella* Dublin control study in 2003-2006.

<table>
<thead>
<tr>
<th>Herd</th>
<th>Date herd started in project&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Date Level 1 was obtained&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Herd size (#cows) at start and end of study period</th>
<th>Main breed</th>
<th>Sero-prevalence in young stock&lt;sup&gt;c&lt;/sup&gt; at first and last sampling round</th>
<th>Sero-prevalence in lactating cows&lt;sup&gt;d&lt;/sup&gt; at first and last sampling round</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Oct. 2003</td>
<td>April 2004</td>
<td>97 170</td>
<td>Danish Holstein</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>B</td>
<td>Jan. 2004</td>
<td>Feb. 2006</td>
<td>112 157</td>
<td>Danish Holstein</td>
<td>1%</td>
<td>0%</td>
</tr>
<tr>
<td>C</td>
<td>Feb. 2004</td>
<td>Oct. 2004</td>
<td>65 74</td>
<td>Danish Holstein</td>
<td>4%</td>
<td>0%</td>
</tr>
<tr>
<td>D</td>
<td>Oct. 2003</td>
<td>Mar. 2005</td>
<td>88 91</td>
<td>Danish Holstein</td>
<td>6%</td>
<td>1%</td>
</tr>
<tr>
<td>E</td>
<td>Dec. 2003</td>
<td>May 2006</td>
<td>71 102</td>
<td>Danish Jersey</td>
<td>7%</td>
<td>0%</td>
</tr>
<tr>
<td>F</td>
<td>Oct. 2003</td>
<td>Sep. 2005</td>
<td>98 120</td>
<td>Danish Holstein</td>
<td>9%</td>
<td>1%</td>
</tr>
<tr>
<td>G</td>
<td>Dec. 2003</td>
<td>July 2006</td>
<td>189 201</td>
<td>Danish Holstein</td>
<td>31%</td>
<td>3%</td>
</tr>
<tr>
<td>H</td>
<td>Oct. 2003</td>
<td>Feb. 2008</td>
<td>88 116</td>
<td>Danish Holstein</td>
<td>9%</td>
<td>23%</td>
</tr>
<tr>
<td>I</td>
<td>Dec. 2003</td>
<td>Nov. 2007</td>
<td>96 136</td>
<td>Danish Holstein</td>
<td>21%</td>
<td>3%</td>
</tr>
<tr>
<td>J</td>
<td>Dec. 2003</td>
<td>Oct. 2008</td>
<td>68 67</td>
<td>Danish Holstein</td>
<td>57%</td>
<td>5%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Defined as date of first individual milk sampling.

<sup>b</sup> Level 1 indicates “most likely free from *Salmonella* Dublin-infection” in the national surveillance programme. Herd A returned to level 2 in January 2009 due to increases in bulk-tank milk antibodies.

<sup>c</sup> Based on screening of antibodies in serum of all animals N3 months in small and medium sized herds, and animals 3–7 months and heifers N15 months in large herds.

<sup>d</sup> Based on screening of antibodies in milk of all lactating cows.
### Table 2 Control actions, starting dates and time to success for 10 Danish dairy herds in a *Salmonella* Dublin field study

<table>
<thead>
<tr>
<th>Herd</th>
<th>Start date of intervention actions&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Main intervention actions</th>
<th>Time until effect on young stock&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Time until reaching level 1&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>November 2003</td>
<td>Camera surveillance of calvings with fast removal of calves from high-risk cows. From September 2005 calves moved to outdoor calf hutches, heifers moved to heifer raising facilities off premises. Culling of persistently high-titre cows.</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>B</td>
<td>January 2004</td>
<td>The first 1½ years of the intervention period calves were removed from the dam immediately after birth. No milk used to feed calves from high-risk cows. Heifers moved to heifer raising facilities off premises.</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>C</td>
<td>January 2004</td>
<td>Calves were removed from the dam 1-7 h after birth. Immediate removal of calf if cows appeared on high-risk list.</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>D</td>
<td>January 2004</td>
<td>No milk fed to calves from high-risk cows. Thorough cleaning of pre-weaning calf pens before introducing new calves. Hygiene in pre-weaned calf pens and feeding buckets improved.</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>E</td>
<td>January 2003</td>
<td>Outdoors calf hutches. Removal of calf from dam 1-7 h after birth. Culling of persistently high-titre cows, but not all persistently high-titre heifers from January 2004.</td>
<td>15</td>
<td>41</td>
</tr>
<tr>
<td>F</td>
<td>January 2004</td>
<td>Single calving pens cleaned after every calving. Calf removed immediately after birth. Thorough cleaning of outdoor calf hutches, before new calves were moved in. Culling of high-risk cows when convenient.</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>G</td>
<td>November 2004</td>
<td>Pre-weaning calf pens cleaned between each calf. Discontinued use of high-pressure cleaning indoors. All-in-all-out for all group housing calf sections. Strict management of colostrum with bank of milk from low-risk cows fed via a tube. Common calving area split into two – one for high-risk cows and one for others from July 2005. Culling of persistent high-titre animals in late stages of intervention.</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>H</td>
<td>October 2003</td>
<td>Calves removed immediately after birth. Pre-weaning calf pens cleaned between each calf. Persistently high-titre cows culled if convenient.</td>
<td>N/A</td>
<td>52</td>
</tr>
<tr>
<td>I</td>
<td>July 2004</td>
<td>Heifer calves removed immediately after birth. Only milk replacement fed to calves.</td>
<td>28</td>
<td>41</td>
</tr>
<tr>
<td>J</td>
<td>May 2004</td>
<td>Calving moved to outdoors. Fast removal of calves from high-risk cows at calving. Pre-weaned calves moved to outdoor calf hutches, however, not consistently.</td>
<td>30</td>
<td>53</td>
</tr>
</tbody>
</table>

<sup>a</sup> Estimated from interviews with farmer  
<sup>b</sup> Number of months from intervention started to young stock seroprevalence was below 5%  
<sup>c</sup> Number of months from intervention started to level 1 was obtained. Level 1 indicates “most likely free from *Salmonella* Dublin-infection” in the Danish surveillance programme
Table 3  Example of list of repeated antibody ELISA results from all tested cattle in *Salmonella* Dublin control herds

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Age in years</th>
<th>ELISA 1 (date)</th>
<th>ELISA 2 (date)</th>
<th>ELISA 3 (date)</th>
<th>ELISA 4 (date)</th>
<th>Average^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>934</td>
<td>3.8</td>
<td>4 (02AUG06)</td>
<td>0 (17MAY06)</td>
<td>0 (15FEB06)</td>
<td>0 (04JAN06)</td>
<td>1</td>
</tr>
<tr>
<td>936</td>
<td>3.7</td>
<td>0 (02AUG06)</td>
<td>0 (17MAY06)</td>
<td>0 (15FEB06)</td>
<td>3 (04JAN06)</td>
<td>1</td>
</tr>
<tr>
<td>941</td>
<td>7.4</td>
<td>38 (02AUG06)</td>
<td>6 (17MAY06)</td>
<td>21 (15FEB06)</td>
<td>3 (04JAN06)</td>
<td>17</td>
</tr>
<tr>
<td>954</td>
<td>3.3</td>
<td>15 (02AUG06)</td>
<td>5 (17MAY06)</td>
<td>18 (15FEB06)</td>
<td>3 (04JAN06)</td>
<td>10</td>
</tr>
<tr>
<td>955</td>
<td>3.3</td>
<td>11 (02AUG06)</td>
<td>34 (17MAY06)</td>
<td>10 (15FEB06)</td>
<td>10 (04JAN06)</td>
<td>16</td>
</tr>
<tr>
<td>956</td>
<td>3.3</td>
<td>7 (02AUG06)</td>
<td>0 (17MAY06)</td>
<td>0 (15FEB06)</td>
<td>2 (04JAN06)</td>
<td>2</td>
</tr>
<tr>
<td>983</td>
<td>5.5</td>
<td>9 (02AUG06)</td>
<td>1 (17MAY06)</td>
<td>.</td>
<td>.</td>
<td>5</td>
</tr>
<tr>
<td>984</td>
<td>5.5</td>
<td>25 (02AUG06)</td>
<td>7 (17MAY06)</td>
<td>15 (15FEB06)</td>
<td>6 (04JAN06)</td>
<td>13</td>
</tr>
<tr>
<td>990</td>
<td>6.2</td>
<td>16 (02AUG06)</td>
<td>18 (17MAY06)</td>
<td>10 (15FEB06)</td>
<td>21 (04JAN06)</td>
<td>16</td>
</tr>
<tr>
<td>991</td>
<td>6.2</td>
<td>17 (02AUG06)</td>
<td>6 (17MAY06)</td>
<td>48 (04JAN06)</td>
<td>17 (01SEP05)</td>
<td>22</td>
</tr>
<tr>
<td>993</td>
<td>5.4</td>
<td>3 (02AUG06)</td>
<td>7 (17MAY06)</td>
<td>8 (04JAN06)</td>
<td>1 (01SEP05)</td>
<td>5</td>
</tr>
<tr>
<td>999</td>
<td>5.3</td>
<td>17 (02AUG06)</td>
<td>0 (17MAY06)</td>
<td>18 (15FEB06)</td>
<td>14 (04JAN06)</td>
<td>12</td>
</tr>
<tr>
<td>1001</td>
<td>5.0</td>
<td>30 (17MAY06)</td>
<td>4 (15FEB06)</td>
<td>0 (04JAN06)</td>
<td>.</td>
<td>11</td>
</tr>
<tr>
<td>1012</td>
<td>4.7</td>
<td>0 (02AUG06)</td>
<td>0 (17MAY06)</td>
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<td>0 (21MAR05)</td>
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<tr>
<td>1013</td>
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<td>24 (15FEB06)</td>
<td>27 (04JAN06)</td>
<td>2 (01SEP05)</td>
<td>10 (16JUN05)</td>
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</tr>
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<td>32 (02AUG06)</td>
<td>20 (15FEB06)</td>
<td>22 (04JAN06)</td>
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<tr>
<td>1038</td>
<td>4.6</td>
<td>27 (02AUG06)</td>
<td>18 (15FEB06)</td>
<td>18 (04JAN06)</td>
<td>10 (01SEP05)</td>
<td>18</td>
</tr>
<tr>
<td>1039</td>
<td>4.6</td>
<td>0 (02AUG06)</td>
<td>0 (17MAY06)</td>
<td>0 (15FEB06)</td>
<td>0 (04JAN06)</td>
<td>0</td>
</tr>
<tr>
<td>1043</td>
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<td>1 (02AUG06)</td>
<td>7 (15FEB06)</td>
<td>0 (04JAN06)</td>
<td>0 (01SEP05)</td>
<td>2</td>
</tr>
<tr>
<td>2452</td>
<td>1.7</td>
<td>23 (03APR06)</td>
<td>46 (27OCT05)</td>
<td>85 (03MAY05)</td>
<td>92 (17NOV04)</td>
<td>62</td>
</tr>
<tr>
<td>2455</td>
<td>1.7</td>
<td>0 (03APR06)</td>
<td>6 (27OCT05)</td>
<td>8 (03MAY05)</td>
<td>6 (17NOV04)</td>
<td>5</td>
</tr>
</tbody>
</table>

^a ELISA 1 is the newest sample and ELISA 4 the oldest sample of the ones shown from each animal. The values shown are the ODC% measured by the ELISA on (the sample date).

^b The average ODC% of the last up to four samples is used to identify cattle with persistently high antibody levels in blood or milk.
Table 4 Example of list of repeated antibody ELISA results from cattle categorized as high risk in Salmonella Dublin control herds. Risk group (R) 1 are considered most likely carrier animals whereas risk group 2 have more doubtful results and should be retested. Both groups should be managed to avoid potential spread of infection.

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Age in years</th>
<th>ELISA 1 (date)</th>
<th>ELISA 2 (date)</th>
<th>ELISA 3 (date)</th>
<th>ELISA 4 (date)</th>
<th>Average&lt;sup&gt;b&lt;/sup&gt;</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>2526</td>
<td>2.0</td>
<td>98 (07NOV06)</td>
<td>111 (03APR06)</td>
<td>160 (27OCT05)</td>
<td>8 (03MAY05)</td>
<td>94</td>
<td>1</td>
</tr>
<tr>
<td>2545</td>
<td>1.9</td>
<td>103 (07NOV06)</td>
<td>91 (03APR06)</td>
<td>0 (27OCT05)</td>
<td>3 (03MAY05)</td>
<td>49</td>
<td>1</td>
</tr>
<tr>
<td>2536</td>
<td>2.0</td>
<td>122 (07NOV06)</td>
<td>2 (03APR06)</td>
<td>0 (27OCT05)</td>
<td>4 (03MAY05)</td>
<td>32</td>
<td>2</td>
</tr>
<tr>
<td>940</td>
<td>7.5</td>
<td>57 (02AUG06)</td>
<td>7 (17MAY06)</td>
<td>49 (15FEB06)</td>
<td>43 (04JAN06)</td>
<td>39</td>
<td>2</td>
</tr>
<tr>
<td>953</td>
<td>3.4</td>
<td>70 (02AUG06)</td>
<td>68 (17MAY06)</td>
<td>33 (15FEB06)</td>
<td>19 (04JAN06)</td>
<td>48</td>
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<tr>
<td>982</td>
<td>6.3</td>
<td>65 (17MAY06)</td>
<td>34 (15FEB06)</td>
<td>28 (04JAN06)</td>
<td>56 (16JUN05)</td>
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<td>2</td>
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<tr>
<td>1001</td>
<td>5.8</td>
<td>95 (02AUG06)</td>
<td>40 (17MAY06)</td>
<td>46 (15FEB06)</td>
<td>25 (04JAN06)</td>
<td>52</td>
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<td>15 (17MAY06)</td>
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<td>26</td>
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</tr>
<tr>
<td>1858</td>
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<td>60 (17MAY06)</td>
<td>13 (15FEB06)</td>
<td>40 (04JAN06)</td>
<td>58 (16JUN05)</td>
<td>43</td>
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<tr>
<td>2002</td>
<td>4.2</td>
<td>54 (02AUG06)</td>
<td>62 (17MAY06)</td>
<td>103 (15FEB06)</td>
<td>48 (04JAN06)</td>
<td>67</td>
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<td>32 (03APR06)</td>
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<td>59 (03APR06)</td>
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<td>14 (03MAY05)</td>
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<td>96 (03APR06)</td>
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<td>54</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>a</sup> ELISA 1 is the newest sample and ELISA 4 the oldest sample of the ones shown from each animal. The values shown are the ODC% measured by the ELISA on (the date).

<sup>b</sup> The average ODC% of the last up to four samples may aid in identifying cattle with high antibody levels in blood or milk.
Figures

![Graph showing antibody ELISA response (ODC%) vs. age in years at sampling used to evaluate progress in control of Salmonella Dublin in young stock and lactating cows. In this particular example there are no signs of infections having occurred in cattle below one year of age suggesting that control actions have had a good effect in this part of the herd.](image)

**Figure 1** Example of antibody ELISA response (ODC%) vs. age in years at sampling used to evaluate progress in control of *Salmonella* Dublin in young stock and lactating cows. In this particular example there are no signs of infections having occurred in cattle below one year of age suggesting that control actions have had a good effect in this part of the herd.
Figure 2 Development in *Salmonella* Dublin seroprevalence from first to last sampling round in a) young stock (biannual sampling) and b) lactating cows (sampling four times per year) during an intervention field study in 10 Danish dairy herds during 2003 to 2006.

Figure 3 Bulk tank milk *Salmonella* Dublin antibody measurements in a) six herds that reached Level 1 in the surveillance programme within the study period 2003-2006. Five of these herds stayed in Level 1 at least until August 2010 and one herd had new or recurrent infection in 2009; b) four intervention herds that reached Level 1 after the study period. The solid line indicates the cut-off value of 25 ODC% used in the surveillance programme.