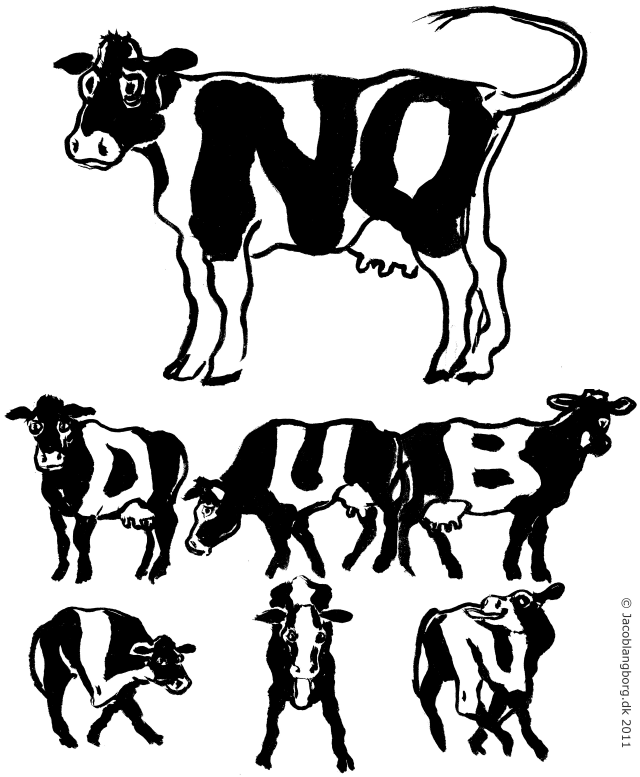
FACULTY OF HEALTH SCIENCES
UNIVERSITY OF COPENHAGEN

Consequences of *Salmonella* Dublin on health and economy in Danish dairy cattle herds



PHD THESIS 2012 · TORBEN DAHL NIELSEN

Consequences of *Salmonella* Dublin on health and economy in Danish dairy cattle herds

PhD thesis

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Consequences of *Salmonella* Dublin on health and economy in Danish dairy cattle herds
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ISBN 978-87-7611-489-3

Printed by SL grafik, Frederiksberg C, Denmark.
www.slgrafik.dk

Contents

Preface

This project was funded from three different sources: i) Faculty of Life Sciences, University of Copenhagen (j.nr. 82-4), ii) Research School for Animal Production and Health (RAPH) which obtained funding through Danish Agency for Science, Technology and Innovation (j.nr. 645-07-0014), and iii) The Danish Dairy Board, Aarhus (today Knowledge Centre for Agriculture, Cattle). The project was furthermore done in collaboration with the project “Salmonella 2007-2011” funded by the Milk Levy Fund and the Cattle Levy Fund.

My sincere gratitude is given to my supervisors. First of all, I would like to thank my main supervisor Associate Professor Liza Rosenbaum Nielsen. Without your extremely dedicated help, the past three years would have been much less rewarding and much more frustrating. I would like to thank Professor Hans Houe for keeping the perspective of the project and for your guidance. I am grateful to Academic Employee Anne Braad Kudahl and Senior Research Scientist Søren Østergaard for discussions at Institute of Animal Science, Aarhus and your commitment to the project. Furthermore, a big thank you to Anne for all the simulations you have performed in the last months, weeks and days.

I would like to thank all the guys in the population biology group but a special thank you to Jeanne for always being helpful. Thank you to fellow PhD students Grethe, Filipa and Francisco Fernando for sharing frustrations, victories and day to day life at the office and to Marshal for all your R help. Professor Nils Toft is thanked for lots of nice meals and keeping daily life in perspective. To Associate Professor Helle Halkjær Kristensen, thank you for proof reading and for acting as dictionary.

During the PhD, I had the opportunity to work at University of Warwick with Professor Laura Green. I am very grateful for this chance and to Laura for showing interest in the project, dedicating a lot of time to me and fast responses during revision of the resulting manuscript. Several people outside the campus have shown interest in and provided help for this project during the last three years. To everyone at Knowledgecentre for Agriculture, Cattle, thank you for providing data, participating in discussions and generally showing an interest in the project and the results from it. I am also grateful to Kvægdyrlægerne Midt and to Jens Phillipsen for allowing me to follow them at work. Thank you to Kristian Kristensen, Department of Animal Science, Aarhus University for support with statistical modelling.

I would like to thank Tina Birk Jensen for inspiring me to apply for this PhD position, general help and proof reading. Thank you to Jakob Langborg Hansen for providing the illustration to the front page of the thesis.

I would also like to thank all my friends and family for showing interest in the project. I am sure the answers that you got to your questions must have sounded like complete rubbish, but thank you for asking. To Mick, Janine and Doreen, thank you for all your help. This has made the PhD period much easier. And last of all, my biggest thank you goes to Simon for making this possible. Without you, I would not have been able to do this.

List of abbreviations

AHE – animal health economics

BTM – bulk tank milk

BVD – bovine virus diarrhoea

CHR – The Central Husbandry Register

DCD – The Danish Cattle Database

DIM – days in milk

ECM – energy corrected milk

ELISA – enzyme-linked immunosorbent assay

GM – gross margin

IG – immunoglobulins

HSe – herd-sensitivity

HSp – herd-specificity

KCAC – Knowledge Centre for Agriculture, Cattle

LPS – lipopolysaccharide

ODC% – optical density corrected %

OR – odds ratio

PAR – population attributable risk

PPV – positive predictive value

S. – *Salmonella enterica* subsp. *enterica*

Se – sensitivity

Sp – specificity

Summary

Salmonella enterica subsp. *enterica* serovar Dublin (*S*. Dublin) is host adapted to cattle, in which it can cause problems through morbidity and mortality. Therefore, it leads to economic losses for farmers. *S.* Dublin is furthermore a serious zoonotic pathogen with higher risk of hospitalisation and mortality in humans than other serotypes. In 2002, the Danish Veterinary and Food Administration and the Knowledge Centre for Agriculture, Cattle initiated a *Salmonella* surveillance programme for all Danish cattle herds. The programme includes monitoring of bulk tank milk (BTM) antibodies from all Danish dairy herds and serological screening of non-dairy herds. The aim of the programme is to eradicate *S.* Dublin from Danish cattle herds by the end of 2014. However, the prevalence of test-positive dairy herds currently remains around 9%, which calls for new ways to motivate farmers to prevent and control the infection.

The production effects of endemic *S.* Dublin infection in the herd are largely unknown, and farmers often report that they believe there are no clinical symptoms or production effects of *S.* Dublin in the herd. This might lead them to accept the presence of the infection in the herd, which compromises the success of the programme. Few prior studies have attempted to quantify the herd level animal health and production effects of *S.* Dublin infection. This lack of knowledge of animal health and production effects means that it is not known what the economic effects of *S.* Dublin herd infection are. Increased knowledge on these subjects could encourage farmers to control *S.* Dublin as well as help them decide on control plans. Moreover, estimates of economic implications of *S*. Dublin in dairy herds can help the dairy cattle sector prioritise and decide on future control strategies.

The aim of the work presented in this thesis was to investigate the animal health economic consequences of *S.* Dublin infection in dairy herds. In order to evaluate the economic consequences, effects on animal health and production as well as effectiveness of control elements for the infection in the herd were investigated. It was decided to focus on two effects of *S.* Dublin herd infection in this project: 1) calf mortality that affects animal health and welfare as well as the farmer's economy (study 1), and 2) milk yield which is a production measure that highly influences the economy of the dairy farmer (study 2). Next, it was investigated which management practices were associated with control of *S.* Dublin transmission among young calves in BTM antibody positive herds (study 3). Finally, results from the three studies were used as part of the parameterisation of a simulation model estimating the animal health economic effects of *S.* Dublin in dairy herds (study 4).

A cross-sectional study of register data including all Danish dairy herds in 2007-2008 showed that *S.* Dublin BTM antibody positive herds had twice as high risk of having calf mortality above the national target (≤ 6.5%) than BTM antibody negative herds. Comparative analyses of milk yield in cows in 28 dairy herds with BTM antibody measurements indicative of new infection and 40 continuously test-negative dairy herds showed, that milk yield was reduced with up to 3 kg energy corrected milk per cow per day for up to 15 months after estimated time of *S.* Dublin herd infection. The reduction in the milk yield was most pronounced for parities 1 and 3 or higher, while parity 2 cows had less reduction in milk yield in infected herds.

In study 3, successful control of *S.* Dublin in a herd was defined as no calves between three and six months of age testing *S.* Dublin serum antibody positive after a one-year

control period. In a questionnaire study, information of management practices were collected by telephone interviews with 84 dairy herd owners. Avoiding purchase of cattle from *S.* Dublin test-positive herds was found to be the management practice most strongly associated with successful control of *S.* Dublin. Furthermore, several examples of good management and housing practices in the calving area and of pre-weaned calves were found to be associated with successful control.

Estimates of *S.* Dublin effect on production, animal health and herd infection dynamics from the three above mentioned studies as well as from literature were incorporated in a simulation model (Dublin-Simherd). This is an age-structured stochastic, mechanistic and dynamic model developed at University of Aarhus. Specifically, milk yield losses in simulated infected herds were calibrated to data from study 2 in this PhD project. Dublin-Simherd simulations were used to estimate the animal health economic consequences of *S*. Dublin herd infection expressed as reductions in gross margin (GM) per stall in infected herds compared to non-infected herds under different herd size and management conditions. GM losses were estimated for 10 years after time of herd infection in herds with 85, 200 and 400 cow stalls for i) very good, ii) good, iii) poor and iv) very poor management.

It was found that both milk yield losses and GM losses increased with herd size and poorer management level. The GM losses in the first year after herd infection were estimated to be higher than the following years for all three herd sizes. Annual GM losses averaged over 10 years was low for very good management, but were high for good to very poor management. E.g. in a 200 cow stall herd it was estimated that the average annual mean loss per stall over the 10 years after herd infection were 9 Euros for very good management and 230 Euros for very poor management. Sensitivity analyses of the included effects of *S.* Dublin herd infection in the 200 cow stall herd estimated that the assumption regarding milk yield losses in *S.* Dublin resistant and carrier cows was the parameter that influenced the estimated GM losses the most, and that this influence increased with poorer management. No effects on estimated GM losses were seen when changing the assumptions regarding *S.* Dublin-related mortality in calves and heifers.

The Dublin-Simherd model can be used to simulate actual control scenarios, including test-and-manage or test-and-cull procedures, and provide decision support on cost-effective ways of controlling *S.* Dublin in herds depending on herd size and other herd specific characteristics. More detailed data are necessary to estimate economic effects of *S.* Dublin herd infection with greater certainty. Further studies on which management practices will control *S.* Dublin in the herd are needed in order to validate the results found in this project, and costs of such control actions should be estimated in order to perform cost-benefit evaluations of different control scenarios.

In conclusion, the results from this PhD project show that *S.* Dublin herd infection is associated with increased calf mortality as well as decreased milk yield, even when the infection has reached the endemic stage in infected herds. It was also shown that exposure of calves to *S.* Dublin can be controlled through appropriate housing and management. It was found that there were potentially high GM losses in *S.* Dublin infected herds in the first year and up to 10 years after herd infection, and the magnitude of GM losses varied widely with management level and herd size.

Sammendrag

Salmonella enterica subsp. *enterica* serovar Dublin (*S*. Dublin) er tilpasset kvæg og kan medføre dødelighed og velfærdsproblemer grundet sygdom, hvilket medfører økonomiske tab for besætningsejerne. *S.* Dublin er desuden en alvorlig zoonose der medfører højere risiko for hospitalsindlæggelse og dødelighed hos inficerede personer end andre *Salmonella*-serotyper. I 2002 indførte Fødevarestyrelsen og Videncentret for Landbrug, Kvæg et *S.* Dublin overvågningsprogram for alle danske kvægbesætninger. Programmet inkluderer monitorering af tankmælksantistoffer af alle danske malkekvægsbesætninger og serologisk screening af andre besætningstyper. Desuden inkluderer programmet en handlingsplan til at udrydde *S.* Dublin i alle danske kvægbesætninger før 2015. I de seneste år er prævalensen af testpositive malkekvægsbesætninger er stagneret omkring 9% hvilket betyder, at der er et behov for nye måder at motivere besætningsejere til at forebygge og bekæmpe infektionen.

Produktionsmæssige følger af endemisk *S.* Dublin besætningsinfektion er langt fra kendt til fulde, og besætningsejere fortæller ofte, at de ikke synes, at der er nogle kliniske symptomer eller produktionseffekter forbundet med *S.* Dublin i besætningen. Dette kan medføre, at de accepterer tilstedeværelsen af infektionen i besætningen, hvilket reducerer chancen for at handlingsplanen får succes. Der er kun få publicerede studier, hvor man har forsøgt at kvantificere produktionseffekterne af *S.* Dublin på besætningsniveau. Denne manglende viden om effekten på dyrenes sundhed og produktion betyder samtidig, at de samlede økonomiske konsekvenser af *S.* Dublin ikke er kendt. Mere viden om disse emner kunne hjælpe med at motivere besætningsejere til at bekæmpe *S.* Dublin, og hjælpe dem med at planlægge bekæmpelsen. Derudover kan økonomiske beregninger af konsekvenserne ved *S.* Dublin i kvægbesætninger hjælpe kvægbranchen med at prioritere og planlægge fremtidige bekæmpelsesstrategier.

Formålet med projektet, var at undersøge sundhedsøkonomiske effekter af *S.* Dublin infektion i malkekvægsbesætninger. Det blev undersøgt, hvilken effekt *S.* Dublin har på dyrenes sundhed og produktion. Desuden blev effektiviteten af bekæmpelsestiltag undersøgt. Det blev besluttet at fokusere på to følger af *S.* Dublin i besætningen: 1) kalvedødelighed, som relaterer til dyrenes sundhed og velfærd samt besætningsejerens økonomi (studium 1), og 2) ydelse, som er et produktionsmål, der har betydelig indflydelse på besætningsejerens indtjening (studium 2). Det blev derefter undersøgt hvilke managementrutiner, der havde betydning for forebyggelse af *S*. Dublin smittespredning mellem kalve i besætninger, der var testpositive i overvågningsprogrammet (studium 3). Endelig blev resultaterne fra studierne 1-3 benyttet til parameterisering af en simuleringsmodel, der blev brugt til at estimere de samlede sundhedsøkonomiske konsekvenser af *S*. Dublin i malkekvægsbesætninger (studium 4).

Studium 1 var et tværsnitsstudium af registerdata, hvor stort set alle danske malkekvægsbesætninger i 2007-2008 var inkluderet. Det viste at besætninger, der var antistofpositive for *S.* Dublin i tankmælken, havde dobbelt så høj risiko for at have en kalvedødelighed, der var højere end det nationale mål på 6,5% i forhold til besætninger, der var antistofnegative i tankmælken.

I studium 2 gennemførtes analyser af køers mælkeydelse i 28 testpositive og 40 testnegative malkekvægsbesætninger. De viste, at ydelsen var reduceret med op til 3 kg energi-korrigeret mælk per ko per dag i op til 15 måneder efter estimeret besætningsinfektionsdato. Ydelsesreduktionen var højest for førstekalvskøer og køer med 3 eller flere kalve, mens andenkalvskøer havde lavere reduktion i ydelsen.

I studium 3 blev succesfuld kontrol af *S.* Dublin defineret ved at der ikke var nogle *S.* Dublin antistofpositive kalve i alderen tre til seks måneder, efter at besætningsejeren havde udført et etårigt bekæmpelsesprogram. I en spørgeskemaundersøgelse blev information om managementrutiner indsamlet via telefoninterview med 84 ejere af malkekvægsbesætninger. Den managementrutine, der var tydeligst forbundet med succesfuld kontrol af *S.* Dublin, var at undlade indkøb af dyr fra *S.* Dublin testpositive besætninger. Derudover blev adskillige 'gode' rutiner og opstaldningsforhold i forhold til kælvning og mælkefodrede kalve fundet at være forbundet med forebyggelse af smittespredning med *S.* Dublin.

Estimater af effekten af *S.* Dublin på produktionen, dyrenes sundhed og dynamikken i besætningsinfektionen, baseret på de tre ovennævnte studier og litteraturen, blev bygget ind i en simuleringsmodel (Dublin-Simherd). Modellen er en aldersstruktureret, stokastisk, mekanistisk og dynamisk model, som er udviklet på Århus Universitet. Ydelsestabet i de simulerede besætninger blev kalibreret til data fra studium 2. Effekten blev målt i dækningsbidrag (DB) per staldplads til køer i inficerede besætninger sammenlignet med DB i ikke-inficerede besætninger for forskellige besætningsstørrelser og managementforhold. DB-tabene blev estimeret for 10 år efter introduktion af S. Dublin til besætningen i malkekvægsbesætninger med 85, 200 og 400 staldpladser for i) meget godt, ii) godt, iii) ringe og iv) meget ringe management.

Simuleringerne viste, at ydelses- og DB-tab steg jo større besætningerne blev og jo ringere management blev. Tabet i DB blev estimeret til at være højere i det første år efter infektion af besætningen end i de efterfølgende år for alle tre besætningsstørrelser. Gennemsnitlige årlige DB-tab over 10 år var lave for meget godt management, men tabene var store for godt, ringe og meget ringe management. For eksempel blev de gennemsnitlige DB-tab per staldplads estimeret til 9 Euro pr. år i besætninger med meget godt management, og 230 Euro pr. år i besætninger med meget ringe management over 10 år i en besætning med 200 staldpladser. Sensitivitetsanalyser af de inkluderede *S.* Dublin effekter viste, at det antagne ydelsestab for *S.* Dublin resistente og kronisk inficerede køer havde størst indflydelse på det estimerede DB-tab, og at denne indflydelse blev øget ved ringere management.

Dublin-Simherd modellen kan bruges til simulering af forskellige kontrolscenarier, som management- eller udsætningsstrategier, og derved kan den bruges til at understøtte beslutninger om cost-effektiv bekæmpelse af *S.* Dublin i malkekvægsbesætninger. Der er brug for mere detaljerede data, hvis de sundhedsøkonomiske følger af besætningsinfektion med *S.* Dublin skal estimeres mere nøjagtigt. Der bør foretages yderligere studier af, hvilke managementrutiner der kan bruges til at bekæmpe *S.* Dublin i besætninger for at bekræfte resultaterne fra dette projekt. Desuden er der brug for at udgifterne til eventuelle kontrolrutiner estimeres og inkluderes i cost-benefit analyser af forskellige kontrolscenarier.

Der blev i dette ph.d. projekt vist, at *S.* Dublin infektion af besætninger er forbundet med forøget kalvedødelighed og ydelsestab, selv i besætninger, hvor infektionen har nået det endemiske stadium. Det blev også vist, at succesfuld forebyggelse af *S.* Dublin-spredning til kalve i inficerede besætninger kan opnås ved passende opstaldning og management. Det blev vist, at der potentielt var store DB-tab i *S.* Dublin-inficerede besætninger i det første år efter introduktion af infektionen til besætningen uanset managementniveau, og i de 10 år efter infektion for alle andre managementniveauer end meget godt management. Resultaterne kan bruges til at hjælpe besætningsejere, rådgivere og kvægbranchen med udryddelse af *S.* Dublin i Danmark.

1 Introduction

1.1 Background

1.1.1 *Salmonella enterica* subsp. *enterica* in humans and cattle

More than 2,400 serovars of *Salmonella enterica* exist (Parry, 2006), of which all are considered zoonotic pathogens. In 2009, there were more than 108,000 reported human cases of salmonellosis in the European Union (European Food Safety Authority, 2011b). Clinical signs in humans mainly include diarrhoea, abdominal pain, fever, nausea, muscle pain and death (Humphrey, 2006). The overall yearly economic burden of *Salmonella* in the member states of the European Union has been estimated at approximately 3 billion Euros (European Food Safety Authority, 2011a). It is thus a major zoonotic pathogen and the European Union has focused on decreasing the number of human *Salmonella* cases, mainly by reducing *Salmonella* in meat and egg products (European Commision, 2005).

Salmonella enterica subsp. *enterica* serovar Dublin (*S*. Dublin) is host adapted to cattle (Wray and Sojka, 1977), in which it causes animal welfare problems through morbidity of symptoms and mortality. It is furthermore a serious zoonotic pathogen with higher risk of hospitalisation and mortality in humans than other serotypes (Helms et al., 2003; Jones et al., 2008). In 2009 and 2010, a total of 95 people were diagnosed with *S.* Dublin infection in Denmark and more than 90% of these were believed to be infected domestically. Since *S.* Dublin is host adapted to cattle, beef and milk are the dominating sources of this pathogen but infection upon direct contact with infected cattle or manure from these also occurs. *S.* Dublin is the most frequently isolated serotype in meat from cattle both in Denmark and Europe (Anonymous, 2011; European Food Safety Authority, 2011b). Hence, there is a desire to control *S.* Dublin in the cattle production.

1.1.2 The Danish *Salmonella* Dublin surveillance

In 2002, the Danish Cattle Federation (now: Knowledge Centre for Agriculture, Cattle (KCAC)) began monitoring all Danish cattle herds for *S.* Dublin antibodies with the purpose of preventing non-infected herds from becoming infected (Anonymous, 2003). Five years later an actual *Salmonella* surveillance programme for *S.* Dublin was initiated. All herds are divided into three overall levels in the *Salmonella* surveillance programme based on movement data and antibody levels in routinely collected bulk tank milk (BTM) samples for dairy herds or movement data and blood samples for non-dairy herds. Level 1 is considered most likely free of *S.* Dublin, in level 2 *S.* Dublin is most likely present in the herd (or herd status is unknown), and in level 3 *S.* Dublin has been isolated from the herd (Anonymous, 2011).

The development over time of dairy herds in level 2 and 3 since 2002 can be seen in Figure 1.1. The prevalence of level 2 and 3 dairy herds December 2011 was approximately 9%.

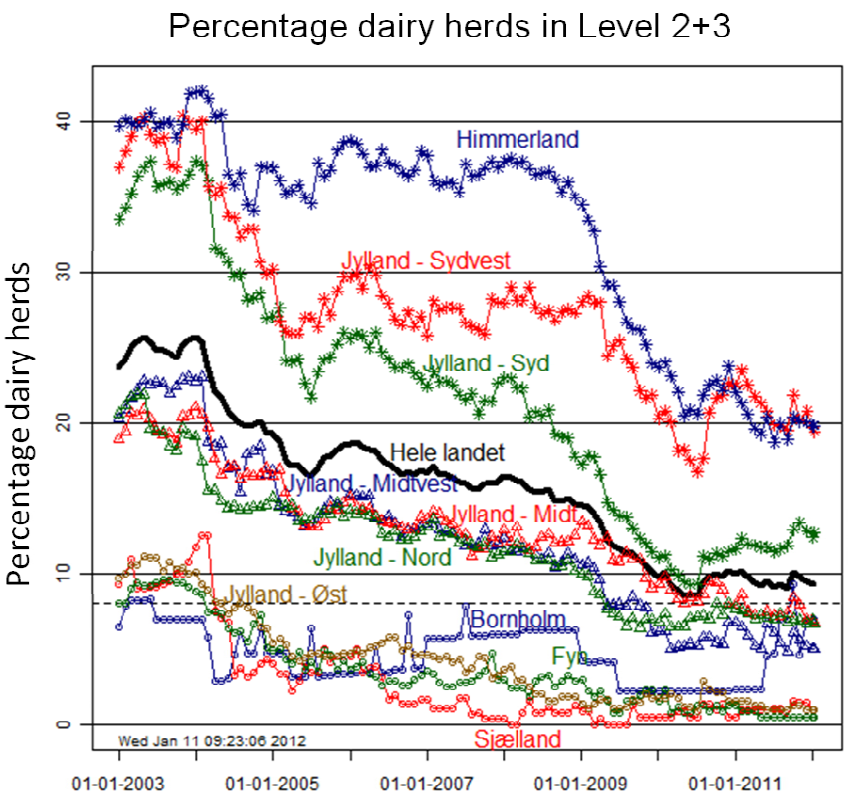

Figure 1.1 Percentage of Danish dairy herds in level 2 and 3 since 2003 for 10 regions and the entire country (solid line) (Knowledge Centre for Agriculture, Cattle, 2011).

The overall aim of the *Salmonella* surveillance programme is to eradicate *S.* Dublin by the end of 2014 (LandbrugsInfo, 2007). The eradication plan consists of three phases:

I. 2007-2009: Voluntary eradication encouraged by KCAC for level 2 and level 3 herds. Level 1 herds were encouraged to prevent introduction of *Salmonella*.

II. 2010-2012: Subdivision of level 2 herds into regular level 2 herds and high risk level 2 herds (level 2R). Level 2R herds experience movement restrictions of animals, although calves can be sold to specialised veal calf producers. In this case, the veal calf producer has to sign a contract agreeing to receive animals from level 2R herds. The restrictions are enforced by the veterinary authorities. To have the restrictions lifted, the herd owner will have to demonstrate that spread of *Salmonella* within the herd is under control. This can be done by testing the 10 youngest calves over three months of age and if the serum antibody level in all these animals does not indicate exposure to *S.* Dublin, then the restrictions can be lifted.

III. 2013-2014: It is likely that further restrictions for level 2 and possible level 3 herds will be implemented if needed, but these have not been decided yet.

Implementation of the *Salmonella* surveillance programme urges farmers to control and eradicate *S.* Dublin, but as can be seen from Figure 1.1 the proportion of herds in level 2 and 3 has been stabilising since the beginning of 2010. Hence, there is a need for further knowledge about the best control strategies at herd level. A complicating factor to the eradication plan is that the exact quantitative effects of *Salmonella* infection in the herd are largely unknown, and farmers often report that they believe there are no clinical symptoms or production effects of *Salmonella* in the herd. This might lead them to accept the presence of the infection in the herd. Furthermore, few herd level production effects of infection have been quantified, both regarding introduction of infection and when the infection is endemic in the herd. This lack of knowledge of effects on animal health and production means that economic effects of *S.* Dublin also are largely unknown. Increased knowledge on these subjects could further encourage farmers to control *S.* Dublin as well as help them decide on a control plan.

1.2 Aim of thesis

The aim of the work presented in this thesis was to investigate the animal health economic consequences of *S.* Dublin infection in dairy herds. In order to evaluate the economic consequences, effects on animal health and production as well as effectiveness of control elements for the infection in the herd were investigated. The following hypotheses were pursued in this thesis:

I. It was hypothesised that *S.* Dublin has an effect on animal health in dairy herds
II. It was hypothesised that *S.* Dublin has an effect on production in dairy herds
III. It was hypothesised that *S.* Dublin can be controlled effectively through management changes in dairy herds
IV. It was hypothesised that *S.* Dublin has a an animal health economic effect in dairy herds

The hypotheses were pursued through four specific objectives:

Objective 1: Investigate the association between calf mortality and *S.* Dublin BTM antibodies in dairy herds

Objective 2: Investigate changes in milk yield following *S.* Dublin bulk tank milk antibody level increase

Objective 3: Identify which dairy herd management practices are associated with preventing exposure of calves to *S.* Dublin

Objective 4: Investigate animal health economic effects of introduction and spread of *S.* Dublin in dairy herds

Objectives 1 and 2 were register-based studies; Objective 3 was pursued in a field study including a questionnaire and collection of serum samples whilst the simulation study for Objective 4 was based on results from Objective 1, 2 and 3 as well as literature.

1.3 Outline of thesis

In Chapter 1, background for the project, hypotheses and objectives are presented while Chapter 2 describes the pathogenesis, epidemiology, clinical signs and diagnostic tests relevant for estimating the effects and control of *S.* Dublin in dairy cattle herds. Chapter 3 gives a brief introduction to animal health economy and how it is used in this thesis. Materials and methods (Chapter 4) describes Danish dairy cattle data sources and gives an overview of study designs and statistical methods used in the studies. A summary of main findings are presented in Chapter 5 and materials and methods as well as results are discussed in Chapter 6. Chapter 7 includes the perspectives and Chapter 8 the references used in this thesis. The four manuscripts of the thesis are presented in Chapter 9 while Chapter 10 includes the appendix, which contain the questionnaire used in Objective 3 in Danish (version used for data collection) and English (version submitted online with Manuscript 3).

1.4 Definitions of concepts used in this thesis

Control: “The reduction of disease incidence, prevalence, morbidity or mortality to a locally acceptable level as a result of deliberate efforts; continued intervention measures are required to maintain the reduction” (Dowdle.W.R., 1998).

Eradication: “Reduction of herd prevalence close to zero and hence no spread of bacteria between herds” (Andrews and Langmuir, 1963).

Monitoring: “The intermittent performance and analysis of routine measurements and observations, aimed at detecting changes in the environment or health status of a population” (Office International des Epizooties, 2011).

Surveillance: “Systematic ongoing collection, collation and analysis of data and the timely dissemination of information to those who need to know so that action can be taken” (Office International des Epizooties, 2011).

Economic concepts and definitions are described in Chapter 3.

2 *Salmonella* Dublin in cattle

2.1 Pathogenesis

The primary route of uptake for *S.* Dublin is via oral intake of contaminated feed, water and milk or via uptake from an infected environment (Hardman et al., 1991). Other ways of transmission, such as aerosols or intrauterine transmission are possible as well, but these are considered less important (Richardson, 1973; Nazer and Osborne, 1977; Wathes et al., 1988). Furthermore, experimental *S.* Dublin infection has been caused by injecting bacteria into the teat canal (Spier et al., 1991).

The infectious dose of *S.* Dublin depends on age. Animals below 196 days need a peroral dose of at least 10^6 bacteria to show symptoms (Nazer and Osborne, 1977; Robertsson, 1984; Segall and Lindberg, 1991) while heifers display a varying response to peroral doses of 10^9 to 10^{11}. This varies from no symptoms at all to symptoms such as abortion, dysentery and pyrexia (Hall and Jones, 1979; Smith et al., 1989). The higher the infectious dose, the more consistent clinical symptoms are displayed (Taylor, 1973; Nazer and Osborne, 1977).

Once ingested, the bacteria colonises the intestinal lumen if they survive the environment in the rumen and overcome the intestinal limiting factors such as peristalsis, competing microflora, effects of bile etc. They can then adhere to and cross the intestinal wall where they multiply in the gut-associated lymphoid tissue (Bäumler et al., 2000). *Salmonella* infected macrophages are drained to the local lymph nodes which serve as an important barrier towards further spread in the host. However, if *Salmonella* break through this barrier they can cause bacteraemia, where the bacteria in particular spread to the liver, tonsils, spleen, lungs and lymph nodes (Fenwick and Collett, 2004). Bacterial proliferation primarily happens within macrophages from which the bacteria can be released into the intestinal lumen and in that way cause contaminated meat through faecal contamination during slaughter (Humphrey, 2006). Infected asymptomatic animals most often shed bacteria intermittently (Richardson, 1973; Smith et al., 1989; Spier et al., 1990). Sick calves also appear to shed *S.* Dublin in saliva (Richardson and Fawcett, 1973).

S. Dublin is host adapted to cattle, which means that this is the most often infected species (Wray and Sojka, 1977; Uzzau et al., 2000). It has been isolated from other species such as humans (Helms et al., 2003, Jones et al., 2008), mice (Tablante and Lane, 1989) as well as pigs and sheep (Sojka et al., 1977). Following infection cattle can become carriers and these can excrete 10^2 to 10^4 bacteria per gram faeces (Sojka et al., 1974). This is important when considering the control of *S.* Dublin in the herd. The carrier state can occur in all age groups, both for animals with and without clinical signs. Carriers of *S.* Dublin can be divided into three different types (Richardson, 1973; Wallis, 2006):

I. Active carriers: Animals show no symptoms but have active infection (often convalescing animals) and they may excrete *S.* Dublin continuously for years or even for life.

II. Passive carriers: These animals ingest *S.* Dublin orally and pass the bacteria in faeces but have no active infection of intestines. The animals will stop excreting once they stop ingesting bacteria.

III. Latent carries: *S.* Dublin is present in tissues in these animals, but they only excrete *S.* Dublin intermittently in faeces, most often in connection with stress such as when moved to another herd, affected by other diseases or at calving (Spier et al., 1991). However, there are also indications that some latent carriers do not shed bacteria at all (Lomborg et al., 2007).

2.1.1 Clinical signs

Clinical disease is most often seen in young calves. Among these, disease can occur from only a few sporadic cases up to major outbreaks with up to 100% morbidity and 50% mortality (Hughes and Jones, 1973). In older animals, sporadic cases are more often seen (Vandegraaff and Malmo, 1977) but larger outbreaks in naïve herds can also occur. Clinically, *S.* Dublin infections can be divided into four different stages (Wray and Davies, 2000):

I. Peracute infection: Animals will die within 1-2 days of infection due to septicaemia and endotoxic shock. Often there are none or few clinical signs prior to death. Peracute infection is most often seen in young calves but it can also be seen in older naïve animals.

II. Acute infection: In young calves, the main clinical signs are diarrhoea, anorexia, weight loss and dehydration, although pneumonia, central nervous system symptoms and death also occur (Grønstøl et al., 1974a; Greene and Dempsey, 1986; Jarveots et al., 2003). In cows, sudden onset of clinical signs is seen with depression, anorexia and fever. Milk yield is reduced (Vandegraaff and Malmo, 1977; Bazeley, 2006) and these symptoms are followed by diarrhoea which can last for 10-14 days although complete recovery may take months. Mortality can be as high as 75% in untreated cows but is about 10% if treated. Abortion can occur in pregnant animals, often without any other signs although decreased milk yield might be seen (Morton, 1996; Carrique-Mas et al., 2010).

III. Subacute infection: Animals can display the same symptoms as in acute salmonellosis but they are less severe. Mortality is low even without treatment.

IV. Chronic infection: Is most often seen in calves older than 6-8 weeks that have survived acute infection. The calves appear unthrifty and can have poly-arthritis, osteomyelitis and gangrene of ears, tail and distal limbs (O'Connor et al., 1972; Rings, 1985; Mee, 1995).

2.1.2 Immune response

Both the cellular and humoral parts of the specific immune system react to *Salmonella* infection. In response to the infection, the animal will produce immunoglobulins (IG) and specific IGs towards *S.* Dublin lipopolysaccharide (LPS) antigens are produced by the humoral immune system. At birth, calves can have maternally derived circulating antibodies directed against *S.* Dublin for the first few weeks to months (Barrington and Parish, 2001), but they have reduced ability to produce IGs directed against *S.* Dublin LPS antigens until the age of approximately 11 weeks (Roden et al., 1992). Experimental oral infections of adult cattle have shown that antibody titres in milk and serum peak at around day 76 after infection and then fall back to the level of non-infected cows between day 90 and 140 (Smith et al., 1989). Studies of carrier animals have shown that three samples over 120 days are needed to distinguish between carriers and transiently infected animals (Smith et al., 1992). In calves experimentally infected at 6-7 weeks of age, antibody titres peak at 40 days post infection (Robertsson, 1984). If an animal is continuously or frequently exposed to *S.* Dublin, antibody level remains higher than for non-exposed animals (similarly to persistently infected active carriers) (Smith et al., 1989; Spier et al., 1990).

2.2 Risk factors for transmission of infection

Several factors relating to *S.* Dublin in cattle influence both the introduction and persistence of the bacterium in the herd. These factors can be divided into host, agent and environmental related risk factors. Most important risk factors for transmission of bacteria include direct contact between animals or indirect vehicle born transmission.

2.2.1 Host related risk factors

S. Dublin carrier animals can cause infection to persist in a herd by re-infecting herd mates or introduce infection to a herd if a carrier animal is introduced.

Immune status of the animal affects its ability to control infection and is a risk factor for both introduction and persistence of infection in the herd. Young calves are more susceptible to infection and more at risk of experiencing clinical disease due to *Salmonella* than older animals, due to the reduced ability to produce antibodies (Roden et al., 1992).

Stressed animals have been reported to be more at risk of infection with *S.* Dublin and to shed more bacteria than non-stressed animals. Hence, stressed animals are more likely to introduce the infection into the herd as well as maintain the infection in the herd through increased shedding. Spier et al. (1991) found that cows injected with dexamethasone after intramammary injection of *S.* Dublin displayed clinical symptoms, such as raised temperature and mastitis three weeks after *S.* Dublin challenge. Furthermore, *S.* Dublin shedding in milk increased significantly after dexamethasone injection. Reactivation of *S.* Dublin shedding has also been reported after transport in experimentally infected calves that had previously been faecal culture negative for 5

weeks (Grønstøl et al., 1974b) and Beach et al. (2002) reported that prevalence of *Salmonella* shedding adult cattle increased from 6% to 21% after transportation.

Stress caused by concurrent infectious diseases has been reported to influence introduction and persistence of *S.* Dublin infection. Herd infection with *Fasciola hepatica* has been reported to be associated with infection with *S.* Dublin (Vaessen et al., 1998), and Aitken et al. (1981) reported that animals harboured *S.* Dublin longer in the tissue and shed it longer if they were also infected with *Fasciola hepatica*. Calves infected with bovine virus diarrhoea (BVD) virus showed more severe symptoms if they were also infected with *S.* Dublin in an experimental study (Wray and Roeder, 1987), but BVD is rare in Denmark with only a few cases per year (Dansk Kvæg, 2009), so this infection is unlikely to play an important role in *S.* Dublin infected herds in Denmark today.

Volatile fatty acids in rumen fluid are part of the host's defence mechanism towards *Salmonella.* Chambers and Lysons (1979) found that survival of *S.* Typhimurium in rumen fluid was increased after the cow had been starved for 48 hours compared to 4.5 hours after regular feedings. Hence, diseases causing anorexia or reduced food intake in connection with e.g. transport could increase the risk of an animal and thereby the herd getting infected.

2.2.2 Agent related risk factors

S. Dublin can survive for a long time in the environment which increases the risk of maintaining infection in the herd. Findley (1972) showed that *S.* Dublin could survive for 33 weeks in slurry and survival has been reported for up to 68 months in dry faeces (Plym-Forshell and Ekesbo, 1996). Wray and Callow (1974) studied survival of *S.* Dublin in colostrum collected four days post partum. They found that *S.* Dublin survived for 62 and 46 days in colostrum stored at 5-11^{o}C and 16-21^{o}C respectively, when inoculated at a concentration of 10^6 pr ml colostrum. When inoculated at 10^4 cells pr ml the survival was reduced to 21 and 2 days respectively. This survival in the environment complicates control in the herds.

2.2.3 Environment related risk factors

2.2.3.1 Management

Several management factors have been reported to be associated with the introduction and maintaining of *S.* Dublin in the herd. For introduction of infection into the herd, one of the most common reported risk factors is purchase of animals, most likely latent carriers (Morton, 1996; Vaessen et al., 1998; Nielsen et al., 2007). Other routes of contact between herds can introduce infection. Adhikari et al. (2009) reported that use of heifer raising facilities ("heifer hotels") was associated with introduction of multiresistant *Salmonella* strains into cattle herds and shared grazing has been reported to increase the risk of *S.* Dublin outbreak in the herds (Schaik et al., 2002).

Risk factors for maintaining the infection in the herd have been reported to be associated with the management procedures within the herd. The calving area is a high risk area for

spread of bacteria and practices related to this have been shown to be associated with *Salmonella* isolation in the herd. Among these practices are: using calving pens for recovering animals (Losinger et al., 1995; Fossler et al., 2005), allowing cows to calve outside calving pens (Weber et al., 2009) and not providing a clean calving pen (House and Smith, 2004). Management related to young calves has also been reported to be associated with *Salmonella*. Poor handling of colostrum such as pooling of milk from several cows (House and Smith, 2004) and not feeding hay to calves from 24 hours after birth (Losinger et al., 1995) has been associated with isolating *Salmonella* from calves. Furthermore, lack of isolation facilities for diseased animals has been associated with presence of clinical disease caused by *S.* Typhimurium (Evans, 1996).

2.2.3.2 Hygiene

Wildlife, such as rodents and birds, might play a role in spreading *Salmonella* (Tablante and Lane, 1989; Evans and Davies, 1996; Warnick et al., 2001; Boqvist and Vågsholm, 2005) as well as cats (Veling et al., 2002b). Utensils for feeding have also been suggested as possible vehicles for *Salmonella* (Hardman et al., 1991).

2.2.3 Herd characteristics

Increasing herd size has been reported to be associated with increasing risk of isolating *Salmonella* from cattle herds (Vaessen et al., 1998; Kabagambe et al., 2000; Cummings et al., 2009) and can be seen as a risk factor for maintaining *Salmonella* in the herd. *S.* Dublin infected (or test-positive) neighbour herds can also increase the risk of infection (Wedderkopp et al., 2001; Nielsen et al., 2007), presumably by increasing the risk of introduction of *Salmonella*.

2.3 Diagnostic tests

There are two main methods to identify *S.* Dublin infection, bacteriological culture which detects the agent, and serology (i.e. antibody detection by the use of enzyme-linked immunosorbent assay (ELISA)).

2.3.1 Bacteriological culture

Bacteriological culture has traditionally been regarded as the gold standard method to detect *S.* Dublin infected animals and herds. Tissue samples from aborted foetuses or dead animals as well as faecal samples from live animals have been used. However, as mentioned above, animals often shed the bacteria intermittently. This is one of the reasons that the sensitivity (Se) for this method is low. Nielsen et al. (2004) estimated Se of faecal culture to be 6-14% at animal level in dairy cattle, and Nielsen et al. (2011) estimated Se to be 5-17% in faecal samples from veal calves at slaughter in abattoirs. In one study, 30 of 78 known *S.* Dublin infected herds were found positive when all animals with current or earlier symptoms were tested by faecal culture (Veling et al., 2002a). In contrast to this, experimental studies have reported sensitivity of up to 80% of single

bacteriological tests on faecal samples which were infused with *S.* Dublin (Baggesen et al., 2007).

2.3.2 ELISA

The other main way to diagnose infection is by measuring *Salmonella* antibodies in serum or milk by ELISA. *S.* Dublin belongs to the D_1-serogroup of *Salmonella*, which means that they have O1, O9 and O12 antigenic factors (Konrad et al., 1994). The ELISA test is an indirect test based on measuring antibodies directed towards *S.* Dublin O-antigens of the LPS. In Denmark, the majority of ELISA tests are analysed at Eurofins Steins Laboratory A/S or at the National Veterinary Institute (Technical University of Denmark) by the method described by Warnick et al. (2006) and Nielsen and Ersbøll (2004). The result of the most often used ELISA test is measured in optical density corrected % (ODC%) which is compared with a known positive control sample. Antigens from other *Salmonella* serotypes might cross-react with the test (Konrad et al., 1994), and in Denmark this is most often *S.* Typhimurium (Anonymous, 2011). A herd is considered test-positive if the average of four BTM tests is ≥ 25 ODC% and a serology test for an individual animal is positive at a value ≥ 50 ODC%.

2.4 Danish test strategy for herd level diagnosis

2.4.1 Bulk tank milk antibodies

Dairy herds have BTM antibody levels measured every 3 months in the *Salmonella* surveillance programme (Anonymous, 2011). A herd is placed in level 1 if the mean of the last 4 samples is < 25 ODC% and there is no increase of > 20 ODC% in the last sample compared to the mean of the three previous samples. If a level 1 herd comes into contact with level 2 or 3 herds (i.e. through recorded common pastures, markets or purchase), the herd is automatically locked in level 2 for at least three weeks. To return to level 1 the herd will have to be retested.

When true prevalence of infected herds is between 8 and 15%, the herd sensitivity (HSe) of the *Salmonella* surveillance programme has been estimated to be approximately 0.95 (95% CI: 0.92-0.96), and the herd specificity (HSp) has been estimated at around 0.96-0.97 (95% CI: 0.95-0.97). The negative predictive value has been estimated at above 0.99 (95% CI: 0.99-1) meaning that less than 1% of the herds in level 1 are truly infected. However, the positive predictive value (PPV) has been estimated to be between 0.75 and 0.80 (95% CI: 0.64-0.84), so between 20 and 25% of the herds in level 2 are not infected at the time of herd classification (Warnick et al., 2006). Jordan et al. (2008) developed a hierarchical model of *S.* Dublin and control in Denmark. They found that there was a 'lag' period from when the herd was cleared of infection until it reached level 1 in the *Salmonella* surveillance programme. This is the likely reason for the lower positive predictive value.

2.4.2. Serology

Serology is also used to identify *S.* Dublin infected animals and herds. In Denmark, this has mainly been used for the surveillance of non-dairy herds, where animals are tested at slaughter or for blood samples collected from the herd on request of the owner. Since the introduction of level 2R dairy herds, serology has been used to test if these herds were in control of the within-herd spread of *S.* Dublin (see Chapter 1.1.2).

The best performance of the test is reached when testing calves and young stock between 100 and 299 days, which gives a test Se at animal-level of 0.77 (95% CI: 0.66-0.88) and a specificity (Sp) of 0.95 (95% CI: 0.93-0.98) at cut-off of 50 ODC% (Nielsen et al., 2004). Testing of calves younger than approximately three months of age results in more false negative animals than testing of older calves does because they have reduced ability to produce the antibodies. False positives may result from circulating maternal antibodies. HSe has been reported to be 91% and HSp to 99.3% when all calves between 4 and 6 months in the herd were tested (Veling et al., 2002a) using an LPS ELISA used in Holland.

2.5 Control options at herd level

There is no single method to control *S.* Dublin in cattle herds. The research so far indicates that several separate initiatives in the herd over a prolonged period of time are needed to control *Salmonella*. Bergevoet et al. (2009) reported from a simulation study that testing and culling of suspected carriers could reduce the within herd prevalence, but not eradicate *S.* Dublin from the herd. However, in another study by Nielsen and Nielsen (2011), ten herds enrolled in a control programme. They all changed or implemented new management routines to control *Salmonella* and nine of the ten herds managed to control *Salmonella* within an average of 13 months. The management changes mainly involved calving pen area, housing/management of young calves and culling of suspected carriers. Jensen et al. (2004) reported on a control programme for *S.* Dublin including 6 dairy herds. The control programme was mainly based on implementing management changes of calving and young calves. The risk of being seropositive fell from 24.8% to 2.2% for cows and from 34.7% to 1.3% for heifers over three years which is indicative of effective control of *S.* Dublin.

The KCAC has encouraged farmers to control *Salmonella*. This has been done through several initiatives such as knowledge dissemination and experience groups, information via newsletters and meetings. However, it is still the herd owner's responsibility to undertake a control programme. A manual is available for farmers and advisors to assist farmers when performing systematic risk scoring to detect open transmission routes within the herd and determine an action plan (Nielsen and Nielsen, 2007).

In addition to this, there is a need for studies on which management routines need to be implemented or avoided in herds trying to control *Salmonella* including larger study populations.

3 Animal health economics

3.1 Definition and concepts

3.1.1 Epidemiology and animal health economics

Animal health economics (AHE) is a field that combines epidemiology which is a natural science and economics which is a social science. Economics can be defined as the study of how people make choices under conditions of scarcity and of the results of these choices for society such as human wellbeing (Frank and Bernanke, 2007). Disease in farm animals is an economic problem relating to efficient use of resources (McInerney, 1996). The underlying problem for an animal health economist is that there is scarcity of resources and this makes it impossible to do all activities at every level that everyone wants (Rushton, 2009). AHE is the area of economics that applies the principles and methods of economic analysis to animal health problems and the role of AHE is to analyse the consequences of a change, e.g. control efforts like introduction of vaccination or to make a judgement on how desirable such a change would be (Mlangwa and Samui, 1996).

3.1.2 Disease effect and control

Disease in an animal population will affect the transformation of resources into products (Dijkhuizen et al., 1995), decrease the output and thereby waste scarce resources (Figure 3.1).

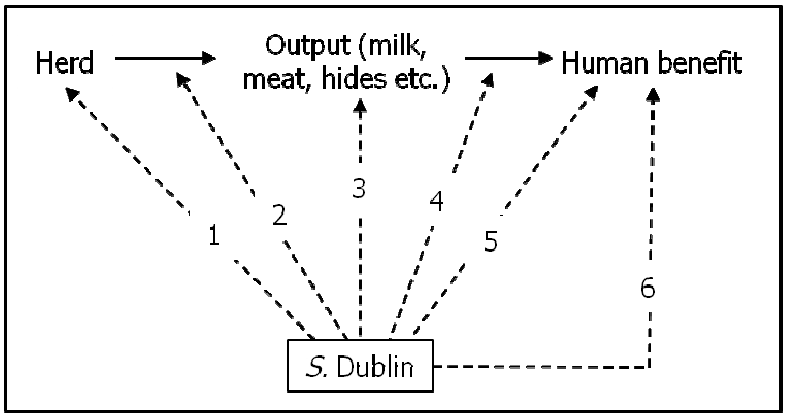

Figure 3.1 Economic implications of *S* Dublin: 1: destroys basic resources, 2: lowers efficiency of production process, 3: reduces physical output or value of this, 4: lower products suitability for processing or create additional costs, 5: affect human wellbeing directly and 6: reduce value of society gains from livestock. Based on McInerny (1996).

Control efforts are implemented to counteract this waste of resources. Hence, disease in an animal population can decrease the output level as well as increase the input level (e.g. medication, farmer's time, extra feed) (Bennett, 2003). The extent to which a disease should be controlled from an AHE perspective is limited by the marginal returns. Disease control should be increased until the marginal benefit of the control equals the marginal cost for disease control (Dijkhuizen et al., 1995). That is when the extra benefit of control equals the extra cost of control (Tisdell, 2009). This means that it is not always financially beneficial to eradicate the disease but other factors, such as legislation, animal welfare or export considerations might influence the decision to eradicate a disease.

Figure 3.1 can be used at the level of the individual herd, country, region or even worldwide. If viewed at herd level, the framework illustrated by McInerny (1996) suggested that there are several economic implications of *S.* Dublin in a Danish dairy herd. Destruction of basic resources can happen through mortality of infected animals and abortions, lowering of the efficiency of the production process e.g. through reduced weight gain of affected calves and reduction of physical output, e.g. through decreased milk yield.

Total costs of disease in an animal population are the sum of losses and control expenditures (McInerney et al., 1992; Rushton et al., 1999). Losses can be defined as missed benefits (e.g. discarded milk or reduced milk yield due to disease), which are the direct effects caused by the disease in the production system. Expenditures are the extra resources utilised as a consequence of the disease (e.g. veterinary fees, disease control measures etc.).

3.2 Modelling approaches

Estimating disease effects and cost-benefit potential of control strategies can be done by controlled intervention studies. However, these can be time consuming, expensive and difficult to perform, since it can be difficult to control other factors that could influence the results. Normative modelling is an alternative to this which is cheaper, faster and where other factors can be controlled. When modelling animal disease in AHE, it needs to be considered which type of model to use, since several different types are available. Dynamic models can simulate effect over time, while this is not possible in static models that do not contain a time variable (Dijkhuizen et al., 1995). Deterministic models generally use fixed input parameter values and generate point estimates of outcome as opposed to stochastic models that incorporate random variation in processes or parameters and produce probability distributions of the outcome (Bishop, 2010). Stochastic models can thus incorporate uncertainty and variability. Furthermore, a choice has to be made between optimisation versus simulation models (Dijkhuizen et al., 1995). Optimisation models identify a solution to a problem within a system that is optimal with respect to a set objective. A pre-defined set of input variables (a plan) is used in simulation models, which then determines the outcome (Rushton et al., 1999). Simulation models are appropriate when the system under study involves highly dynamic

relationships (possibly over many time periods) and contains many subsystems that cannot easily be controlled and studied simultaneously, such as biological systems and processes. Computer simulation models are also referred to as mechanistic models (Dijkhuizen et al., 1997).

3.2.1 The Simherd model

The "Simherd" model has been developed at Institute of Animal Science, Aarhus University and it is a dynamic, stochastic and mechanistic Monte Carlo model that simulates the dairy herd (including young stock) in time steps of one week. It was originally developed to simulate and analyse production as well as animal health in dairy herds and it incorporates the complex feedback mechanisms between replacement, culling and feeding (Østergaard et al., 2000; Østergaard et al., 2003). Discrete events (e.g. oestrous detection, conception, foetal death, sex and viability of the calf, disease as well as involuntary culling and death) and individual variation at cow level (such as milk yield) are triggered stochastically using random numbers from relevant distributions (Østergaard et al., 2003). Thus, for each time step of one week each animal is allocated different states. For a cow this can be level of milk yield, being in oestrus if non-pregnant or being culled etc. Different management scenarios can be simulated by changing model input parameters. The model has subsequently been developed to include other diseases, such as pathogen specific mastitis, paratuberculosis and recently *S.* Dublin (Østergaard et al., 2005; Kudahl et al., 2007; Nielsen et al., 2012). The "Dublin-Simherd" model is described in more detail in Manuscript 4 including how the effects of *S.* Dublin were incorporated into the model.

3.3 Animal health economics in this thesis

This thesis includes investigations of all parts of the biological model presented in Figure 3.2. The first three manuscripts lie within one or more of the elements of the biological model, and this should provide information to improve the livestock disease information constraints which limits application of economic models (Bennett, 2003). In contrast to the first three manuscripts, Manuscript 4 is placed within the economic part. The Dublin-Simherd model, which is used as the economic model in this PhD project, includes costs related to treatment of *S.* Dublin infection and the replacement of animals but relates mainly to losses for *S.* Dublin infected dairy herds that are recently infected and where there is spread of the pathogen. Key disease control measure costs associated with e.g. management changes or test-strategies were not included and assessed in the Dublin-Simherd model in this project. Hence, issues relating to part 1, 2 and part of 3 and 4 in Figure 3.1 will be addressed, in essence that is what can be described as direct impact of disease (McInerney, 1996). No attempts will be made on estimating the effects of human wellbeing (part 5 and 6 of Figure 3.1) or any other intangible values which also can be a part of an economic analysis. Hence, money is the only reported utility from the analyses performed in this project.

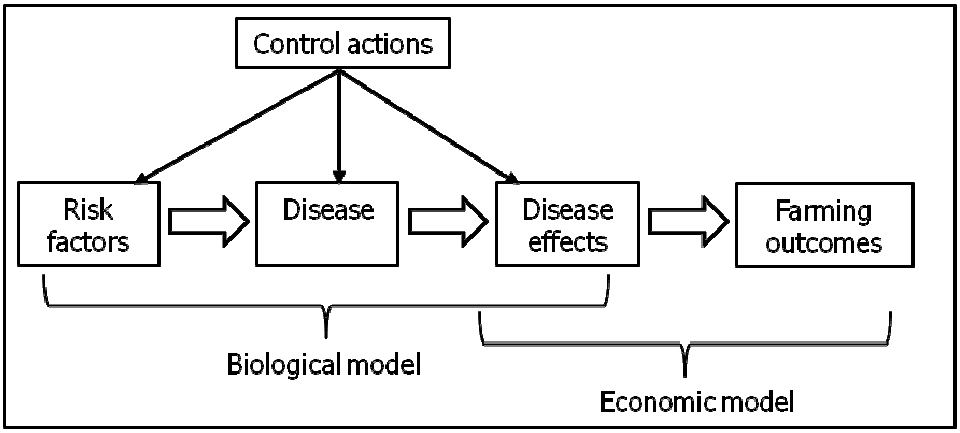

Figure 3.2 From McInerney (2001) Basic components of a model for animal health economics.

4 Materials and Methods

Three different datasets were collected in order to pursue Objectives 1 to 3. In Objective 1, mainly effects of endemic *S.* Dublin were evaluated, while in Objective 2 the effect of introduction and subsequent spread of *S.* Dublin was evaluated. Data for these two objectives were gathered from The Central Husbandry Register (CHR) and The Danish Cattle Database (DCD). To pursue Objective 3, a questionnaire study was performed through telephone interviews and blood samples were tested for *S.* Dublin antibodies. For Objective 4, a simulation study was performed partly based on results from Objective 2 and logical reasoning from Objectives 1 and 3 as well as literature. This chapter provides an overview of the data sources and methods used for the study objectives addressed in this thesis.

4.1 Databases

Large amounts of register data are available for the Danish cattle population. The Danish dairy cattle population included in 2010/2011 4,138 herds, of which 422 herds were organic (Danish Milk Board, 2011). The average herd size was 127 cows in the autumn 2010 (Videncentret for Landbrug, 2011a), and the overall yearly mean yield per cow was 9,308 kg energy corrected milk (ECM) (Videncentret for Landbrug, 2011b). Danish Holsteins was the most frequent breed and accounted for 73% of the dairy cattle population in 2009 (Anonymous, 2009).

4.1.1 Central Husbandry Register

The CHR is owned by the ministry of Food, Agriculture and Fisheries and is the central database for registration of animals and holdings (The Danish Veterinary and Food Administration, 2011b). The register is public and contains herd identification numbers, herd owners' and their veterinarians' names and addresses, the size and the type of the herds, location of the herds and *S.* Dublin status in the *Salmonella* surveillance programme. Furthermore, all births, deaths and animal movements are recorded at animal level (Nielsen, 2011). This is possible because all cattle must be ear tagged with an ID-number within 20 days of birth and before leaving the farm of birth (The Danish Veterinary and Food Administration, 2011a).

Validation of the register data includes automatic control systems with procedures including follow-ups on missing, inconsistent or late notifications. Farmers will be asked to correct data and may face legal actions if they fail to do so (The Danish Veterinary and Food Administration, 2011a).

4.1.2 Danish Cattle Database

The DCD contains a large amount of data from different sources (Figure 4.1). In Table 4.1 it can be seen what type of data and who is responsible for recording this. Calvings as well as movement (including culling and slaughter) of animals are required by law to be reported and the farmer is responsible for this. Data in the DCD consist hence of both public data (from the CHR register) and data owned by the farmer (e.g. production and

slaughter data). To access the private data for e.g. research, an agreement has to be obtained from the farmer.

Control points are also here used in data validation, among other things the control ensures that there is no registration of disease or calving of a cow that was not present in the herd on the date of the event and that two calvings by the same cow is not recorded in a period of less than 243 days (Bundgaard, 2005). Printouts of errors are sent to the persons/units reporting it, who are then responsible for correcting them.

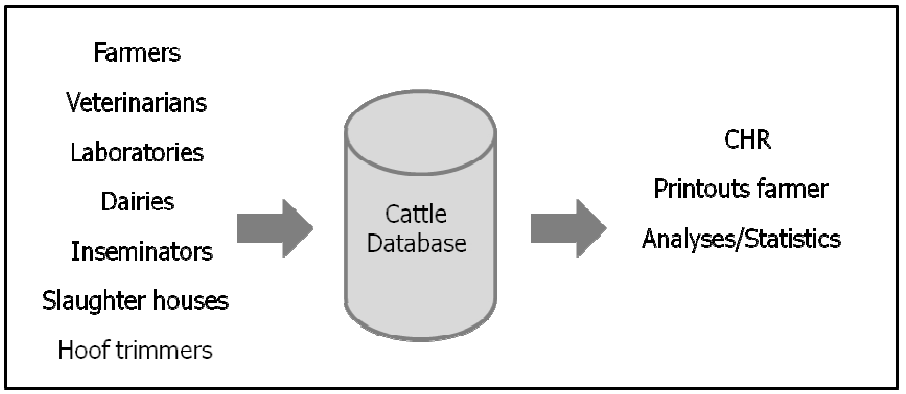

Figure 4.1 Data input sources for the Danish Cattle Database. Data are automatically transferred to the Central Husbandry Register (CHR), but can furthermore be used by researchers as well as farmers who can access data from their own farm. Modified from Bundgaard (2005).

4.2 Studies

Description of the four studies included in this thesis can be seen in Table 4.2, including objective, study design, data sources and methods of analysis.

In brief, the first 3 objectives were pursued by epidemiological observational studies of either the cross-sectional or case-control study designs. Objectives 1 and 2 were based solely on register data, whereas Objective 3 included additional data collection. Objective 4 was pursued by theoretical modelling using simulation.

Table 4.1 Information registered in the Danish Cattle Database and who is responsible for the registrations. Modified from Bundgaard (2005).

Information	Responsible
Calving and status of calf[a] Gender[a] Breed[a] Parents[a] Movements into/out of herd[a] Culling[a] Service by bull Drying off date Body condition score Weight recordings	Farmer
Milk yield	Electronically/Technician/Farmer[b]
Insemination Pregnancy test	Inseminator/Farmer
Disease Treatments Death/Euthanasia[a]	Farmer/Veterinarian/Hoof trimmer
Milk/blood analysis results	Laboratories
Slaughter results	Slaughter houses
Milk delivered	Dairies
Animal show results	Officials

[a]Required by legislation [b]Most often registered electronically or by technician.

4.2.1 Sampling considerations

The target population for this PhD thesis was Danish dairy herds. All herds with more than 20 cows in August 2008 were included in Objective 1, whilst smaller study populations were used for Objectives 2 and 3. In total, 46 herds were eligible to be included in the study for Objective 2 (i.e. had BTM *Salmonella* antibody increase) between January 2005 and December 2009. The largest group of these herds was selected (conventional farming, Danish Holstein) resulting in 28 case herds included in this study. For Objective 3, study herds were selected based on delivering calves raised for slaughter to 21 veal calf herds included in a pilot study. Furthermore, they had to be classified as level 2 (or 3) in the *Salmonella* surveillance programme. Based on these two selection criteria, 88 herds were eligible to be included and of these 86 agreed. By the end of the study, there were blood test and questionnaire results from 84 herds. Hence, in both these studies all herds fulfilling the inclusion criteria were evaluated to potentially be included in the analyses.

The study unit for Objectives 1, 3 and 4 was the herd, and for Objective 2 it was cow.

Table 4.2 Overview of objectives, study designs, materials and methods used in this thesis.

Obj. #	Objective description and study design	Data sources and types	Method of analysis	Comments
1	Association between *S.* Dublin antibodies and calf mortality at herd level. *Study design:* Register-based cross-sectional study.	**Data:** DCD calf mortality data at herd level calculated for the period 01.08.2007-01.08.2008 for all dairy cattle in Denmark (n=4,488). For further analysis data from 4,315 dairy herds with at least 20 cows in August 2008 were used. **Outcome variable**: Dichotomised calculated calf mortality (high ≥ 6.5%, low < 6.5%). (6.5% was selected by KCAC as the target in a calf mortality reduction campaign). **Risk factors:** *S.* Dublin test status from the *Salmonella* surveillance programme, herd size, main breed in the herd, cattle herd density, purchase pattern, production type (organic or conventional).	Multivariable logistic regression analysis with backward stepwise elimination of confounding variables using 1% significance level.	All details provided in Manuscript 1. Days at risk of dying for calves is explained in 4.2.2.1 and Figure 4.2
2	Changes in milk yield following *S.* Dublin BTM antibody level increase. *Study design:* Register-based case-control study.	**Data:** DCD cow test day ECM for 28 Danish Holstein case herds with an increase in BTM *S.* Dublin level and 40 Danish Holstein control herds with consistently low BTM antibody level. **Outcome variable:** Test day ECM before and after estimated herd infection in case herds. **Risk factor:** Increase in *S.* Dublin BTM antibody level. **Confounding variables:** Season of test day ECM, year, days in milk, log to somatic cells count, Wilmink's function, herd size, time from estimated herd infection.	Multilevel model using iterative generalized least square means procedure. Variables selected by forward selection using 5% significance level. The lactation curve was modelled by days in milk and Wilmink's function: $\exp(ECM)^{(-0.05*DIM)}$ (Wilmink, 1987).	All details provided in Manuscript 2.

3	Association between management and *S.* Dublin control in dairy calves. *Study design:* Observational cross-sectional study.	**Data:** Blood test results from 84 dairy herds in autumn 2009 were used to determine if herd managers were successful in controlling *S.* Dublin exposure of calves. A telephone interview was used to collect information of management practices. **Outcome variable:** Successful control of *S.* Dublin exposure of calves between three and six months of age in autumn 2009 (Success = no calves antibody level ≥ 50 ODC%, no success = one or more calves antibody level ≥ 50 ODC%). **Confounding variables:** Management routines included in the interview and herd demographics.	Multivariable logistic regression analysis with backward stepwise elimination of confounding variables using 5% significance level. Discriminant analysis using stepwise selection and inclusion criteria set at 15% significance[a].	All details provided in Manuscript 3 and below for discriminant analysis. The questionnaire in Danish and English provided in Appendix 1.
4	Estimate economic effects of introduction and spread of *S.* Dublin in dairy herds. *Study design:* Simulation study.	**Data:** Input parameters based on literature studies including results from Objectives 1, 2 and 3 as well as expert opinions. **Outcome variables:** Yearly estimated gross margin per cow stall and milk yield for the first ten years after herd infection.	Simulation using an age-structured stochastic, mechanistic and dynamic model of *S.* Dublin in dairy herds (Nielsen et al., 2012).	All details provided in Manuscript 4 and 4.2.2.2.

[a] Discriminant analysis is a method to investigate if two groups are different and if independent variables can be used to develop a prediction equation (Sharma, 1996). Discriminant analysis conducts F-tests to decide which variables are significant to differentiate between groups (Li, 2006). It places several assumptions on data such as multivariate normal distribution of variables but performs quite well when the majority of variables are dichotomous (Lachenbruch, 1975)

4.2.2 Specification and definitions of variables

Most variables included in the studies were clearly defined in the databases. However, the calculation of calf mortality used as the outcome variable in Objective 1 is illustrated below. Furthermore, use of Objectives 1 to 3 in Objective 4 is described briefly in 4.2.2.2.

4.2.2.1 Calculation of calf mortality for Objective 1

Calf mortality was calculated as a function of dead calves and number of calf-days at risk in the study period by as specified in equation 1. Explanation of calf days at risk can be seen in Figure 4.2.

$$\text{Calf mortality}_{1\text{-}180} = 100*(1 - \prod_{i=1}^{180}(1 - \frac{D_i}{B + I[<180] - D^{i-1} - C^{i-1} - E\ [i>1]})) \qquad \text{Eq. 1}$$

$\prod_{i=1}^{180}$ is the product of days alive *i* from *i* = 1 to 180 days of '1-D/N' and gives P(survival day 1:180).

D_i is number of dead or euthanized calves on day *i*:
The denominator was number of calves at risk of dying on day *i*:
B is number of live born calves in the study period.
I[<180] is number of calves introduced to herd before 180 days of age.

From this was subtracted calves that died, were euthanized in the herd or were censored before the start of day *i*:

D^{i-1} is the sum of dead calves including day *i* -1, if D^0 =0 then *i* =1.
C^{i-} is the sum of censored calves including day *i* -1, if D^0 =0 then *i* =1.
E(i>1) is the number of calves euthanized as newborn which were not deducted until day *i* =2.

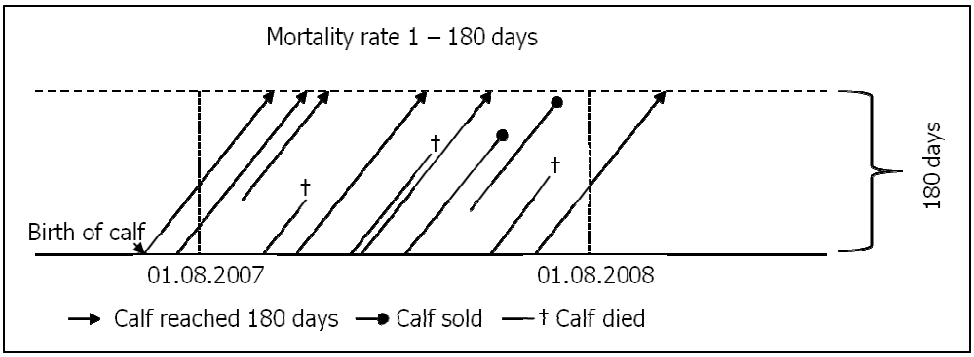

Figure 4.2 Calf days included in the calf mortality calculations for dairy herds in 2008. Calves born before 01.08.2007 were only considered at risk of dying after this date. Similarly, calves in the herd at the end of the study were only considered at risk until 01.08.2008.

4.2.2.2. Variables for Dublin-Simherd model

Milk yield was modelled for 18 months after estimated herd infection in Manuscript 2. There were subsequent indications that the herd infection date could be set at an earlier date than was the case in Manuscript 2. Hence, a new estimated date of herd infection was set and milk yield in infected herds was calibrated for 24 months after this date for the Dublin-Simherd model.

Calf mortality parameters in the Dublin-Simherd model were based on literature which included results presented in Manuscript 1 (Nielsen et al., 2012). Likewise, the management practices identified in Manuscript 3 were used when estimating herd hygiene levels, indicating infectious contact parameters. They were not included directly in the estimation of parameters in the Dublin-Simherd model but did support the estimates.

5 Results

The main results from the work conducted during this PhD project is presented according to the four studies (Figure 5.1).

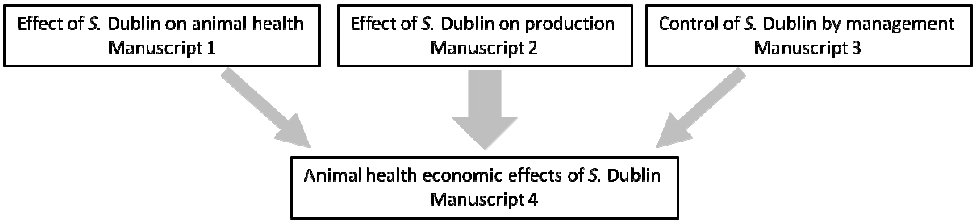

Figure 5.1 Sub-projects included in this PhD project.

5.1 Association between *S.* Dublin and calf mortality

A total of 4,315 Danish dairy herds were included in this study. In August 2008, 14.3% of Danish dairy herds were considered possibly infected or confirmed infected (level 2 and 3 in the *Salmonella* surveillance programme). The national average calf mortality was found to be 8.6%, and in 11% of the herds, calf mortality of 0% was recorded in the period August 2007 to August 2008. It was found that *S.* Dublin test status in the *Salmonella* surveillance programme was significantly associated with calf mortality when taking into account other risk factors such as herd size, main breed in the herd, cattle herd density, purchase pattern, production type (organic or conventional). Herds categorised as level 2 or 3 in the *Salmonella* surveillance programme had an odds ratio (OR) of 2.0 (95% CI: 1.7-2.4) of having high calf mortality (≥ 6.5%) compared to level 1 herds. Evaluation of the effect of *Salmonella* on calf mortality at population level showed that the population attributable risk (PAR) was 2.2% meaning that if all herds changed to level 1, the proportion of herds with high calf mortality would only be reduced from 38.7% to 36.5%. The low PAR is due to the low proportion of herds in level 2 and 3. The population attributable fraction was 5.6%, meaning that 5.6% of herds had high mortality due to some herds being categorised as level 2 or 3 in *Salmonella* surveillance programme.

5.2 Effects of *S.* Dublin on milk yield

Results from this study showed that first parity cow milk yield was reduced by on average 1.4 (95% CI: 0.5 to 2.3) kg ECM/cow per day from seven to 15 months after the estimated herd infection date, compared with first parity cows in the same herds in the 12 month-period before the estimated herd infection date. Milk yield for parity 3+ was reduced by on average 3.0 (95% CI: 1.3 to 4.8) kg ECM/cow per day from seven to 15 months after herd infection compared with parity 3+ cows in the 12 month period before the estimated herd infection. In contrast to this, only minor differences in yield in second parity cows before and after herd infection was found.

Analyses of test day ECM before and after estimated date of herd infection resulted in the same variables being associated with test day ECM for all parities. However, the milk yield varied between parities in different time periods compared to estimated date of herd infection (Figure 5.2).

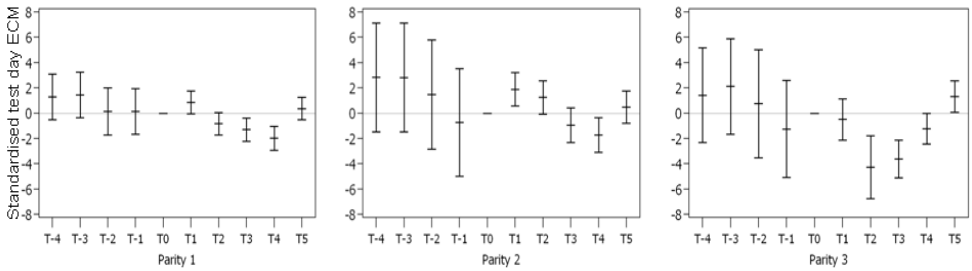

Figure 5.2 Standardised test day energy corrected milk yield (ECM) in 28 Danish Holstein herds before and after estimated herd infection time point (T0). T0 is zero to three months after estimated herd infection, T1 is four to six months after infection, T-1 is one to three months before infection etc. The bars represent 95% confidence interval of the standardised milk yield.

5.3 Management practices associated with control of *S.* Dublin in calves

The two methods of analysis used for this objective identified different management practices associated with successful control of *S.* Dublin in calves (Table 5.1). However, purchase of animals from test-positive herds was identified as the variable with the highest coefficient by both methods, i.e. it was most strongly associated with lack of successful control to purchase cattle from a test-positive herd.

Table 5.1 Management practices found to be associated with successful prevention of *S.* Dublin exposure of calves in Danish dairy herds in 2008 to 2009.

Variable describing management practice	Found to be associated with probability of successful control by:		Comments
	Logistic regression analysis[a]	Discriminant analysis[b]	
Purchase of animals from test-positive herds	+	+	Purchase associated with decreased probability of success
Calving area management[c]	+	n/a[d]	Poor calving area management associated with decreased probability of success
Separation of pre-weaned calf pens	+	-	Separation by bars rather than by solid walls associated with decreased probability of success
Biosecurity routines between barns	+	-	Biosecurity routines between barns associated with decreased probability of success
Number staff responsible for colostrum handling	-	+	More than one person responsible associated with decreased probability of success
Number cows calved before moved to calving area in the past 12 months	-	+	More than four cows calved before moved to the calving area associated with decreased probability of success
Poorer quality colostrum for bull calves than for heifers	-	+	Poorer quality colostrum used for bull calves associated with decreased probability of control

[a]Based on 84 herds. [b]Based on 81 herds. [c]Acceptable calving area management generally included: one person responsible for calving and colostrum handling, allowing a maximum of four cows in the calving area at any time, not using the calving area for sick animals, applying new bedding in calving area at least once a week, cleaning calving area at least twice a month and allowing a maximum of four cows to calve before they were moved to the designated calving area during the previous year. [d]Not included in discriminant analysis

5.3.1. Validity and reliability of questionnaire data

Follow-up visits were performed in nine herds to estimate the validity of questionnaire responses in the interview. It was possible to evaluate nine questions from the

questionnaire by these visits (Table 5.2). These were the questions that could be answered by observations in the herd. Only one of the questions validated at the herd visits was found to be associated with successful control in the two analyses and this was: 'Separation of pre-weaned calf pens'. For this question, the herd owner's response in the telephone interview was different from what was observed at the consecutive herd visit in one of the nine visited herds.

Table 5.2 Management practices from questionnaire (Manuscript 3) validated at herd visit.

Management practice	Answer from questionnaire and herd visit same	Answer from questionnaire and herd visit differed
Barn type lactating cows	9	0
Barn type dry cows	9	0
Barn type heifers	9	0
Number of cows in calving pen at any time	8	1
Number calves in each pen/calf hut	9	0
How are pens/calf huts separated	8	1
Number of contact calves in pre-weaned area	6	3
Separate barn area for pre-weaned calves	7	2
Number of calves in a section	8	1

Reliability of the interview results was assessed approximately three weeks after the first interviews. To test the reliability, the telephone interview was repeated with nine herd owners/managers. Thirty of the 45 management questions included in the interview were answered differently in none or one case in the first and second interview by the nine herd owners who were interviewed twice. In seven questions, the nine herd owners answered differently between the two interviews in two or three cases. In one question, four of the nine herd owners answered differently in the two interviews. However, this was probably related to the question itself: 'Are any neighbour herds *Salmonella* positive'. All four herd managers had answered 'Do not know' in the first interview, but had found an answer for the second interview.

5.4 Economic effects of introduction and spread of *S.* Dublin

The Simherd model estimated the economic effects of *S.* Dublin herd infection as annual mean GM per cow stall for the 10 years after introduction of *S.* Dublin. Mean number of infected animals was highest in the first year after introduction of infection (Figure 5.3). The number of infected animals could be higher than the number of cow stalls in the herd due to: i) young stock acquiring the infection and ii) individual animals becoming

infected more than once in a year. The number of infected animals was higher and the infection persisted for longer in the herd in larger herds and decreasing quality of management than in smaller herds and herds with better management. The ECM milk yield per cow was lower in the *S.* Dublin infected herds compared to the non-infected herds in the 10 years after infection (Figure 5.4). Milk yield was generally decreased for longer with larger the herd size and poorer management.

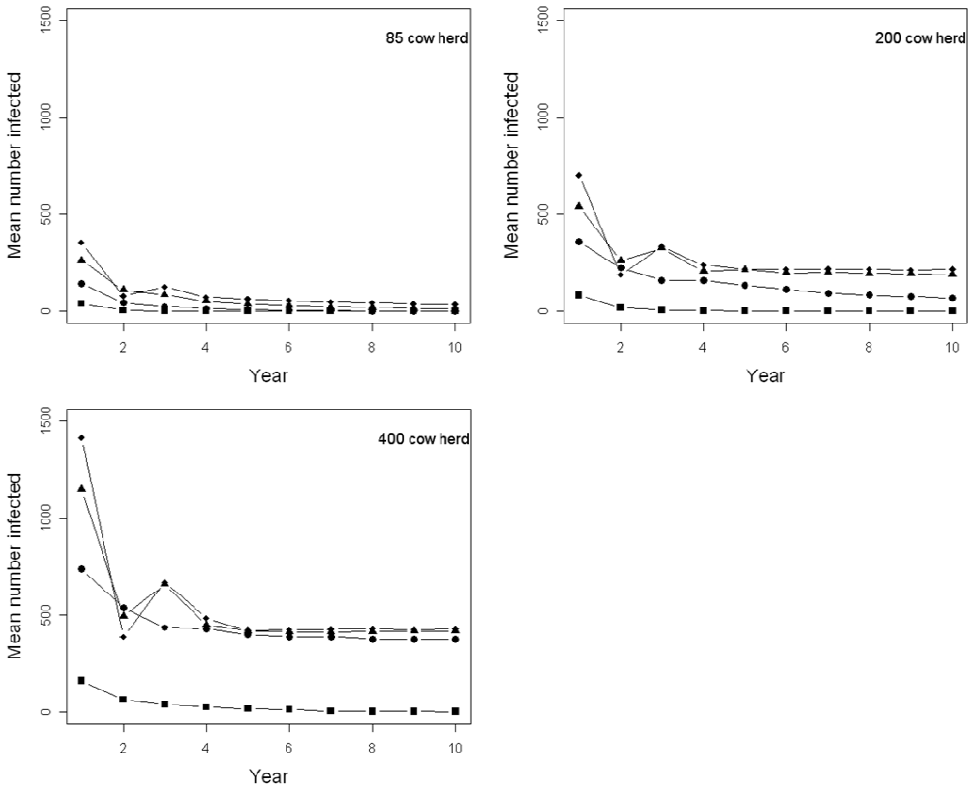

Figure 5.3 Model predicted mean annual infected animals in the 10 years following introduction of one infectious heifer with *S.* Dublin into 85, 200 and 400 cow stall herds. Estimates were derived from 1,000 iterations and results based on iterations where infection spread. ■ corresponds to very good, ● to good, ▲ to poor and ♦ to very poor management.

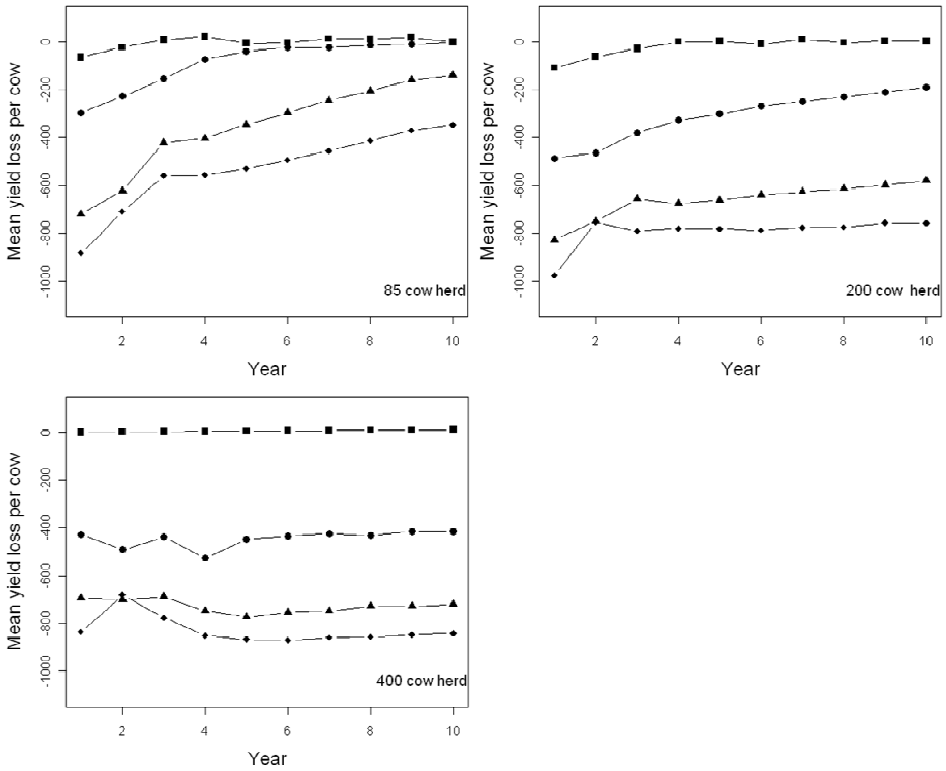

Figure 5.4 Model predicted difference between the annual milk yields (kg ECM) per cow stall (Mean yield loss per cow) in the 10 years following introduction of one infectious heifer with *S.* Dublin into 85, 200 and 400 cow stall herds compared to reference herds without *S.* Dublin infection. Estimates were derived from 1,000 iterations and results based on iterations where infection spread. ■ corresponds to very good, ● to good, ▲ to poor and ♦ to very poor management.

The averaged annual mean simulated GM per stall over 10 years for the reference herds without *S.* Dublin infection was 1,319 (5^{th}-95^{th} percentiles: 1,170 to 1,460), 1,370 (1,254 to 1,477) and 1,344 (1,266 to 1,417) Euros for 85, 200 and 400 cow stall herd, respectively. Simulation of herd infection resulted in different estimated losses in GM per stall depending on herd size and management level in the herd. Lower averaged losses in GM per stall were simulated for the 10 year period for *S.* Dublin in the 85 cow stall herd compared to the 200 and 400 cow stall herd (Figure 5.5). For very good management, GM differences reached 3 (-41 to 35), 9 (-35 to 16) and 12 (-43 to 11) Euros compared to non-infected herds for 85, 200 and 400 cow stall herd, respectively. For very poor management the losses per stall for the 85 cow stall herd were -164 Euros (-238 to -52) which was lower than losses for the 200 and 400 cow stall herds, where the losses reached -230 Euros (-272 to -197) and -232 Euros (-255 to -207), respectively.

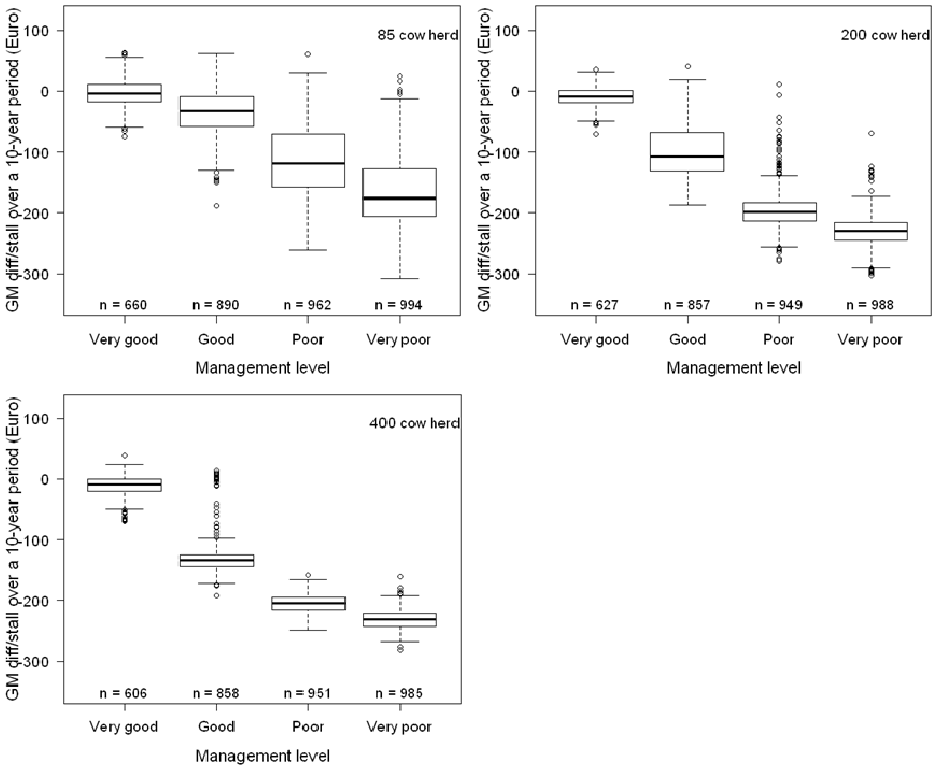

Figure 5.5 Averaged annual model predicted difference in gross margin (GM) per cow stall in Euros over the 10 years following introduction of one infectious heifer with *S.* Dublin into 85, 200 and 400 cow stall herds compared to reference herds without *S.* Dublin infection. Estimates were derived from 1,000 iterations and n represents the number of iterations where infection spread within the herd.

Losses in GM per stall were highest in the first year after infection (Figure 5.6). Losses in year 1 were highest in the 85 cow stall herd, but they declined faster than for the 200 and 400 cow stall herds. From year 2 to year 4, the GM losses per stall increased for poor and very poor management for 200 and 400 cow stall herds. This corresponded to the second peak in number of infected animals in these simulations (Figure 5.3).

The sensitivity analyses of the Simherd simulations showed that assumptions about milk yield losses in the resistant and carrier cows generally had the highest influence on GM (Figure 5.7). The worse the management levels the more influential this assumption was. Assuming milk yield effects to be 50% of the best estimate resulted in accumulated mean losses for the 10 years after herd infected of 18,000 Euros for very good management and 120,000 for very poor management. For very good management, only relative minor changes in GM effects were seen compared to best estimate regardless of the simulation scenario. Assuming no mortality in calves and heifers only had very minor effects on GM per stall compared to best estimate.

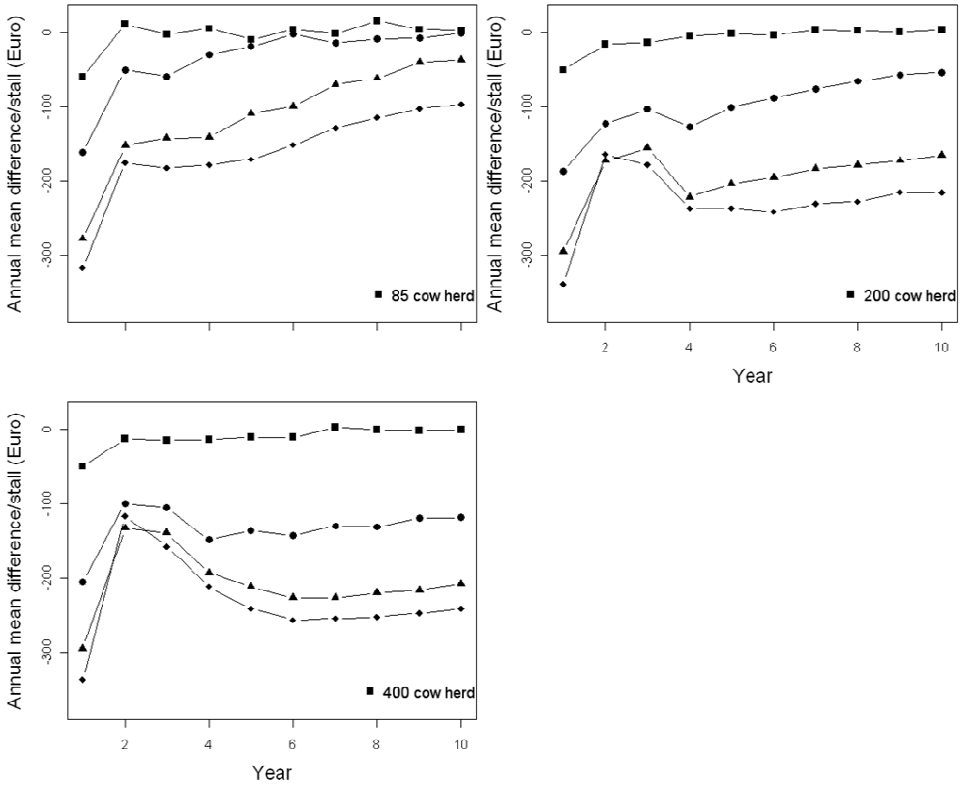

Figure 5.6 Annual model predicted difference in gross margin (GM) per cow stall in Euros over the 10 years following introduction of one infectious heifer with *S.* Dublin into 85, 200 and 400 cow stall herds compared to reference herds without *S.* Dublin infection. Estimates were derived from 1,000 iterations and n represents the number of iterations where infection spread within the herd.

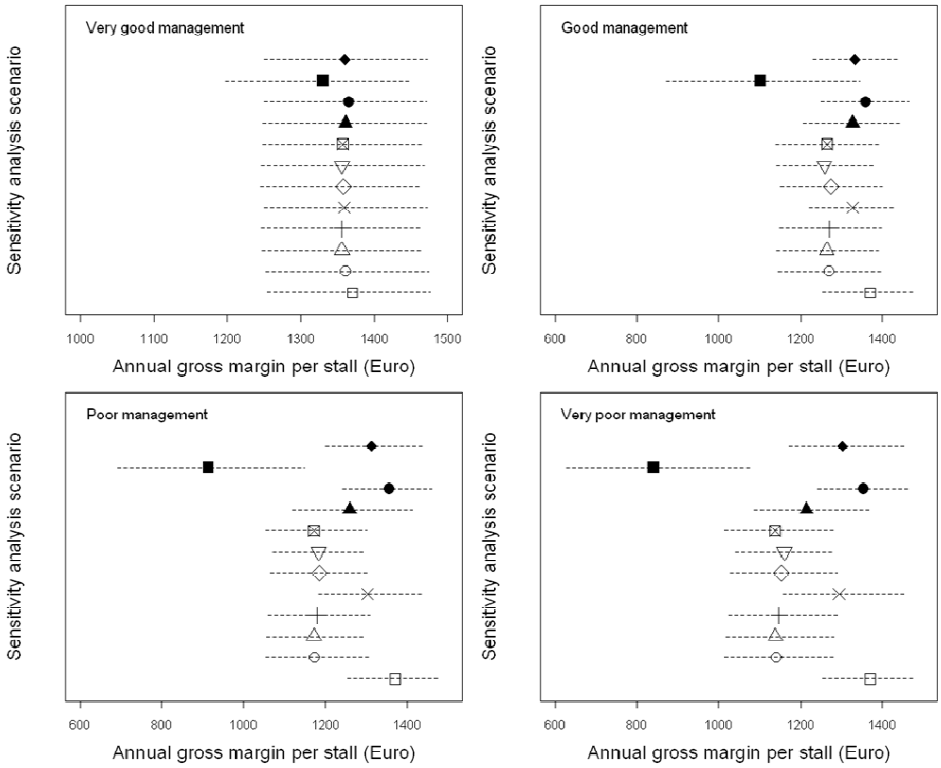

Figure 5.7 Sensitivity analysis results for model predicted annual 5th and 95th percentiles of mean gross margin (GM) per stall in Euros in the 10 years following introduction of one infectious heifer with *S.* Dublin into 200 cow stall herd. Estimates were derived from 1,000 iterations and results based on iterations where infection spread. □ is GM for uninfected herd, ○ best estimate (presented in Figure 5.5), Δ no milk loss in acutely infected and diseased, + no milk loss in acutely infected not diseased or supershedders, x no milk loss in resistant or carriers, ◊ no abortions, ∇ no mortality in calves/heifers, ☒no mortality in cows ▲no clinical effects of infection,● all effects reduced, ■ all effects increased, ♦ all milk yield effects reduced by 50%.

6 General discussion and conclusions

6.1 Discussion

The main aim of this PhD project was to investigate animal health economic consequences of *S.* Dublin in dairy herds. *S.* Dublin's effects on calf mortality and milk yield as well as control elements for the infection were investigated. Farmers often report that they do not notice any effects of *S.* Dublin in the herd. In combination with farmers often having to bear the cost of the control actions this makes them unlikely to comply with recommendations to control the infection on their own accord as planned by the centrally organised eradication programme (Andersen and Christensen, 2008). Results from this project showed that there is an effect of *S.* Dublin infection of in many dairy cattle herds. It was found that *S.* Dublin BTM antibody positive herds had higher calf mortality than BTM antibody negative herds (Objective 1), milk yield decreased after *S.* Dublin herd infection (Objective 2) and high losses in GM per stall were estimated after introduction and within-herd spread of *S.* Dublin (Objective 4). Specific management practices (especially avoiding purchasing animals from *S.* Dublin antibody positive herds) were identified to be associated with preventing exposure of calves to *S.* Dublin (Objective 3). Dissemination of these results can be used to inform farmers and farmer's organisations of the potential benefits of controlling and preventing *S.* Dublin infection in dairy herds as well as identify management practices that potentially affect the success of control efforts in *S.* Dublin infected dairy herd.

6.1.1 Data quality and availability

In this project the antibody levels of either animals (Objective 3) or BTM (Objectives 1 and 2) were used to define whether a herd was considered infected or not. The *Salmonella* surveillance programme is based on BTM antibodies and has a relative low PPV between 0.47 and 0.88 with true herd prevalence below 30% (Warnick et al., 2006). Hence, it is likely that some herds have been included as infected with *S.* Dublin in the studies when in fact they were not (i.e. misclassification bias). Furthermore, some herds that were regarded *S.* Dublin infected might have been infected with other serotypes of *Salmonella.* The ELISA most often used in Denmark identifies LPS O-antigens and can react with all *Salmonella* serotypes carrying these antigens. This could bias the results, but it is most likely that non-infected herds have been included in the studies as presumably infected, than the other way around. Hence, it is likely that effects have been diluted and that estimates of *S.* Dublin effect on calf mortality and milk yield are conservative. In Objective 2, the inclusion criteria for infected herds were much stricter than for the level 2 classification of herds in the *Salmonella* surveillance programme. The risk of misclassification of the herds is therefore assumed to be lower than in the *Salmonella* surveillance programme. Traditionally, faecal culture has been used as the gold standard for identifying infected animals and herds. However, due to the intermittent and low excretion of bacteria from non-clinical animals, the sensitivity of this method has been estimated as low as 5-17% (Nielsen et al., 2004; Nielsen et al., 2011a). Hence, antibody measurements are assumed to be the preferred method of identifying infected animals and herds for studies like these.

Register data were used for Objectives 1 and 2. Register data are cheap and readily available since they are already collected. However, they cannot provide detailed information of e.g. management practices used in the herd. It was possible to include nearly all Danish dairy cattle herds in Objective 1 due to the use of register data. In contrast to this, only few herds fulfilled the inclusion criteria for Objective 2 and were included in this study. Detailed information of management practices and *S.* Dublin antibody status of calves were necessary to answer Objective 3 and hence, new data were collected for this study. The 84 herds included in this study resulted in wide confidence intervals for parameter estimates of management practices found to be significant by logistic regression modelling in this study. This indicated that a larger number of observations (i.e. more herds) would increase the confidence in the results.

Cross-sectional studies were used in Objectives 1 and 3. In these studies the prevalence of the outcome can be compared across sub-populations with different exposure status although it is usually not possible to determine causality (Ersbøll et al., 2004). Simulation was used for Objective 4 in order to estimate selected economic effects of *S.* Dublin herd infection and compare these effects for different herd sizes and under different management conditions. This would not have been feasible by observational or register data studies.

The study population in Objective 1 included most of the target population (i.e. Danish dairy herds). In Objective 3, the study population was only 84 herds, but these included the most common breeds in Danish dairy herds (Danish Holstein and Jersey) as well as both organic and conventional herds. These two study populations are therefore expected to be representative of the target population. In contrast to this, only conventional Danish Holstein herds were included in Objective 2 and results from this study were used for Objective 4. Conventional Danish Holstein herds is the most commonly found type of dairy herd in Denmark (Dansk Kvæg, 2009), but care should be taken interpreting the results for other herd types e.g. Jersey or organic herds.

Other *Salmonella* serotypes than *S.* Dublin might cross-react with the ELISA-test used in the *Salmonella* surveillance programme (Konrad et al., 1994). In Denmark this would most often be *S.* Typhimurium. However, *S.* Dublin was the most frequently isolated serotype from Danish beef in 2010 with more than twice as many isolates than *S.* Typhimurium (Anonymous, 2011) and *S.* Dublin has the potential to remain longer in cattle herds than other serotypes (Boqvist and Vågsholm, 2005, Nielsen et al., 2011b). It is therefore reasonable to assume that the majority of the *Salmonella* infected herds in the studies included in this thesis were infected with *S.* Dublin.

6.1.2 Effects of *S.* Dublin in dairy herds on health and production

It was hypothesised that *S.* Dublin has an effect on animal health and production in dairy cattle herds. Several such effects have been reported, e.g. diarrhoea, pneumonia and death in calves and adult cows (Vandegraaff and Malmo, 1977; Greene and Dempsey, 1986) as well as abortion and decreased milk yield in cows (Vandegraaff and Malmo, 1977; Morton, 1996; Carrique-Mas et al., 2010), although few of the effects have been quantified and some of the studies are case reports rather than systematic analyses of the effects of *S.* Dublin. It was decided to concentrate on two effects in this project: 1) calf mortality that can affect animal health and welfare as well as the farmer's economy,

and 2) milk yield which is a production measure that influences the economy of the dairy farmer.

High BTM *S.* Dublin antibody levels were associated with high calf mortality (Manuscript 1). *S.* Dublin has been reported to be associated with calf mortality in several other studies (Hughes and Jones, 1973; Forbes et al., 1977; Gitter et al., 1978; Greene and Dempsey, 1986; Anderson and Blanchard, 1989; Morton, 1996). However, these studies reported from herds where animals displayed clinical signs. Hence, there would be selection bias if these studies were to demonstrate the effect in infected herds on average. The herds included in Manuscript 1 as *S.* Dublin infected were all dairy herds in level 2 and 3 in the *Salmonella* surveillance programme, i.e. the majority of these were expected to include few animals showing clinical signs of *S.* Dublin infection. Furthermore, it must be expected that the majority of *S.* Dublin infected herds in this study was endemically infected, although some would have been recently infected. This indicates that even endemic *S.* Dublin herd infection can affect animal health through increased calf mortality.

Data for the calf mortality study included 4,315 of 4,488 Danish dairy herds in August 2008. Most of the excluded herds were small herds with less than 20 cows (151 herds) and owners of these herds were probably not full-time dairy farmers. Hence, the management in these herds could vary widely and affect the calf mortality. Furthermore, with the small herd sizes, one or a few dead calves could result in a very high calculated mortality. Thus, data used in this study are very likely to represent Danish dairy herds well.

In contrast to *S.* Dublin's effect on calf mortality, effect on production was estimated in recently infected herds, i.e. herds with a sudden high increase in BTM antibodies. Decreased milk yield was observed from seven months to 15 months after estimated herd infection for first and third or higher parity cows (Manuscript 2). Previous studies have reported decreased milk yield in cows displaying clinical symptoms of *S.* Dublin infection lasting days from onset of symptoms (John, 1946; Vandegraaff and Malmo, 1977). Bazeley (2006) reported that herd milk yield returned to pre-infected levels after a period of two months after the first clinical symptoms of *S.* Dublin were observed in the herd.

Since the yield losses were estimated at cow level in Manuscript 2, while the infection was determined at herd level, it is possible that effects on milk yield were not detectable until a certain proportion of the cows had been infected. Test day ECM was modelled as lactation curves, i.e. as a function of DIM and Wilminks' function. Another variable (T) was included in the model to investigate the effect of *S.* Dublin on milk yield by in three-monthly periods. This means that a certain proportion of the individual test day ECM observations in a three-monthly period had to be decreased, before the overall yield in this period was significantly lower than before estimated herd infection. It is possible that this influenced the time when decreased milk yield could be identified compared to estimated time of herd infection.

Another reason for the late milk yield effects of *S.* Dublin infection could be wrong estimation of herd infection date. In Manuscript 2, the infection date was set to the actual

date that the first very high BTM measurement was recorded minus 2 months. It is possible that the duration of time from introduction of infection of the herd to the increase of antibodies above 70 ODC% is in fact longer than that (Jordan et al., 2008). Data indicate that estimated herd infection date was estimated later than was actually the case, due to milk yield decreasing before estimated herd infection date. This would mean that milk yield in infected herds was compared to the wrong basis level and would possibly not show a reduction even if present. If the estimated herd infection date was estimated later than was actually the case and if, as the data indicate, the milk yield decreased earlier than modelled in Manuscript 2, the overall reductions in milk yield could have been higher than what was modelled.

Due to the fact that *S.* Dublin herd infection was defined based on BTM antibody level in Manuscript 2, it was not known if and when cows showed clinical symptoms of infections.

It is noticeable that estimated reduction in milk yield was much less in second parity cows compared to the other parities. Different management strategies in case and control herds could possible cause this pattern in milk yield. The ratio between first and second parity observations remained constant in control herds over time but decreased in case herds. This could indicate that farmers in case herds culled a larger proportion of parity 2 cows due to poor milk production and that this might explain why there appear to be less milk yield reduction after estimated herd infection in this parity compared to parity 1 and 3+.

Other confounding variables than was included in the study can influence milk yield. Age at first calving has been reported to affect milk yield in first parity cows (Ettema and Santos, 2004; Svensson and Hultgren, 2008). This was not included in the model and it is possible that this would have affected milk yield.

6.1.3 Control of *S.* Dublin

Current advice to farmers from the KCAC concerning how to control *S.* Dublin infection is focused on management; hence the hypothesis that *S.* Dublin can be controlled by management was investigated. It was evaluated which management practices were associated with controlling calves' exposure to *S.* Dublin. Calves younger than 6-8 weeks are highly susceptible to *S.* Dublin (Nazer and Osborne, 1977; Segall and Lindberg, 1991), which makes *S.* Dublin control particular important in this age group.

The results showed that not purchasing animals from herds that were test-positive in the *Salmonella* surveillance programme was the management routine most strongly associated with all calves being test-negative in the herd. Other studies have reported an association between purchasing animals and *S.* Dublin infection in the herd (Morton, 1996; Nielsen et al., 2007). Avoiding purchase of cattle has also been reported to be associated with control of *Salmonella* in both observational (Nielsen and Nielsen, 2011) and simulation studies (Bergevoet et al., 2009). It is likely that purchasing animals from *S.* Dublin infected herds could increase the infection load in the herds, making control of the infection difficult. Other management practices related to the calving area and management and housing of young calves were also found to be associated with exposure of young calves. Preventive practices included allowing a maximum of four

cows in calving pen, not using calving pen for sick animals, making sure that a maximum of four cows calved before being moved to the calving area, and separation of pens for pre-weaned calves with solid walls rather than bars. The mentioned practices prevent the calf's exposure to large groups of potentially infected animals or manure, which could expose the calves to *S.* Dublin.

It was not a specific purpose of this PhD to investigate the costs of control actions. However, farmers participating in the study for Objective 3 were asked how much time and money they spent on each specific management practice that was introduced in order to control *S.* Dublin (data not shown). Most farmers were unable to provide good information on how much of either time or money had been spent on control efforts, but often reported that very little money was spent. Even when changing stable systems for e.g. pre-weaned calves, farmers would often use old building material present on the farm and they did not feel that they had spent any money. This makes it difficult to estimate the costs of control actions implemented in the herd and will complicate future economic analysis involving control strategies but both time and money spent on control actions need to be considered.

Only 88 herds were eligible to be included in the study for Objective 3 and efforts were made to include all these in the study. Data collection on management practices were done by telephone interviews by the same experienced interviewer. This was the only possible way to get detailed information of management practices within the time frame. Data could also have been collected by postal questionnaires, but this would likely have resulted in fewer responses and longer response time than the telephone interviews. Improved data quality could have been expected by performing herd visits in all herds, but this was not possible due to time and financial constraints. However, the results of this study need to be validated by other studies, preferably with larger sample sizes.

The questionnaire used in Objective 3 was not pre-tested which could have improved the responses, but due to the small sample size, no observations could be "wasted" on pre-testing. Furthermore, the questionnaire was validated by herd visits and reliability interviews were performed. These did not reveal any major problems with reliability and validity of the collected data, although it was only possible to validate nine questions.

6.1.4 Economic effects of *S.* Dublin in dairy herds

Results in this study estimated higher economic losses than what has been reported in previous studies. The Dublin-Simherd model was calibrated to data estimating that milk yield was affected for up to 21 months after herd infection, and simulations estimated that milk yield was decreased even longer than this for many of the scenarios. Hence, milk yield was affected much longer than the two months that Bazeley (2006) used for estimating losses and this resulted in higher economic losses. Visser et al. (1997) included herds after isolating *S.* Dublin from samples, which means that they did not necessarily include recently infected herds like we simulated in this study. The longer estimated decrease in milk yield and the inclusion of herds immediately after herd infection would result in expected higher losses in Objective 4 than what has previously been reported.

Milk yield was calibrated for an 85 cow stall herd (mean herd size of herds included in Manuscript 2) and management level defined as poor in the Simherd model. It was not possible to assess the management of the herds in Manuscript 2, but it was assumed that since the BTM antibody levels increased steeply to a high level that the infection dynamics in these herds were close to what would be represented by poor management settings in the Dublin-Simherd model (Nielsen et al., 2012). Calibrations for the Dublin-Simherd simulations estimated that proportionate highest milk yield loss for the cow was in the acute infected stage, but that the quantitatively highest milk yield loss for the herd was observed in cows in the resistant stage, i.e. cows less likely to display symptoms. This was due to the short period a cow was assumed acutely infected compared to the longer period, it was assumed resistant. Unlike the observational study, the milk yield for the simulated herd in Manuscript 4 did not reach the pre-infected level of the herd within two years of herd infection. This again was due to the modelling of the yield in the resistant stage and the length of this in the Dublin-Simherd model. It is possible that this resulted in overestimation of the GM losses if the time period that yield losses occurred after herd infection was overestimated. On the other hand, it is likely that herd owners for herds included in Manuscript 2 would introduce control actions and hence reduce the time period that large effects of *S.* Dublin was observed. This cannot be assessed since data in Manuscript 2 were register data. In the simulations no actions were taken to control the infection during the simulation period.

The sensitivity analysis showed that milk yield was the most influential single factor on estimated GM losses due to *S.* Dublin and that it became more influential the poorer the management. It also showed that if the effects of *S.* Dublin on milk yield were overestimated by 50%, effects over 10 years of the infection would still be sizeable. There were no practically relevant changes in estimated GM losses if the model assumptions were changed to no calf and heifer mortality effect of *S*. Dublin. This was due to the costs of feed for young stock until they start producing milk. If calves or heifers died, these costs would be saved. The costs of raising and purchasing a heifer were similar in the Dublin-Simherd model. Secondary benefits of the farmer raising his own young stock and avoiding to purchase animals, such as no introduction of infectious diseases and genetic improvement, were not included in the model.

The Simherd model includes around 2,140 parameters that are used to design the virtual herd (Nielsen et al., 2012). This makes the model able to simulate real life in dairy herds. For example, the costs of replacing a cow is not a defined set cost, but rather the cost is included as foregone revenue of the herd owner either not being able to sell a pregnant heifer or having to purchase one. This younger replacement animal will in addition produce less milk and through this create less income for the farmer. Hence, the Simherd model is a realistic representation of real life dairy herds, but of course is highly dependent on being correctly specified with current market prices etc.

Results from Manuscript 4 can be used to inform stake holders of potential costs related to *S.* Dublin infection. In order to perform a full economic analysis of *S.* Dublin effects, effects on human welfare of the infection should be included. Even though few people are hospitalised on a yearly basis in Denmark due to *S.* Dublin infection (Anonymous, 2011), the increased risk of invasive disease and case fatality associated with this serotype (Helms et al., 2003; Jones et al., 2008) makes it a serious health risk and

therefore it has high impact on human wellbeing. Bennett and IJpelaar (2005) estimated *Salmonella* to have the highest impact on human welfare out of 34 endemic livestock diseases in cattle. Wells et al (1998) prioritised *Salmonella spp.* second highest after mastitis when ranking key dairy health concerns. The ranking was done based on production losses, animal welfare, zoonotic potential and international trade effects. They assumed only minor losses due to production losses, but due to the zoonotic potential, *Salmonella* was ranked high. Interestingly enough, no animal welfare effects of *Salmonella* were assumed in the ranking. Results from the studies included in this PhD has shown that *S.* Dublin has the potential to cause large production losses and affects animal welfare in addition to the effects on human wellbeing that other authors assume, which makes it even more important to control the infection in cattle herds.

6.2 Conclusions

S. Dublin is associated with compromised animal health and production in infected herds. *S.* Dublin is associated with high calf mortality and losses in milk yield. GM losses due to *S.* Dublin herd infection were estimated to be higher and longer lasting in large herds than in small herds although GM loss per stall was estimated to be highest the first year after introduction of infection in the small herd where very good management practices were implemented. Furthermore, it was shown that GM losses were higher in herds with poor management than in herds with good management suggesting that it is worth obtaining a high standard of external and internal biosecurity.

It was shown that it is possible to prevent calves from being exposed to *S.* Dublin through appropriate management practices such as avoiding purchase of cattle from test-positive herds, good calving management and separation of pre-weaned calves.

7 Perspectives

KCAC's goal of eradicating *S.* Dublin by the end of 2014 might be difficult to achieve due to the lack of compliance by some owners of infected herds. The results from this PhD project can be used as incentive to convince farmers that they would benefit from eradicating *S.* Dublin from their herd and help them select which management practices to pay special attention to in order to achieve this.

More detailed data are necessary to estimate economic effects of *S.* Dublin herd infection more precisely. Abortion rate in both recently and endemically infected herds is one of the effects where more data are needed. Milk yield effects of *S.* Dublin infection should also be estimated more precisely for the different infection stages of the cow. To estimate these things, more knowledge on the infection dynamics within the herd is necessary. This will require prospective studies with repeated testing of for example serum antibodies of individual cattle within the herd and will hence be both time consuming and expensive. The difficulty in performing studies that can provide sufficient data for such studies are evident from the Kongeå-project run by the Danish Dairy Board in 2000-2003. In that project, antibody levels of cows were tested monthly but this did not provide sufficient data to estimate the milk yield losses in different infection stages in the tested cows in that study.

Further studies on which management practices will control *S.* Dublin in the herd are needed in order to validate the results found this project. Again, it would be beneficial to test repeatedly to validate the effects of the control efforts.

Simherd simulations of control strategies should be performed to aid farmers trying to control *S.* Dublin. This could include test- and management strategies or test- and cull strategies. The results from this project have furthermore made it possible to simulate the control of *S.* Dublin and paratuberculosis simultaneously. These control strategies could be included in cost-benefit analyses for the dairy sector, which could evaluate the economy of controlling *S.* Dublin for the whole dairy sector as well as economic analyses for Denmark. The results from this PhD can be used in future economic analyses which represent the entire economy such as input-output models or computable general equilibrium models (Rich et al., 2005) as well as to motivate farmers to control *S.* Dublin.

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9 Manuscripts

Manuscript 1
Nielsen, T. D., L. R. Nielsen, N. Toft, and H. Houe. 2010. Association between bulk-tank milk *Salmonella* antibody level and high calf mortality in Danish dairy herds. Journal of Dairy Science. 93:304-310.

Manuscript 2
Nielsen, T. D., L. E. Green, A. B. Kudahl, S. Østergaard, and L. R. Nielsen. 2012. Evaluation of milk yield losses associated with *Salmonella* antibodies in bulk-tank milk in bovine dairy herds. Submitted to Journal of Dairy Science.

Manuscript 3
T. D. Nielsen, I. L. Vesterbæk, A. B. Kudahl, K. J. Borup and L. R. Nielsen. 2012. Effect of management on prevention of *Salmonella* Dublin exposure of calves during a one-year control programme in 84 Danish dairy herds. Preventive Veterinary Medicine. In Pres: doi:10.1016/j.prevetmed.2012.01.012.

Manuscript 4
Nielsen, T. D., A. B. Kudahl, S. Østergaard, and L. R. Nielsen. 2012. Gross margin losses due to *Salmonella* Dublin infection in Danish dairy cattle herds estimated by simulation modelling. Submitted to Preventive Veterinary Medicine.

9.1 Manuscript 1

Association between *Salmonella* and high Calf Mortality in Danish Dairy Herds

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J. Dairy Sci. 93:304–310
doi:10.3168/jds.2009-2528

Association between bulk-tank milk *Salmonella* antibody level and high calf mortality in Danish dairy herds

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ABSTRACT

Salmonella enterica ssp. *enterica* Dublin is the most common *Salmonella* serotype found in the dairy sector in Denmark. *Salmonella* antibody level in bulk-tank milk (**BTM**), indicative of *Salmonella* Dublin infection in the herd, has been recorded regularly in all Danish dairy herds through a surveillance program since 2002. The objective of this study was to investigate whether high BTM *Salmonella* antibody level was associated with high calf mortality at herd level. Other risk factors for high calf mortality were also investigated: breed, production type (organic vs. conventional), number of animals purchased, herd size, and number of neighbor herds within a 4.9-km radius. Data from the Danish Cattle Database including the *Salmonella* surveillance program from September 2007 through August 2008 were used. Dairy herds with more than 20 cows were included (n = 4,337). Because of a highly right-skewed distribution of calf mortality with many zero values, calf mortality had to be dichotomized for the analysis. Therefore, in this study, high calf mortality was defined as calf mortality of more than 6.5% for calves aged 1 to 180 d. A logistic analysis was performed to identify risk factors associated with the probability of a herd having high calf mortality. The following factors were significantly associated with high calf mortality: high BTM *Salmonella* antibody level, odds ratio (OR) = 2.0 (95% confidence interval = 1.6–2.4), organic production OR = 1.4 (95% confidence interval = 1.1–1.7) for organic versus conventional production, and breed. Purchase of 8 or more animals increased the OR of high calf mortality more than purchase of 1 to 7 animals, which again had a higher OR compared with purchase of 0 animals. Because only 14.3% of the population consisted of herds with high BTM *Salmonella* status, the estimated proportion of herds with high calf mortality could only be reduced from 38.7 to 36.5% by eradicating *Salmonella* from the Danish cattle population (i.e., a population attributable risk of 2.2%). This showed that although there is a strong association between BTM *Salmonella* status and calf mortality, the problem with high calf mortality will not be solved by eradicating *Salmonella*. All other things equal, a population with more *Salmonella*-infected herds would gain a larger reduction in calf mortality from a *Salmonella* control campaign. Nevertheless, individual herds with a high within-herd prevalence of *Salmonella* are likely to benefit, both economically and regarding animal welfare, from controlling pathogenic *Salmonella* types in cattle.
Key words: *Salmonella* Dublin, risk factor, calf mortality, dairy cattle

INTRODUCTION

Mortality in dairy calves aged 1 to 180 d was, on average, 8.6% in Denmark in 2007. This was considered a welfare problem, and therefore the Danish Cattle Federation started a campaign to reduce calf mortality to an average of 6.5% by the end of 2009.

Salmonella enterica ssp. *enterica* Dublin (*Salmonella* Dublin) is host-adapted to cattle (Wray and Sojka, 1977) and can cause both economic losses and reduced welfare in cattle herds through abortions, reproductive problems, and decreased milk yield in adult cows, as well as calf hood disease (Peters, 1985; Visser et al., 1997). *Salmonella* Dublin primarily affects calves less than 6 mo old (Peters, 1985; Clegg et al., 1986). Clinical signs include diarrhea, fever, dehydration, lethargy, pneumonia, and death. Therefore, controlling *Salmonella* Dublin might decrease mortality in calves at herd, regional, and national levels.

Salmonella Dublin is endemic in cattle in Denmark with herd seroprevalences ranging from 0 to 30% in different regions of the country, making it the most common serotype of *Salmonella* isolated from cattle (Ministry of Food, Agriculture and Fisheries, 2009). The bacteria is introduced to a herd mainly by either purchase of infected animals (Vaessen et al., 1998) or direct contact with infected animals, for example, by sharing pastures (van Schaik et al., 2002). *Salmonella* Dublin can survive for several years in the environment, which can act as a source of reinfection for the herd (Plym-Forshell and Ekesbo, 1996).

Received June 30, 2009.
Accepted September 20, 2009.
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A national surveillance program was started in 2002 in Denmark to monitor *Salmonella* status of cattle herds. Since 2007, there has been a campaign in place to encourage eradication of *Salmonella* Dublin from cattle herds and thus improve animal health and welfare as well as food safety for consumers. Monitoring is based on testing of antibodies directed against *Salmonella* Dublin lipopolysaccharide antigens (O:9,12) in either bulk-tank milk (**BTM**) for dairy herds or blood for non-milk-producing herds by ELISA (Hoorfar et al., 1995). Other serotypes of *Salmonella* may cross-react with this antigen (Konrad et al., 1994); hence, *Salmonella* in this study refers to all serotypes of *Salmonella* that result in a positive ELISA response. The cross-reacting serotype of *Salmonella* in Denmark is mainly *Salmonella* Typhimurium. Samples of BTM are collected every 3 mo and a mean value is calculated for the last 4 measurements. Background-corrected optical density value of the sample to a known positive control sample (**ODC%**) is calculated and herds are separated into 3 categories in the surveillance program (Ministry of Food, Agriculture and Fisheries, 2003). These categories are publicly available, so farmers have the opportunity to avoid buying animals from farms that are not considered free of *Salmonella* infection.

Other factors have been reported to affect calf mortality, such as breed (Weigel and Barlass, 2003). Andrews (1999) reported that calves bought into the herd had higher mortality than homebred calves. Furthermore, because *Salmonella* Dublin is a contagious disease, there is a risk that herd density will affect the distribution of *Salmonella*-infected herds (Nielsen et al., 2007). No previous studies have examined whether there is an association between BTM *Salmonella* antibody level and mortality in calves. Nielsen et al. (2007) investigated whether high calf mortality in previous year-quarters could be used as an early indicator of a change in *Salmonella* status of herds, but found no clear associations.

The objective of this study was to investigate whether there was an association between *Salmonella* infection in dairy herds (based on BTM antibody testing) and calf mortality during a period of 1 yr while taking into account other possible risk factors for calf mortality such as breed, production type, purchase of animals, herd size, and herd density.

MATERIALS AND METHODS

Data Sources

Registry data from the Central Husbandry Register and the Danish Cattle Database (**DCD**), including data from the National Surveillance Program for *Salmonella* Dublin, were collected for this study. These databases are well integrated. All cattle are ear-tagged within a few days of birth and the recording of all movements and deaths is compulsory and reinforced by the European Union's cross-checking and reimbursement systems. *Salmonella* laboratory results from cattle samples are sent to the DCD from the Danish laboratories.

Study Herds

Herd was the study unit. In total, 4,488 herds were recorded as milk-producing in Denmark in September 2008. The study population consisted of all Danish dairy herds containing a minimum of 20 cows in August 2008 (n = 4,337).

Description of Variables

Variables on risk factors for high calf mortality were constructed from registry data extracted from the DCD. Included risk factors were BTM *Salmonella* antibody level, breed, production type, number of purchased animals, neighbor herds within a 4.9-km radius, and herd size. Because of skewed distributions, all variables other than herd size were categorized before inclusion in the logistic analysis model as potential risk factors.

BTM Salmonella Antibody Level. This variable was recorded as the mean BTM ODC% of *Salmonella* antibody level from September 2007 through August 2008. It was dichotomized using the same cut-off used in the surveillance program, with high BTM *Salmonella* status being herds with an average $\geq$25 ODC% and low BTM *Salmonella* status being herds with an average $<$25 ODC% in BTM samples from the study period.

Breed. Breed included all cattle in the herd (all age groups, both sexes, and both dairy and beef cattle). For this study, breed was classified as Jersey if the herd consisted of more than 80% Jersey animals, large breed if it consisted of more than 80% large dairy breed animals (primarily Danish Holsteins), and the rest of the herds were classified as mixed breed.

Production Type. Production type was either organic or conventional production as recorded in August 2008 according to the rules for organic production in Denmark. It was assumed to be constant for a herd in the study period.

Purchased Animals. Purchased animals was the number of cattle moved into the herd from September 2007 through August 2008. More than half the herds did not purchase any animals in the study period, but the remaining herds were categorized into 3 approximately equally sized categories, which were 1 to 7, 8 to 40, and $>$40 animals purchased.

Neighbor Herds Within 4.9 Kilometers. Number of neighbor herds within a 4.9-km radius included all cattle herds (e.g., heifer raising facilities, beef, dairy–beef, dairy, hobby, and mixed herds) in August 2008. This distance was used because it was shown previously to be the range that *Salmonella* status of a herd can influence the status of neighboring herds (Ersbøll and Ersbøll, 2007). The variable for the analysis was categorized into 4 categories of approximately equal size, which were ≤25, 26 to 49, 50 to 75, and >75 neighbor herds within a 4.9-km radius.

Herd Size. Herd size was recorded as total number of animals in the herd in August 2008. This was a discrete continuous variable.

Calf Mortality. Calculation of calf mortality was based on number of dead calves per day divided by total number of calf days at risk in the herd. If a calf died within 24 h of birth, it was classified as stillborn and not included in the study. Calf mortality was calculated using the formula below. A calf was censored from the herd if it was sold for export, slaughter, or to another herd. In this case it would not count as being at risk of dying from the day it was removed from the herd. Thus, the probability (Pr) of a calf dying d 1 through 180 was

$$\Pr(\text{Calf mortality}_{1-180}) = 100 \times \left\{1 - \prod_{i=1}^{180}\left(1 - \frac{D_i}{B + I\left[< 180\right] - D^{i-1} - C^{i-1} - E\left[i > 1\right]}\right)\right\},$$

where D_i is number of dead or euthanized calves on day i; the denominator is number of calves at risk of dying on day i; B is number of liveborn calves in the study period; and I [<180] is number of calves introduced to herd before 180 d of age. From this was subtracted calves that died, were killed in the herd, or were censored before the start of day i. D^{i-1} is the sum of dead calves including day $i - 1$; if $D^0 = 0$ then $i = 1$. C^{i-1} is the sum of censored calves including day $i - 1$. $E[i > 1]$ is the number of calves euthanized as newborn, which were not deducted until day $i = 2$.

Calf mortality was measured in percent and was dichotomized with a cut-off level between high and low in the logistic analysis set to 6.5%. This cut-off is the aim of the campaign initiated by the Danish Cattle Federation.

Statistical Analysis

Data were analyzed using SAS (version 9.1.3, SAS Institute, Cary, NC). Outlier detection and correlation between variables was assessed using scatter plots and descriptive statistics. A logistic analysis with the dichotomized calf mortality variable as outcome was performed using backward stepwise elimination in PROC GENMOD of SAS (SAS Institute). The main risk factors (BTM *Salmonella* status, breed, production type, number of purchased animals, herd size, neighbor herds within a 4.9-km radius, and their 2-way interactions) were tested as variables in the model. The criterion for risk factors and interactions to remain in the model was set at 1% significance level. Nonsignificant interactions were removed first, followed by nonsignificant main effects. After initial reduction of the model, main effects and their 2-way interactions were reinserted one at a time into the model to test for changes in significance by evaluating the *P*-value and confounding by evaluating the changes in estimates for the variables. Furthermore, odds ratios (**OR**) for risk factors and 95% confidence intervals were calculated, as well as population attributable risk (**PAR**) and population attributable fraction (**PAF**) for BTM *Salmonella* status. The model was validated by evaluating the goodness of fit-estimate Pearson chi-square value divided by degrees of freedom.

RESULTS

Descriptive Results

Of the 4,337 herds originally included in the study, 3 herds consisted of more than 50% beef cattle but were recorded as dairy herds in the original data set. Four herds had a calf mortality of 100%. However, they had few dead calves relative to the number of cows, most likely because heifers were removed from the premises soon after birth. Thus, the 100% calf mortalities were misleading numbers. One herd had missing data on calf mortality. Thus, these 8 herds were excluded from the data set. Five herds had no recordings of number of purchased animals, but were kept in the data set.

Another 2 herds were excluded because they were extremely big and not representative in an analysis with herd size as a continuous variable. They consisted of 3,059 and 2,514 animals, respectively. This was much more than the mean herd size of 251 animals in the rest of the data set, and over 1,000 animals more than any other herd. Another 12 herds consisted of more than 1,000 animals. Because there were relatively few large herds, the analysis was performed using herd size as well as herd size truncated at 1,000 animals. When performing the logistic analysis on the full data set, an interaction was found between herd size and purchased animals. It showed that when purchasing more than 40 animals, calf mortality decreased with increasing herd size, whereas calf mortality increased with herd size when purchasing 40 or fewer animals. This interaction

Table 1. Classification and distribution of categorized variables used for logistic analysis

Variable	n of risk factor	Classification	n (%)
Calf mortality			
Low	4,327	≤6.5%	2,654 (61.3)
High		>6.5%	1,673 (38.7)
Bulk-tank milk *Salmonella* Dublin status			
Low	4,327	<25	618 (14.3)
High		≥25	3,709 (85.7)
Dominant breed			
Large	4,327	>80% large breed	3,267 (75.5)
Mixed		Mixed breed	633 (14.6)
Jersey		>80% Jersey	427 (9.9)
Production type	4,327	Conventional	3,908 (90.3)
		Organic	419 (9.7)
No. of animals purchased from Sept. 2007 through Aug. 2008	4,322	0	2,184 (50.5)
		1–7	692 (16.0)
		8–40	703 (16.3)
		>40	743 (17.2)
No. of neighbor herds within 4.9 km	4,327	≤25	862 (19.9)
		25–50	1,269 (29.3)
		50–75	1,071 (24.8)
		>75	1,125 (26.0)

was not found when removing herds with more than 1,000 animals. Because there were only 12 of these herds, the interaction was considered an artifact of data rather than a biologically plausible effect. Estimates and *P*-values for the other variables did not change when removing these herds. Thus, only 4,315 observations were used for herd size and the interaction was not considered further. Table 1 shows categorized variables used in the logistic analysis and their respective categories together with the distribution of observations in each category.

Analytical Results

The significant risk factors and significance levels in the final logistic analysis model for high calf mortality are given in Table 2. Breed, BTM *Salmonella* status, and purchased animals all had *P*-values below 0.0001, whereas production type had a *P*-value of 0.004.

The OR and 95% confidence interval for each risk factor in the final model are also given in Table 2. Herds with high BTM *Salmonella* status had an OR of 2.0 of high calf mortality in the study period compared with herds with low BTM *Salmonella* status. The highest OR of the study was found for Jersey compared with large breed (OR = 3.3), whereas mixed breed was in between the 2 other breeds (OR = 1.6). Herds where no animals where purchased had the lowest risk of high calf mortality, followed by herds that purchased 1 to 7 animals. There was no difference in calf mortality when purchasing 8 to 40 animals or more than 40 animals,

Table 2. Risk factors associated with calf mortality above 6.5% in Danish dairy herds at 1% significance level from September 2007 through August 2008, as well as odds ratios (OR) and 95% confidence intervals (CI)

Risk factor	Coefficient	SE	*P*-value	OR	95% CI
Intercept	−1.05	0.05			
Bulk-tank milk *Salmonella* antibody status			<0.0001		
High	0.69	0.09		2.0	1.7–2.4
Low	0	0		—[1]	—
Breed			<0.0001		
Jersey[c]	1.20	0.11		3.3	2.7–4.1
Mixed[b]	0.44	0.09		1.6	1.3–1.9
Large[a]	0	0		—	—
Production type			0.0040		
Organic	0.31	0.11		1.4	1.1–1.7
Conventional	0	0		—	—
Purchased animals			<0.0001		
>40[c]	0.58	0.09		1.8	1.5–2.1
8–40[c]	0.71	0.09		2.0	1.7–2.4
1–7[b]	0.24	0.09		1.3	1.1–1.5
0[a]	0	0		—	—

[a–c]Groups with different superscripts differed significantly ($P < 0.01$).

[1]Dash indicates referent.

but both groups had significantly higher calf mortality than the 2 groups with few purchases.

The effect of *Salmonella* herd status on calf mortality at population level was estimated. The PAR was 0.022 for high BTM *Salmonella* status, which, combined with the prevalence of high BTM status herds (14.3%), resulted in a PAF of 5.6%. Pearson chi-square value divided by degrees of freedom was 1.0017, suggesting a good fit for the final model.

DISCUSSION

Main Findings

This study showed an overall association between calf mortality in dairy herds and BTM *Salmonella* status, production type, breed, and number of purchased animals.

High BTM *Salmonella* status, indicative of *Salmonella* Dublin infection, was associated with increased risk of high calf mortality in dairy herds. Others have found increased calf mortality with clinical salmonellosis (Lance et al., 1992a; Rice et al., 1997). Gay and Hunsaker (1993) isolated multiple *Salmonella* serovars from a dairy herd 2 yr after clinical symptoms of salmonellosis had ceased. This herd had high calf mortality (54 of 308 heifer calves died), but the study did not show whether this was a result of *Salmonella* being present in the herd. In another study, clinical disease was not observed in a dairy herd where *Salmonella* Dublin was isolated from BTM (Anderson et al., 2001).

The Jersey breed was found to be associated with high calf mortality. Weigel and Barlass (2003) let farmers score the calf mortality for Jerseys, Holsteins, and cross-breeds, and farmers gave Jerseys the highest mortality score. Increased number of purchased animals had an association with calf mortality. In this study, it was not possible to distinguish which animals were purchased (age, sex, and so on), but Andrews (1999) found that purchased calves had higher mortality than homebred calves. We found no association between calf mortality and herd size, which corresponded with what others have found previously (Martin et al., 1975; Mee et al., 2008). Gulliksen et al. (2009) found increasing calf mortality with increasing herd size, but they investigated Norwegian dairy herds, which on average are much smaller than Danish herds.

The study showed that, taking into account the differences in calf mortality between different breeds, production types, and purchase patterns, the risk of high mortality that was attributed to high BTM *Salmonella* status was 2.2% (PAR). Hence, if all dairy herds would achieve low BTM *Salmonella* status, we would expect the proportion of herds with calf mortality above 6.5% to decrease from 38.7 to 36.5% under conditions where 14.3% of the herds were infected with *Salmonella*, as in this study. The PAF value suggested that 5.6% of the herds in the dairy herd population had a high mortality as a result of some herds having high BTM *Salmonella* status. This shows that eradication of *Salmonella* from dairy herds is likely to decrease calf mortality to some extent (both at herd level and at national level) but, because of the low prevalence of *Salmonella*, it is unlikely to reduce calf mortality noticeably at national level. This study investigated mainly endemic *Salmonella* Dublin infection in the herds. The benefit of *Salmonella* control may be higher in higher prevalence regions or herds with clinical outbreaks.

Statistical Analysis

The nature of the data suggested an ANOVA as best choice of analytical method. However, calf mortality was not normally distributed as a continuous variable and the herds with zero mortality for calves (12% of the study population) had to be removed to perform an ANOVA. Even without these observations, assumptions of normal distribution could not be fulfilled and so data were dichotomized and analyzed by logistic analysis. To underline the logic in using categorical variables, an attempt was made to find natural cut-off values (e.g., breed, purchased animals) or values used elsewhere in official programs (e.g., BTM *Salmonella* status, calf mortality).

The logistic analysis was performed at 5 different cut-offs for calf mortality (data not shown): 2.3% (the 25% quartile for the study data), 2.9% (the 25% quartile for Danish dairy herds), 10%, <5% compared with >10%, and 6.5% (aim of calf mortality campaign). Main risk factors found to be significant in the final models were identical at all cut-off levels except production type, which had significance levels between 0.05 and 2%, depending on the cut-off level. This led us to conclude that the model was robust with respect to cut-off for calf mortality and, thus, that our findings represented real effects rather than artifacts of the chosen method of analysis.

Data Quality and Availability

This study was based solely on registry data. These contain information on all herds in Denmark, which gives a unique opportunity to evaluate risk factors for calf mortality at herd level. Because *Salmonella* is a contagious disease, observations at herd level were needed to assess the risk factors. However, the use of registry data excluded the possibility of including factors related to management such as grazing, treat-

ments, calving management, hygiene, barn structures, and so on, which limited the conclusions that could be made. Calf management has been found to affect calf mortality (Lance et al., 1992b; Losinger and Heinrichs, 1997). Lance et al. (1992b) found that housing type and dipping of navels with disinfectant affected the mortality of preweaned calves. It could improve the model if management practices were included. However, we were not able to assess management of calves from the registry data. Fossler et al. (2005a,b) investigated associations between SCC and production level measured as rolling herd average on *Salmonella* shedding in calves and cows. These measures can be seen as indirect measures of management of the herds. They found no association between SCC and shedding, but found that production level was associated with shedding of *Salmonella* in calves but not in cows. The effect of *Salmonella* herd status on calf mortality may to some extent be explained by underlying management and hygiene factors, and organic producers are subjects to rules that lead to different management than in conventional herds, in particular regarding feed, medication, and contact between individual animals in the herd.

Farmers themselves had to record dead calves, so there was a risk of errors in the calf mortality data. However, recordings of dead animals are accurate in Denmark because all dead animals have to undergo destruction and destruction centers record which animals they receive. Very few herds (n = 10) had to be removed from the data set because of missing or unrealistic recordings. A total of 6 missing values were found among the different variables. This study contained 4,337 out of 4,488 Danish dairy herds, and the study population can be assumed to be representative of dairy herds in Denmark.

Salmonella Herd Classification

Bulk-tank milk *Salmonella* status is an indirect measure of infection in the herd. Misclassification could have biased the results of the model. In 2007, *Salmonella* Dublin accounted for 52% of *Salmonella* serotypes isolated from dairy herds in Denmark, and it has the potential to persist longer in the herds than other types of *Salmonella* (Boqvist and Vågsholm, 2005). However, results for this study included other types of *Salmonella* because there was a risk of cross-reaction in the testing program (Konrad et al., 1994). Warnick et al. (2006) evaluated the classification accuracy of the surveillance program. They found that at a prevalence of 15% *Salmonella* positive herds in the study population, the negative predictive value for category 1 (expected free of *Salmonella*) was estimated to be 99%, whereas the positive predictive value for category 2 (possibly *Salmonella* infected) was estimated to be 80%. The surveillance program is constructed to ensure that herds classified as *Salmonella*-free really are free. The high negative predictive value means that only 1% of herds classified as category 1 are likely to be false negative. However, the low positive predictive value means that around 20% of herds classified as possibly *Salmonella*-infected could be free of infection. This may have led to underestimation of the association between high BTM *Salmonella* status and high calf mortality in this study if herds wrongly classified had low calf mortality, or overestimation of the association if herds wrongly classified had high calf mortality.

It was not possible to estimate the number of calves that could be saved on a national level by eradicating *Salmonella* Dublin because the outcome of the model is percent calf mortality at herd level and because we do not know the exact distribution of other serotypes in the population. The number of calves that can be saved on a yearly basis is probably fairly limited as expressed by the PAR. However, individual herds with a high within-herd prevalence of *Salmonella* are likely to benefit, both economically and regarding animal welfare, from controlling pathogenic *Salmonella*-types.

ACKNOWLEDGMENTS

The authors thank Jørgen Nielsen and Peter Stamp Enemark from Danish Cattle Federation (Aarhus, Denmark) for providing data.

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9.2 Manuscript 2

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 the duration of any effect.

Longitudinal data from 2005 through 2009 were used, with data from 12 months before until 18 months after the estimated date of infection. Twenty-eight case herds were selected based on an increase in the level of *Salmonella* specific antibodies in bulk-tank milk from < 10 corrected optic density percentage (**ODC%**) to ≥ 70 ODC% between two consecutive 3-monthly measurements in the Danish *Salmonella* surveillance program. All selected case herds were conventional Danish Holstein herds. Control herds (n = 40) were selected randomly from Danish Holstein herds with *Salmonella* antibody levels consistently < 10 ODC%. A date of herd infection was randomly allocated to the control herds. Hierarchical mixed effect models with the outcome test day energy corrected milk yield (**ECM**)/cow were used to investigate the daily milk yield before and after the estimated herd infection date for cows in parity 1, 2 and 3+. Control herds were used to evaluate whether the effects in the case herds could be reproduced in herds without *Salmonella* infection. Herd size, days in milk, somatic cell count, season, and year were included in the models.

The key results were that first parity cow yield was reduced by a mean of 1.4 kg (95% CI: 0.5 to 2.3) ECM/cow per day from seven to 15 months after the estimated herd infection date, compared with first parity cows in the same herds in the 12 months before the estimated herd infection date. Yield for parity 3+ was reduced by a mean of 3.0 kg (95% CI: 1.3 to 4.8) ECM/cow per day from seven to 15 months after herd infection compared with parity 3+ cows in the 12 months before the estimated herd infection. There were minor differences in yield in second parity cows before and after herd infection, and no difference between cows in control herds before and after the simulated infection date. There was a significant drop in milk yield in affected herds and the reduction was detectable several months after the increase in bulk-tank milk *Salmonella* antibodies. It took more than a year for milk yield to return to pre-infection levels.

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

1 INTRODUCTION

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

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

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

2 MATERIALS AND METHODS

2.1 Salmonella Status of Herds

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

2.2 Selection of Herds

The study was based on registry data from the Danish Cattle Database (Knowledge Centre for Agriculture, Cattle) from January 2005 to December 2009. Selection of herds was based on their BTM *Salmonella* ODC%-measurements from the Danish surveillance program. A herd was included as a case herd, if it had an antibody response < 10 ODC% in at least three samples over a minimum of one year followed by an increase to ≥ 70 ODC% and the test following the initial high test was ≥ 25 ODC% to exclude potentially false positive. Out of approximately 3300 dairy herds, 44 herds fulfilled these criteria. Two herds had an antibody response < 25 ODC% in the test following the initial test, but antibody response ≥ 25 ODC% in subsequent tests. This indicated that they were infected with *Salmonella* and they were also included as case herds. The 46 herds were stratified on main breed, farming type (conventional or organic), and herd size and were analyzed descriptively. The largest group consisted of conventional Danish Holstein dairy herds and 28 herds with a minimum of 40 cows in the study period were selected as case-herds. The following herds were excluded from the model: five herds with no

milk yield recordings around the estimated time of infection, four herds not consisting of Danish Holsteins (one Jersey, two Danish Reds and one Crossbreed), one herd consisting of < 40 cows in the study period and eight organic herds. Forty control herds were randomly selected from conventional Danish Holstein herds with > 40 cows in the study period and antibody response < 10 ODC% throughout the study period.

2.3 Test day energy corrected milk yield (Test day ECM)

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

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

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

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

2.4 Time Period (T)

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

2.5 Season

Test day ECM displayed a marked seasonality with highest yield in spring and lowest in fall. A sine curve was created for each parity with amplitude depending on the difference between year-quarter with highest and lowest yield for the control herds, where year-quarters were January to March, April to June, July to September and October to

December. This difference in yield between spring and fall was 1.5, 1.5 and 1.9 kg test day ECM for parity 1, 2 and 3+ respectively. The sine curve was given by:

Sine = difference in milk yield*sine (2*π*year-quarter/4) Eq. (2)

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

2.6 Other Confounding Variables

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

2.7 Data Analysis

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

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

For parity 3+ the final model was:

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

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

Test day ECM was modeled from 12 months (T_{-4}) before to 18 months (T_5) after the estimated infection date for the herd. Control and case herds were modeled separately. The final models for control herds were applied to the respective parity case herd data to assess associations between test day ECM and *Salmonella.* Year 2005 was used as baseline in the model, and data were centered on mean of logSCC (4) (corresponding to

a cell count of approximately 55,000 per ml). Fall was used as baseline for parity 3+. Standard residuals for each level in the model and predicted vs. observed test day ECM were plotted to asses model fit.

3 RESULTS

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

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

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

[1]Q1=1st quartile and Q3= 3rd quartile

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

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

[1]Standard deviation

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

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

[1]Time period in 3-month intervals

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

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

in parity 1, 2 and 3+ respectively, the mean loss in milk production would be more than 40,000 kg ECM (95% CI: 8,000-153,000) in the first year after infection.

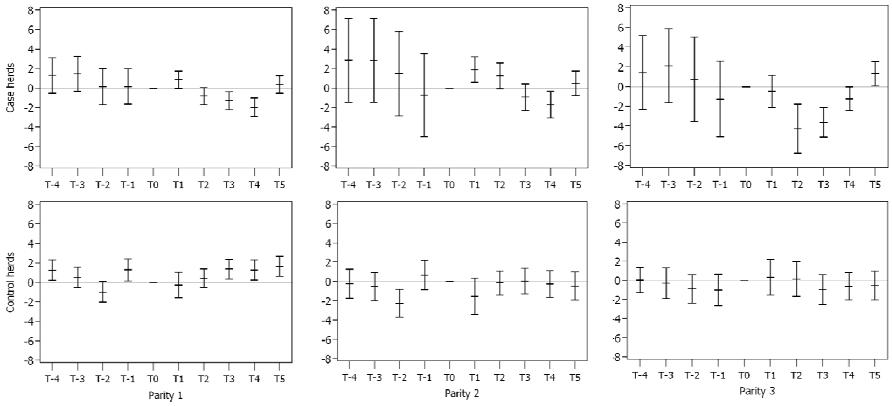

Figure 1. Predicted daily milk yield mean (Kg Energy corrected milk) compared to one to three months after *Salmonella* infection (T_0) for case (n=28) and control herds (n=40). T_1 is four to six months after infection, T_{-1} is one to three months before infection etc. The bars represent 95% confidence interval of mean milk yield

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

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

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

[1]Standard error of the mean [2]Lower confidence limit [3]Upper confidence limit [4]Days in milk [5]Log somatic cell count [6]Test day

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

Variable		Mean	s.e.[1]	LCLM[2]	UCLM[3]
Intercept		39.24	1.97	35.39	43.10
DIM[4]		-0.05	0.00	-0.06	-0.05
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					
(months relative to estimated herd infection)					
-4	(-12 through -10)	1.42	1.91	-2.33	5.18
-3	(-9 through -7)	2.12	1.93	-1.66	5.90
-2	(-6 through -4)	0.75	2.18	-3.52	5.01
-1	(-3 through -1)	-1.24	1.95	-5.07	2.59
0	(1 through 3)	0	-	-	-
1	(4 through 6)	-0.49	0.83	-2.12	1.14
2	(7 through 9)	-4.27	1.27	-6.75	-1.79
3	(10 through 12)	-3.62	0.76	-5.12	-2.12
4	(13 through 15)	-1.22	0.62	-2.43	-0.01
5	(16 through 18)	1.33	0.64	0.08	2.57
Random effects					
Herd level variance		7.98	2.92		
Cow level variance		27.75	1.02		
Test day ECM level variance		26.02	0.30		

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

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

4 DISCUSSION

4.1 Results

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

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

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

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

4.2 Herd classification

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

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

months. Because increase in BTM antibodies happened prior to reduction in milk yield, it is likely that introduction of *Salmonella* to the herd caused the reduction in yield.

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

4.3 Infection date

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

4.4 Strength and limitation of study

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

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

Control herds were selected randomly from all conventional Danish Holstein dairy herds with consistently low BTM antibody levels. Case herds in the period 2005-2009 with conventional farming practice and Danish Holstein cows were included in the study, and

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

5 CONCLUSIONS

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

ACKNOWLEDGEMENTS

Jørgen Nielsen from the Danish Cattle Federation is thanked for providing data. This study was funded by the Danish Cattle Federation and the Faculty of Life Sciences, University of Copenhagen.

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APPENDIX 1

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

	Parity 1				Parity 2			
Variable	Mean	s.e.[1]	LCLM[2]	UCLM[3]	Mean	s.e.	LCLM	UCLM
DIM[4]*T[5]								
DIM*T_{-4}	0.005	0.002	0.001	0.009	-0.007	0.003	-0.013	-0.001
DIM*T_{-3}	0.001	0.002	-0.003	0.005	-0.010	0.003	-0.016	-0.004
DIM*T_{-2}	0.003	0.002	-0.001	0.007	-0.006	0.002	-0.010	-0.002
DIM*T_{-1}	0.003	0.001	0.001	0.005	0.003	0.002	-0.001	0.007
DIM*T_0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
DIM*T_1	0.001	0.001	-0.001	0.003	-0.007	0.002	-0.011	-0.003
DIM*T_2	0.005	0.002	0.001	0.009	-0.003	0.002	-0.007	0.001
DIM*T_3	0.006	0.002	0.002	0.010	0.003	0.002	-0.001	0.007
DIM*T_4	0.003	0.002	-0.001	0.007	-0.001	0.002	-0.005	0.003
DIM*T_5	-0.003	0.002	-0.007	0.001	-0.005	0.003	-0.011	0.001
Sine*T								
Sine*T_{-4}	0.23	0.22	-0.19	0.66	0.42	0.33	-0.21	1.06
Sine*T_{-3}	-0.74	0.26	-1.25	-0.22	-1.63	0.40	-2.41	-0.86
Sine*T_{-2}	0.20	0.21	-0.21	0.61	0.03	0.30	-0.56	0.63
Sine*T_{-1}	0.60	0.22	0.16	1.03	0.70	0.33	0.06	1.34
Sine*T_0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sine*T_1	-0.29	0.24	-0.76	0.17	-0.50	0.37	-1.21	0.22
Sine*T_2	0.34	0.21	-0.08	0.76	-0.34	0.32	-0.97	0.29
Sine*T_3	0.97	0.23	0.52	1.41	1.03	0.34	0.36	1.69
Sine*T_4	0.19	0.21	-0.22	0.60	-0.88	0.31	-1.48	-0.28
Sine*T_5	-0.07	0.28	-0.61	0.47	-1.01	0.40	-1.79	-0.23
Year*T								
2006*T_{-4}	-0.56	0.92	-2.37	1.25	-0.31	2.18	-4.58	3.96
2006*T_{-3}	0.48	0.91	-1.30	2.27	1.63	2.18	-2.64	5.89
2006*T_{-2}	0.62	0.94	-1.23	2.47	0.92	2.19	-3.36	5.21
2006*T_{-1}	-0.56	0.92	-2.37	1.25	0.84	2.16	-3.39	5.07
2006*T_0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2006*T_1	-0.51	0.46	-1.41	0.39	-1.29	0.68	-2.62	0.04
2006*T_2	-0.21	0.52	-1.22	0.81	2.91	0.79	1.37	4.45
2006*T_3	-0.02	0.58	-1.16	1.12	-0.38	0.84	-2.03	1.26
2006*T_4	2.28	0.70	0.91	3.64	1.80	0.93	-0.03	3.63
2006*T_5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2007*T_{-4}	-0.47	0.95	-2.33	1.39	-0.53	2.20	-4.85	3.79
2007*T_{-3}	0.10	0.95	-1.77	1.96	0.22	2.20	-4.10	4.54
2007*T_{-2}	1.10	-0.96	2.99	-0.79	1.82	2.21	-2.51	6.14
2007*T_{-1}	0.69	0.93	-1.14	2.51	1.70	2.17	-2.55	5.96
2007*T_0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2007*T_1	-1.06	0.43	-1.90	-0.21	-1.30	0.62	-2.52	-0.08
2007*T_2	-1.12	0.40	-1.91	-0.33	-2.31	0.59	-3.46	-1.16

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

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

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

		Parity 3+			
Variable		Mean	s.e.[1]	LCLM[2]	UCLM[3]
DIM[4]*T[5]					
	DIM*T_{-4}	-0.003	0.002	-0.007	0.001
	DIM*T_{-3}	-0.008	0.002	-0.012	-0.004
	DIM*T_{-2}	-0.006	0.002	-0.010	-0.002
	DIM*T_{-1}	0.003	0.002	-0.001	0.007
	DIM*T_0	0.000	0.000	0.000	0.000
	DIM*T_1	-0.004	0.002	-0.008	0.000
	DIM*T_2	0.000	0.002	-0.004	0.004
	DIM*T_3	0.001	0.002	-0.003	0.005
	DIM*T_4	-0.003	0.002	-0.007	0.001
	DIM*T_5	-0.008	0.002	-0.012	-0.004
Year*T					
	2006*T_{-4}	1.45	1.92	-2.30	5.21
	2006*T_{-3}	0.74	1.91	-3.00	4.47
	2006*T_{-2}	2.00	1.98	-1.88	5.89
	2006*T_{-1}	0.99	1.93	-2.80	4.77
	2006*T_0	0.00	0.00	0.00	0.00
	2006*T_1	2.12	0.68	0.78	3.46
	2006*T_2	1.81	0.94	-0.03	3.65
	2006*T_3	3.79	0.94	1.95	5.63
	2006*T_4	2.61	0.97	0.71	4.50
	2006*T_5	0.00	0.00	0.00	0.00
	2007*T_{-4}	0.36	1.93	-3.42	4.13
	2007*T_{-3}	0.62	1.93	-3.17	4.40
	2007*T_{-2}	4.07	2.01	0.13	8.01
	2007*T_{-1}	1.34	1.96	-2.51	5.18
	2007*T_0	0.00	0.00	0.00	0.00
	2007*T_1	0.65	0.67	-0.65	1.95
	2007*T_2	0.66	0.65	-0.61	1.93
	2007*T_3	0.21	0.64	-1.05	1.47
	2007*T_4	0.14	0.63	-1.10	1.38
	2007*T_5	-1.02	0.65	-2.29	0.24
	2008*T_{-4}	0.00	0.00	0.00	0.00
	2008*T_{-3}	1.17	1.94	-2.63	4.96
	2008*T_{-2}	2.38	2.01	-1.56	6.33
	2008*T_{-1}	0.24	1.96	-3.61	4.08

	2008*T_0	0.00	0.00	0.00	0.00
	2008*T_1	1.90	0.57	0.79	3.01
	2008*T_2	2.59	0.60	1.40	3.77
	2008*T_3	3.85	0.59	2.70	5.00
	2008*T_4	1.30	0.52	0.28	2.32
	2008*T_5	0.00	0.00	0.00	0.00
	2009*T_{-4}	0.00	0.00	0.00	0.00
	2009*T_{-3}	0.00	0.00	0.00	0.00
	2009*T_{-2}	0.00	0.00	0.00	0.00
	2009*T_{-1}	0.00	0.00	0.00	0.00
	2009*T_0	0.00	0.00	0.00	0.00
	2009*T_1	0.00	0.00	0.00	0.00
	2009*T_2	0.00	0.00	0.00	0.00
	2009*T_3	0.00	0.00	0.00	0.00
	2009*T_4	0.00	0.00	0.00	0.00
	2009*T_5	0.00	0.00	0.00	0.00
Season*T					
	Spring*T_{-4}	-2.70	1.27	-5.19	-0.21
	Spring*T_{-3}	-2.27	1.03	-4.29	-0.26
	Spring*T_{-2}	-3.93	1.66	-7.19	-0.67
	Spring*T_{-1}	-0.60	1.19	-2.92	1.73
	Spring*T_0	0.00	0.00	0.00	0.00
	Spring*T_1	-3.31	1.08	-5.43	-1.20
	Spring*T_2	0.10	1.53	-2.90	3.10
	Spring*T_3	-0.28	1.11	-2.45	1.90
	Spring*T_4	-2.14	1.20	-4.48	0.21
	Spring*T_5	-1.48	1.02	-3.49	0.52
	Summer*T_{-4}	-1.23	0.60	-2.40	-0.05
	Summer*T_{-3}	-0.68	1.13	-2.89	1.54
	Summer*T_{-2}	0.07	1.23	-2.34	2.48
	Summer*T_{-1}	0.33	0.64	-0.92	1.59
	Summer*T_0	0.00	0.00	0.00	0.00
	Summer*T_1	-0.88	1.22	-3.26	1.50
	Summer*T_2	1.41	1.07	-0.68	3.50
	Summer*T_3	1.34	0.64	0.08	2.59
	Summer*T_4	0.83	0.60	-0.35	2.00
	Summer*T_5	0.44	1.19	-1.90	2.78
	Winter*T_{-4}	0.33	0.55	-0.75	1.41
	Winter*T_{-3}	-0.68	1.13	-2.89	1.54
	Winter*T_{-2}	-0.15	1.38	-2.85	2.56
	Winter*T_{-1}	2.04	1.02	0.04	4.05

Winter*T_0	0.00	0.00	0.00	0.00
Winter*T_1	0.27	0.67	-1.03	1.58
Winter*T_2	3.52	1.22	1.13	5.91
Winter*T_3	0.86	1.29	-1.66	3.39
Winter*T_4	0.49	0.52	-0.52	1.50
Winter*T_5	0.57	0.54	-0.50	1.63

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

9.3 Manuscript 3

Effect of management on prevention of *Salmonella* Dublin exposure of calves during a one-year control programme in 84 Danish dairy herds

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Abstract

Studies reporting on how to control *Salmonella* in cattle herds have mainly been theoretical simulation models or case reports describing control of clinical salmonellosis outbreaks. The objective of this observational study was to investigate which management routines were associated with successful control of *Salmonella* Dublin in calves in dairy herds with previous signs of endemic infection. A total of 86 bulk-tank milk *Salmonella* Dublin antibody-positive bovine dairy herds were enrolled in the study in September 2008 and were all encouraged to control spread of the infection. One year later it was assessed if they were successful. The criterion for successful control was defined as the 10 youngest calves above three months of age testing *Salmonella* Dublin antibody-negative, indicating that exposure to *Salmonella* of these calves from birth until close to the day of testing had been successfully prevented. Management routines were registered through telephone interviews based on a questionnaire resulting in 45 variables for analysis.

By the end of the study, a total of 84 herds had completed the interviews and had serum samples collected from calves. Data were analysed using two statistical methods: logistic regression analysis and discriminant analysis. Both analyses identified that increased probability of successful control was strongly associated with avoiding purchase of cattle from test-positive herds. Additionally, ensuring good calving area management, separating calf pens by solid walls rather than bars and not introducing biosecurity routines between the barn sections (e.g. boot wash, change of clothing) were associated with increased probability of successful control in the logistic analysis. The latter may seem illogical, but may be explained by successful herds already having good hygienic routines in place and therefore not having introduced new routines between barn sections in the study period. The discriminant analysis furthermore identified successful control to be associated with preventing cows from calving before being moved to the designated calving pen, by only letting one person be responsible for colostrum management and by not feeding poorer quality colostrum to bull calves than to heifer calves.

The results are useful for dairy cattle producers and veterinary authorities to substantiate advice on management practices that are likely to lead to successful control of *Salmonella* Dublin.

Key words: *Salmonella*; cattle; control; management; dairy; field study

1. Introduction

Salmonellosis is a problem in infected cattle herds due to abortion, increased calf mortality, enteritis and decreased milk yield (Carrique-Mas et al., 2010). *Salmonella* (*S.*) Dublin is the serotype most frequently isolated from Danish cattle (Anonymous, 2010). Furthermore, *S.* Dublin is a zoonosis, and though rare in humans, it is more invasive and leads to higher case fatality rates than other serotypes found in hospitalised patients (Helms et al., 2003). Therefore, it is desirable to be able to control the infection in cattle herds.

S. Dublin is endemic in Denmark and in 2007, the Danish Cattle Federation initiated a campaign to eradicate *S.* Dublin from the Danish cattle population by the end of 2014 (Anonymous, 2009). As part of this programme, all dairy herds are tested every three months for antibodies in bulk-tank milk directed against serogroup D antigens by an in-house ELISA test (Eurofins Steins Laboratory A/S, Holstebro, Denmark). Test-negative herds are classified as level 1 'most likely free of *S.* Dublin' and test-positive are classified as level 2 'most likely infected with *S.* Dublin'.

Studies reporting on *Salmonella* control in cattle herds have either been case reports describing outbreak control of clinical salmonellosis or simulation studies investigating the effect of hypothetical control scenarios (Bergevoet et al., 2009; Jordan et al., 2008). A case report from a calf rearing unit described control of a *S.* Newport outbreak (Gardner et al., 2004). The outbreak was only controlled after they stopped receiving calves from affected source farms, cleaned and disinfected the rearing barns and changed several routines such as not bringing calf carts into barns and using separate coveralls and boots in each barn as well. Another case report from a calf rearing unit described the control of a *S*. Dublin outbreak (Greene and Dempsey, 1986). The calf barn was vacated, cleaned and disinfected, diseased calves were isolated and treated with antibiotics and only designated personnel with protective clothing was allowed into the isolation area. Disinfecting boot wash was used and newly introduced calves were vaccinated with a live vaccine. Jensen et al. (1994) investigated eight dairy herds involved in a control programme of *S*. Dublin over three years. The herds were all endemically infected before the start of the study and were advised on how to control *Salmonella*. The advice was mainly concentrated on calving and management of calves up to three months of age as well as preventing introduction of *Salmonella* from outside sources. The within-herd prevalences of seropositive animals were reduced from between 8% and 60% to below 5% at the end of the study. *Salmonella* control efforts and results in 10 Danish dairy herds over a three and a half year period were described by Nielsen and Nielsen (2011). In that study, control strategies included: no animals purchased from *Salmonella* test-positive herds, more focus on hygiene in the calving area and pre-weaned calf area as well as improved handling and feeding of colostrum, and testing and culling of potential carriers in some of the herds. Nine of the 10 herds managed to control *Salmonella* and prevent the calves from being exposed within an average of 13 months from implementing the control strategies.

Boqvist and Vågsholm (2005) investigated potential risk factors associated with length of restriction periods due to *Salmonella* in 112 cattle herds. They found that abundant

presence of vermin and birds and herd size to be associated with longer restriction periods. However, few other studies have included a large group of infected herds to investigate which management factors were important for successful control of *Salmonella* in dairy cattle herds, and none have included detailed management factors for different age groups. Such knowledge would benefit individual farmers, advisors and cattle associations in targeting control efforts towards those with substantiated effects. The objective of this study was to investigate which management routines were associated with prevention of *S.* Dublin exposure in calves in infected dairy herds. Furthermore, we investigated if the method of analysis influenced the conclusions of the study.

2. Materials and methods

2.1 Selection of study herds

This was a cross-sectional study with follow-up in *Salmonella* test-positive (level 2) dairy herds as the target group. The dairy herds involved in this study were selected based on two criteria. Firstly, they delivered bull calves to 21 specialised veal calf producers enrolled in a pilot *Salmonella* control project for veal producing herds and were therefore encouraged to control *Salmonella* to avoid delivering infected calves for fattening. Secondly, they had to be *Salmonella* test-positive in the national surveillance programme in September 2008. In total, 88 herd owners were asked to participate in the study and 86 agreed. The blood samples collected in the study were paid by the project funding; otherwise the herds did not receive any compensation for participating. The reason for selecting herds that were associated with veal producing herds in an associated project was that these herds were likely to be highly motivated to participate in the study and provide us with answers to the questionnaire. All herds were situated in Jutland, the main peninsula of Denmark, where most of the dairy cattle herds in Denmark are located.

2.2 Questionnaire and interviews

A questionnaire consisting of 63 questions was developed by the authors. The questions were based on literature describing risk factors for *Salmonella* infection in dairy herds and reports on control of *Salmonella* in infected herds. Questions were related to seven management topics: calving area, colostrum, pre-weaned calves, calves <6 months, heifers, cows and general biosecurity measures. Furthermore, herd demographics (e.g. herd size, number of staff employed, and *Salmonella* positive neighbour herds) were included for all herds. Herd size and purchase patterns were obtained from the Danish Cattle Database prior to interviewing farmers.

The questionnaire was incorporated into SurveyXact® (Rambøll Management Consulting, Aarhus, Denmark) and pre-tested on two people with experience with telephone interviewing and knowledge farming practices in Danish dairy production. All interviews were performed by telephone by the same experienced interviewer in October and November 2009, following specific interview guidelines. Each interview lasted on average 20-25 minutes using closed questions (Vaillancourt et al., 1991), but with opportunity to record comments if none of the provided categories fitted the answer. One

question (if any prevention measures were not possible to implement due to financial constraints) had to be omitted due to erroneous set up in SurveyXact®. The questionnaire is available on request. The interviewer asked to talk with the daily manager of the herd.

2.3 Herd classification according to antibody measurements in calves

Blood samples of calves were collected from September through December 2009 to evaluate whether calves in the study herds had been exposed to *Salmonella* during the first months after calving. The calves were tested at their birth farm and were mainly replacement heifer calves, since the bull calves had been sent off to specialised veal producers from two weeks of age. Blood tests were thus done at around the time of the interview, approximately one year after the herds were enrolled in the study. When evaluating the farmer's control efforts in the Danish control programme, the 10 youngest calves above the age of three months are tested for antibodies towards *S.* Dublin. We used the same procedure here in order to not confuse farmers and local advisors. The 10 youngest calves above the age of three months were identified via the Central Husbandry Register. Blood samples from the identified animals were collected by the farmers' local veterinary advisor, labelled and sent overnight by ordinary mail to be analysed for antibody contents at Eurofins Steins Laboratory (Holstebro, Denmark). At the laboratory the samples were recorded and stored cooled until analysis. The diagnostic test for *S.* Dublin antibodies in serum (serum ELISA) has been described elsewhere (Nielsen and Ersbøll, 2004). Briefly, this is an indirect ELISA. Microtitration plates were coated with *Salmonella* serogroup-D lipopolysaccharide antigen before serum was added to the plate wells in duplicates. To each plate known positive and negative reference serum samples were added. Immunoglobulins bound to the wells were detected by an affinity-purified horseradish peroxidise-labelled goat anti-bovine IgG (H+L) conjugate after incubation. Substrate and indicator solution were added before incubation for approximately 15 minutes in the dark. The reaction was stopped at optical density (OD) of the positive reference well between 1.5 and 2.0 and the resulting optic density of each well was read on an ELISA reader. Background corrected OD values (ODC%) were calculated from the mean OD of the sample wells and related to the mean OD of the positive control samples as described by Nielsen and Ersbøll (2004).

A calf was classified as test-positive, indicating *Salmonella*-exposure, if the antibody level was ≥50 ODC%. Otherwise it was classified as test-negative. The sensitivity of the serum ELISA at cut-off 50 ODC% for calves aged between 100 and 300 days has been estimated to be approximately 0.77 and the specificity has been estimated to approximately 0.95 (Nielsen et al., 2004a). This gives a herd sensitivity (HSe) of 0.93 (Eq. 1 and Eq. 2) (Nielsen et al., 2004b) at a given true prevalence of 25% and a herd specificity (HSp) of 0.60 (Eq. 3) (Nielsen et al., 2004b).

$$AP = Se*TP + (1-Sp)*(1-TP) \quad \text{(Eq. 1)}$$

$$HSe = 1-(1-AP)^n \quad \text{(Eq. 2)}$$

$$HSp = Sp^n \quad \text{(Eq.3)}$$

The herds were considered successful at controlling *Salmonella* if none of the tested calves were test-positive. Otherwise, herds were classified as not successful according to previous experience from field studies (Nielsen and Nielsen, 2011; Veling et al., 2002a). Sixty-nine farmers voluntarily asked for more than 10 calves to be sampled. If more than 10 calves were tested in the herd, all results for calves between three and six months were used to classify the herd.

2.4 Data registering and management

Answers were recorded online directly into the SurveyXact® database during the interviews, and a Microsoft Excel® file containing data was extracted from the database. Frequency tables were performed in Microsoft Excel® and the categories for each answer were evaluated. Based on these, the majority of the questions were categorised into two or three level variables. In total, 45 variables were created from the questionnaire and used for statistical analyses. Some questions from questionnaire were omitted since they were related to financial issues regarding *Salmonella* control on the farm while others were combined to create one variable. For example, different biosecurity routines in the cow barn such as boot change/wash, hand wash, change of clothing and other biosecurity routines were individual questions, but they were combined to one dichotomous variable: 'biosecurity routines in cow barn - Yes/No', because few herds had introduced each of the individual routines. Variables were sorted so that risk mitigating behaviour was always assigned the value '0' while risky behaviour was assigned the value '1' for dichotomous variables. For variables with three levels, '1' accounted for less risk mitigating behaviour than '0', and '2' for risky behaviour. As an example, the variables: "Number staff responsible for calving area", "number cows in calving pen at any time", "number of calvings outside calving pen during the last year", "calving pen used for sick animals and if so does it get cleaned before next calving", "frequency cleaning and drying out of calving pen", "frequency new bedding provided", "time from calving to calf removed from cow" were aggregated to one variable with two levels for the calving area management (i.e. poor vs. acceptable).

In total, there was only one missing answer from three different herds in the questionnaires. There were three very large herds (>900 animals) and therefore herd size was log-transformed to avoid over-interpretation of the herd size effect.

2.5 Statistical analyses

All analyses were performed in SAS® v. 9.2 (SAS Institute Inc., Cary, NC, USA). To identify variables that were potentially associated with successful control of *Salmonella* (i.e. with $p < 0.3$), a univariable logistic regression analysis was performed. To investigate which management routines were associated with successfully controlling *Salmonella,* data were analysed in two different ways: logistic regression analysis and discriminant analysis. Correlations between variables were tested before analyses were performed. The correlations were below 0.46 between all variables except between "Number staff employed" and herd size for which it was 0.67. These two variables were not included in the model simultaneously.

2.5.1 Logistic regression analysis

Sets of single variables were further grouped into meaningful group-variables within specific management areas. This was done to allow for inclusion of multi-collinear single variables. The group-variables were constructed as follows: One summarised score was created for each of the five management areas: i) calving area, ii) colostrum, iii) pre-weaned calves, iv) calves <6 months and v) heifers. To attempt to assign scores objectively to each herd, a scoring system was developed ignoring the questionnaire responses. The summarised score was decided by assessing each variable within a management area and assessing how important we assumed it to be for controlling *Salmonella* within the herd based on literature, empirical knowledge and biological plausibility (Nielsen et al., 2007a; Nielsen and Nielsen, 2011; Veling, 2004). If the variable was assumed to be very important, its score would be multiplied by two to increase the weight it had on the final score for the management area. Each herd was then assigned the value '0', '1' or '2' for the entire management area, depending on their summarised score for this management area. '0' represented the herds with the set of management practices least likely to control *Salmonella*, '1' herds with acceptable and '2' herds with the good practices for each of the management areas.

The group variables were used for backward selection in a multivariable logistic analysis with the outcome successful control of *Salmonella* (yes/no). To test if any of the original single variables were associated with control of *Salmonella,* these variables were introduced and tested by forward selection after model reduction for the grouped variables. We tested only single variables with $p < 0.3$ (i.e. variables included in Appendix 1) from the univariable logistic analysis and only if they were not part of any of the grouped variables that remained in the model. Two-way interactions were included for the remaining variables in the reduced model, and the criterion for retaining the variables and interactions in the model was $p < 0.05$. Deleted single and grouped variables were re-inserted in the final model to test for confounding; these were retained in the model if they changed estimates by more than 25%. The simplest model in which all included factors were either statistically significant or confounders of the main effects was chosen. The model fit was evaluated by the goodness of fit-estimate Pearson Chi-square value divided by degrees of freedom.

2.5.2 Discriminant analysis

Discriminant analysis is a method to investigate if two groups are different and if independent variables can be used to develop a prediction equation (Sharma, 1996). The principle of this method is to determine which variable combinations that best discriminate between the outcomes of interest given the data, in this case successful vs. unsuccessful control of *S.* Dublin. A linear discriminant analysis (LDA) was performed, aiming to discriminate between successful and unsuccessful herds (Eq. 4).

$$Y = C_1(V_1 - M_1) + C_2(V_2 - M_2) + \ldots + C_n(V_n - M_n) \quad \text{(Eq. 4)}$$

Where C = coefficient for variable *i*, V= value of variable *i* and M = mean of values for variable *i*, for *i*=1, 2, …, *n*.

Barn type for heifers, dry cows and lactating cows were nominal variables with three or four categories each and they had to be transformed into four dichotomous dummy variables before being included in the analysis. Thus, 49 variables based on the original 45 in the dataset were included in this analysis. Stepwise selection was used to identify the variables, and significance level for retaining the variables in the model was set at 0.15. After the initial identification of discriminating variables, prediction of success for *Salmonella* control for all herds was tested by cross-validation by leaving one herd out at the time, re-run the analysis and test if the analysis would predict success for this herd correctly.

Both analyses were performed on the full dataset as well as a subset consisting of all herds with at least 8 blood sample results (n=78 herds).

3. Results

3.1 Serology results

Two herd owners declined to participate in the study which resulted in available blood test results and completed questionnaires available from 84 herds by the end of the study period ultimo 2009. In 27 herds, there were at least one calf with antibody levels ≥50 ODC% and these were classified as unsuccessful in controlling *Salmonella.* The other 57 herds were classified as successful since all calves had antibody levels <50 ODC%. Even though the aim was to collect 10 samples from every herd this was not possible in all herds (i.e. small herds with too few calves in the right age group), while in other herds the owner requested more samples to be collected as part of their own evaluation of their control efforts. On average 25 (5^{th} to 95^{th} percentiles: 5 - 59) blood tests of calves between three and six months of age were collected per herd and included in the study. The number of blood test sampled from the herds was closely related to herd size.

3.2 Statistical analyses

3.2.1 Logistic analysis

Twelve management practices were identified as potentially associated with successful control of *Salmonella* ($P < 0.3$) by univariable logistic regression (results can be seen in Appendix 1).

Results for multivariate logistic analysis for association between successful control of *Salmonella* and management practices can be seen in Table 1. Calving management was the only group-variable included after model reduction. Originally this variable had three levels, but there was no difference between good and acceptable calving management, hence these two categories were combined to one group. Three other variables identified as potentially associated with *Salmonella* control in the univariable logistic analysis remained in this model after reduction. None of the tested interactions were found significant, and none of the non-significant variables were observed to confound the effect of the remaining variables in the model. This resulted in a simple and

meaningful model although there was indication of overdispersion (i.e. Pearson's Chi-square/df=1.6). It can be seen that the odds for successful control of *Salmonella* were 14 times higher (95% CI: 3.1 - 67) in herds where no animals were purchased from test-positive herds during 2009 compared to herds that had purchased animals from *Salmonella* test-positive herds. Herds that had introduced biosecurity routines between barns appeared to be less likely to be successful at controlling *Salmonella* than herds without biosecurity routines between barns (OR = 0.2, 95% CI: 0.1 to 0.8).

Table 1 Final model results from a multivariable logistic regression analysis of management practices found to be associated with successful prevention of *Salmonella* exposure of calves in 84 Danish dairy herds in 2008 to 2009.

Variable	Level	n	Value	SE	*P-value*	OR	95% CI of OR
Intercept			-2.5	1.1			
Calving area management[1]					0.006		
	Poor	14	Ref	-		-	-
	Acceptable	70	2.0	0.8		7.4	1.6 – 33
Separation of pre-weaned calf pens					0.01		
	Bars/partly bars	58	Ref	-		-	-
	Solid walls	26	1.7	0.8		5.4	1.3 – 24
Biosecurity routines between barns					0.02		
	No	34	Ref	-		-	
	Yes	50	-1.4	0.6		0.2	0.1 – 0.8
Purchase of animals from test-positive herds					<0.001		
	Yes	16	Ref	-		-	-
	No	68	2.7	0.8		14.5	3.1 – 67

[1]Acceptable calving area management generally included: fewer persons responsible for calving and colostrum handling, allowing a maximum of four cows in the calving area at any time, not using the calving area for sick animals, applying new bedding in calving area at least once a week, cleaning calving area at least twice a month and allowing a maximum of five cows to calve before they were moved to the designated calving area during the previous year

3.2.2 Discriminant analysis

There were three herds with missing answers for one variable; hence only 81 herds were included for selection of variables in the discriminant analysis. Class means in the discriminant analysis for successful and unsuccessful herds were calculated from Eq. 1 as the mean for the two. Four variables selected by the stepwise discriminant analysis were found to be useful in classifying successful and unsuccessful herds by cross-validation of all 84 herds (Table 2). Purchase of cattle from test-positive herds was most influential in discriminating between successful and unsuccessful herds since this factor has the highest coefficient, i.e. it contributes most to the discriminant function in Eq. 1. The negative coefficient indicates that herds that purchased cattle from test-positive herds had lower probability of successful *Salmonella* control than herds that did not

purchase animals from test-positive herds. Poorer quality colostrum for bull than for heifer calves, more than one person responsible for colostrum and higher number of cows calving before moved to calving area were also found to decrease the chance of herds being classified as successful. The fraction of correctly classified herds using cross-validation was 76%. The analysis classified 53 of 57 (93%) successful herds correctly but only 11 of 27 (41%) not-successful herds were classified correctly.

Table 2 Variables selected by discriminant analysis as potential predictors of successful prevention of *Salmonella* exposure in calves in 81 Danish dairy herds in 2008 to 2009. The mean value of the discriminant function for successful herds was 0.32 and for unsuccessful herds it was -0.67. Negative coefficient should be interpreted as the factor reducing the probability of successful control of *Salmonella*.

Variable	Coefficient	Mean of variable
Purchase of animals from test-positive herds	-2.32	0.19
Number staff responsible for colostrum handling	0.80	0.77
Number cows calved before moved to calving area	0.65	0.52
Poorer quality colostrum for bull calves than for heifers	1.21	0.76

When using the subset of 78 herds with at least 8 samples in the analyses, the same variables were found to be significant in both the analyses. Similar coefficients were found in the logistic analysis. The classification in the discriminant analysis for successful herds were 92% (47/51 correctly classified herds) while 30% of not-successful herds were classified correctly (8/27) when using the dataset including 78 herds.

4. Discussion

4.1 Results

The study included data from 84 dairy herds which mean that we were able to investigate which management practices were associated with effectively preventing calves from being exposed to *S*. Dublin. Previous observational studies were either case reports of acute *Salmonella* outbreaks in calves (Gardner et al., 2004; Greene and Dempsey, 1986) or case reports of control efforts in few herds (Jensen et al., 1994; Nielsen and Nielsen, 2011) and hence no statistical analyses of the effect of different management practices were possible in those studies.

Independent of the method of analysis, we consistently identified purchase of animals from test-positive herds as a significant risk factor for unsuccessful *Salmonella* control in the herd. A logistic regression analysis of the original 45 ungrouped variables also resulted in this variable associated with successful control of *Salmonella* (results not shown). Purchase of animals has been reported to be a risk factor for introducing *Salmonella* into herds (Morton, 1996; Nielsen et al., 2007b) as well as other infectious diseases (Ortiz-Pelaez and Pfeiffer, 2008), so it is possible that purchase of animals kept re-introducing *Salmonella* to the herds or increased the spread of infection in the herd due to mixing of animals with different immune statuses.

Herds that had not introduced biosecurity routines between barn sections appeared to be more successful preventing *Salmonella* infection in calves born in the herds than herds that had introduced biosecurity routines between barn sections according to the logistic regression analysis. This was also found in logistic regression analysis of ungrouped variables (results not shown). It is not a biological plausible finding, and it might be explained by farmers with more severe *Salmonella* problems in their herds at time of inclusion in the study having implemented more biosecurity routines between barn sections due to the infection. These farmers would also be more aware of the biosecurity routines they have put in place and thus be more likely to answer that they had such measures in place when answering the questionnaire. Furthermore, farmers with severe *Salmonella* problems (i.e. high prevalence of *Salmonella* infected cows) might have reduced *Salmonella* in the herd but not managing to completely control exposure of the calves and hence be classified as unsuccessful in this study.

Recognising that management practices are rarely independent of each other in a dairy herd we grouped variables that arose from single questions posed to the farmers for the different management areas. Management practices of the calving area was significantly associated with the probability of successful control of *Salmonella,* and several of the risk factors included in this variable have been found to increase the risk of *Salmonella* in calves in other studies. These include using the calving pen as recovery pen for sick animals (Fossler et al., 2005; Losinger et al., 1995) and not providing clean environment in calving pen so that calves were born in *Salmonella* infected environment (House and Smith, 2004) and < 90% of cows calving in a designated calving area (Weber et al., 2009). In the study by Jensen et al. (2004) of six herds undergoing control programmes, several routines for prevention in the calving pen were included such as: calves removed immediately after birth, only one cow in calving pen at a time, clean and well bedded calving pen and no use of calving pen as sick pen.

The groupings into management areas in our study were done as objectively as possible although the values assigned to each management routine were weighted by the authors. It is possible that if the weighting of the variables had been done differently other management practices might have been found to be associated with successful control of *Salmonella.*

The discriminant analysis found four variables relevant to discriminate between successful and unsuccessful herds. In addition to purchase from test-positive herds, number of cows calving outside the designated calving area was also included in the calving area variable identified by the logistic analysis. The two other variables were related to colostrum management, namely number of staff responsible and poorer quality colostrum for bull than for heifer calves. Others have found that poor handling of milk and colostrum (including pooling) (House and Smith, 2004) was a risk factor for *Salmonella* in cattle herds. Overall the four variables from the discriminant analysis were better at predicting the correct outcome for successful herds (93%) than unsuccessful herds (41%). Another four variables were selected by the discriminant analysis; Biosecurity routines between barns, heifer barn type, weaning age and number of calves that a pre-weaned calf was able to have physical contact to. Including these four variables in the cross-validation meant that correct classification of unsuccessful herds by cross-validation increased from 11 to 15 of the 27 (56%), but correct classification of

successful herds decreased from 53 to 48 (84%) and overall one herd less was classified correctly. Hence, we chose to only include the four variables. Assessing the variables identified by this analysis might be useful when evaluating if a specific herd is likely to be able to control *Salmonella*, however the results need to be validated by applying them to other datasets where it is known if the farmer is successful or not in controlling *Salmonella*.

4.2 Herd classification with regard to Salmonella control

The ELISA test used in this study detects antibodies against *S*. Dublin although it might cross-react with other *Salmonella* serotypes (Konrad et al., 1994). This is most often *S.* Typhimurium in Denmark. Bacteriological cultures are rarely available from endemically infected herds in Denmark, so we have no way of telling which ones were infected by which serotypes. However, since the most commonly isolated *Salmonella* serotype in Danish cattle is *S.* Dublin (Anonymous, 2010), we expect the majority of herds in this study to have been infected with *S.* Dublin. This serotype is host adapted to cattle (Wray and Sojka, 1977), so it is possible that the management practices identified in this study to be associated with successful control of *Salmonella* are specific for *S.* Dublin. Other management practices might be associated with controlling other, non-host adapted serotypes.

We used antibodies against *S.* Dublin in calves aged three to six months to distinguish between successful and unsuccessful herds. This reduced the risk of false negative herds compared to using faecal shedding, since shedding of *Salmonella* is intermittent (House et al., 1993). However, there is a risk that the herds with the fewest tested calves were false negative. We tried to prevent this by comparing blood results to BTM level and found that the herds with few negative blood results also had low BTM antibody levels, so we feel confident that there was no exposure of calves to *Salmonella.* Furthermore, when excluding herds with fewer than eight samples from the analysis (six herds), the same variables were found to be significant, which increases our confidence in the results. The sensitivity for detecting single *S.* Dublin infected animals by antibodies is 77% with an ELISA cut-off at 50 ODC% (Nielsen et al., 2004a), and Veling et al. (2002a) found a herd sensitivity of 91% when testing all calves between 4 and 6 months of age for antibodies. Because we were interested in *S.* Dublin-exposure of the calves as a group, we would expect a high sensitivity, but there is a small risk that we could have misclassified some herds as successful in controlling *Salmonella* when in fact some calves might have been exposed and become infected. The misclassification could for example be due to the small risk that recent *Salmonella* infected calves were serology positive, since it can take up to two weeks for seroconversion (Da Roden et al., 1992; House et al., 2001). Traditionally, bacteriological culture has been seen as the gold standard method to indentify *Salmonella* infected cattle. However, the sensitivity of this method is as low as 5-17% in infected animals without clinical sign (Nielsen et al., 2004a; Nielsen et al., 2011). Moreover, we were interested in identifying exposed animals, i.e. both previously and currently infected animals, bacteriology would have added limited information to the classification of the herds. The specificity of the ELISA has been reported to be 95% for identifying infected animals (Nielsen et al., 2004a). Thus, the ELISA test will identify some previously infected calves as false positive. As we were

interested in *S.* Dublin exposed and not only currently infected calves, the specificity for the ELISA test in this study is less relevant.

4.3 Data quality

We were unable to pre-test the questionnaire on farmers, because several of the questions were related to the control programme these farmers participated in, and with only 88 potential participants we felt that none of them could be spared for pre-testing. However, the questionnaire was discussed with two persons who had experience with questionnaires and Danish cattle farming. The use of telephone for interviewing meant that we could perform reliability interviews and the majority part of the questions had similar answers in both rounds for the 9 herds included (data not shown). We were only able to validate nine of the 45 questions by herd visits in the 9 herds, namely those that could be visually evaluated at a visit such as type of separations between calf pens. This showed some difference in how many farmers answered correctly for two questions, with three answers in the wrong category (data not shown), but the overall validity and reliability appeared to be acceptable.

The management practices investigated in this study covered a broad range of areas and animals on the farms. Other management practices than the ones investigated by the questionnaire have been reported to be associated with *Salmonella* in cattle and might be relevant for control success. Boqvist and Vågsholm (2005) reported that abundance of vermin was seen on *Salmonella* infected farms, and Tablante and Lane (1989) reported of a closed dairy herd where *S.* Dublin was only isolated from one diseased calf but from several mice. They therefore speculated that mice served as reservoir for *S.* Dublin. Presence of cats on farms has been reported to be associated with increased odds of clinical outbreaks of *S.* Typhimurium in a case-control study (Veling et al., 2002b). Due to the Danish on-farm quality control scheme administered by the dairy organisations, it must be expected, that most of the dairy herds have some sort of rodent control in place, but the presence of cats or dogs in the barns could be a problem in Danish dairy farms. Culling of carriers or even the whole herd to control or eliminate *Salmonella* has been practised as well (Boqvist and Vågsholm, 2005; Sternberg et al., 2008). The overall purpose of the Danish *Salmonella* Dublin programme is to eradicate S. Dublin. However, this study does not allow for conclusions on elimination of *Salmonella* from the herds. In Denmark, potential carriers are typically identified by repeated serological tests and not by bacteriology. The advice for herds undergoing control is not to cull carriers until calves are sero-negative to *Salmonella*, indicating that there is no spread of *Salmonella* from the cows to the calves. We did investigate if any additional tests at animal level were done and if these were used in the management (i.e. to identify and cull carriers). This was not found to be associated with control of *Salmonella* in the analyses.

Herds included in this study had on average 223 cows while the national average was 121 cows in spring 2009 for dairy herds (Knowledge Centre for Agriculture, Cattle, Aarhus, Denmark). Larger herds have increased risk of being *Salmonella* infected (Adhikari et al., 2009; Cummings et al., 2009; Nielsen et al., 2007b), which is a probable reason for the large herds included in this study. Different breeds (mainly Danish Holsteins and Jersey) as well as both organic and conventional herds were included in

this study, so we expect the herds to represent Danish dairy herds well. Some herd owners raise bull calves for slaughter rather than sell them to specialised veal calf producers like in this study and it is possible that management practices are different in these farms. However, we do not suspect this to affect calving area or management of pre-weaned calves, which were selected as associated with successful *Salmonella* control in this study and hence we expect the results to be valid for all Danish dairy farms.

4.5 Statistical analyses

Most of the variables included in the analyses were categorical variables. Linear discriminant analysis assumes that variables are approximately normally distributed, but the method has been shown to be reliable if the majority of independent variables are dichotomous (Lachenbruch, 1975), which is the case in this study. Still, the difference in the results between the two methods illustrates that the method of analysis of data influences the conclusions that can be drawn from the study.

5. Conclusion

The management practice most consistently and strongly associated with successful control of *Salmonella* in dairy herds appeared to be not purchasing animals from *Salmonella* test-positive herds. Other management practices associated with successful control related mainly to the management in the calving area, for example allowing a maximum four cows in the calving area at any time, avoid using the calving pen for sick animals, cleaning calving pen at least twice a month and providing new bedding at least once a week. Management of colostrum and young calves also appeared to be important for successful control. The results in this study were dependent on which method of analysis was applied.

Acknowledgements

The authors would like to thank farmers participating in the study, Mette Høst Hammershøj and Anne Mette Graumann, Agrotech A/S, Kristian Kristensen, Faculty of Agricultural Sciences, Institute of Animal Health and Bioscience, University of Aarhus, Denmark and Anette Boklund, Danish Veterinary Institute.

Conflict of interest

The authors declare they have no conflict of interest.

Funding

Sampling and laboratory tests were funded by The Danish Cattle Federation, Denmark.

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9.4 Manuscript 4

Gross margin losses due to *Salmonella* Dublin infection in Danish dairy cattle herds estimated by simulation modelling

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Abstract

Salmonella Dublin affects production and animal health in cattle herds. The objective of this study was to estimate losses in gross margin (GM) following introduction and spread of *S.* Dublin in dairy herds.

GM losses were estimated using an age-structured stochastic, mechanistic and dynamic simulation model. The model incorporated six age groups (neonatal, pre-weaned calves, weaned calves, growing heifers, breeding heifers and cows) and five infection stages (susceptible, acutely infected, carrier, super shedder and resistant). The effects of introducing one infectious heifer were estimated through 1000 simulation iterations for 12 scenarios. These 12 scenarios were combinations of three herd sizes (85, 200 and 400 cows) and 4 management levels (very good, good, poor and very poor). Input parameters for *S.* Dublin effects on production and animal health were based on literature and calibrations to mimic real life observations. Mean annual GM per stall were compared between herds that experienced spread of *S.* Dublin and non-infected reference herds for 10 years after introduction of infection in the simulation model.

Estimated GM losses were highest in the first year after infection, and increased with poorer management and herd size. E.g. annual GM losses were estimated to on average 57 Euros per stall for the first year after infection, and to 9 Euros per stall averaged over the 10 years after herd infection for a 200 cow stall herd with very good management. In contrast, a 200 cow stall herd with poor management would lose on average 315 Euros per stall in the first year, and 196 Euros per stall per year averaged over the 10-year period following infection. The losses in GM arose from both direct losses such as reduced milk yield, dead animals and abortions as well as indirect losses such as reduced income from sold heifers and calves, and lower milk yield of replacement animals. Sensitivity analyses estimated that assumptions about milk yield losses for cows in the resistant or carrier stage had the highest influence on estimated GM losses, and that this effect was more influential the poorer the management was. This was due to increasing number of cows becoming infected in poorer management scenarios.

Results from this study can be used to encourage farmers to prevent introduction and control spread of *S.* Dublin within the herd. Furthermore, it can be used in future cost-benefit analysis of control actions for *S.* Dublin both at herd and sector level.

Keywords: *Salmonella* Dublin; Economic; Effects; Animal Health Economics; Simulation model; Dairy cattle;

1. Introduction

Salmonella Dublin is a host adapted pathogen of cattle (Wray and Sojka, 1977; Uzzau et al., 2000). It can cause diarrhoea, pneumonia and death in calves and adult cattle (Vandegraaff and Malmo, 1977; Greene and Dempsey, 1986) as well as abortion and decreased milk yield in cows (Vandegraaff and Malmo, 1977; Morton, 1996; Carrique-Mas et al., 2010). Infected animals can become carriers that shed the bacteria intermittently in faeces for prolonged periods (Spier et al., 1990; Wallis, 2006). *S.* Dublin has been reported to survive for long periods in the environment, e.g. in wet and dried faeces (Findlay, 1972; Plym-Forshell and Ekesbo, 1996) and to persist in cattle herds for several years (Clegg et al., 1986; Boqvist and Vågsholm, 2005).

The effects on production and other economic effects of *S.* Dublin in dairy herds are not well specified. Economic effects can be reported as losses, which are missed benefits (e.g. discarded milk or reduced milk yield due to disease) or costs which are the sum of losses and control expenditures (McInerney et al., 1992; Rushton et al., 1999). Expenditures are extra resources used as a consequence of the disease (e.g. veterinary fees and disease control measures). Bazeley (2006a) estimated the costs of a *S.* Dublin outbreak in a dairy herd consisting of approximately 100 cows. Clinical effects such as abortions and decreased milk yield in cows, and diarrhoea and death among calves lasted for approximately two months. During this period, the costs due to the outbreak were estimated to be approximately £7870 of which almost £3600 were due to decreased milk yield. Visser et al. (1997) estimated the average losses due to *S.* Dublin infection in 40 dairy herds to be around 5000 Dutch Guilders for the period of infection. They included extra veterinary and labour costs in the losses. Herds were included in that study following one positive bacteriology culture of blood or tissue from aborted foetuses.

Milk yield in diseased cows has been reported to decrease markedly or even stop entirely in some cases (John, 1946; Vandegraaff and Malmo, 1977), but there are few reports quantifying the yield losses in infected cows without clinical signs. Bazeley (2006a) investigated an outbreak in a 100 cow herd with average yearly milk yield of 7000 L per cow. Abortions were the main clinical sign of *S.* Dublin, and the estimated total herd loss in milk yield was 19430 L over a period of approximately two months. Nielsen et al. (2012b) investigated changes in energy corrected milk yield (ECM) in three-months intervals at cow level for parity 1, 2 and 3 and older cows (3+) following sudden high increases in *S.* Dublin antibodies directed against O-antigens in bulk-tank milk indicative of new *S.* Dublin infection in the herd. In that study, it was found that mean daily milk yield was deceased by 1.4 Kg ECM per cow in the period seven to 15 months after estimated herd infection, while it was reduced by 3.0 Kg ECM per cow per day in the same period for parity 3+ cows. Parity 2 cows mainly had reduced yield 13 to 15 months after estimated herd infection. In another study, a herd with clinical cases of *S.* Anatum for one month was reported to have decreased milk yield for a total of four months after the first case (Glickman et al., 1981). Kahrs et al. (1972) reported that it took six months from the beginning of a *S.* Typhimurium outbreak before herd milk yield was back to pre-infection levels.

In 2007, a Danish *S.* Dublin control programme was initiated in which all dairy herds are tested for *S.* Dublin antibodies in bulk-tank milk every three months and classified into three categories (Anonymous, 2009). The aim of the programme is to eradicate *S.* Dublin from the Danish cattle population by the end of 2014. It requires compliance from farmers in infected herds to reach this goal. As part of the programme, advice on control of *S.* Dublin has been communicated to the farmers. Studies have shown that it is possible to control *S.* Dublin with management changes (Jensen et al., 2004; Nielsen and Nielsen, 2011; Nielsen et al., 2012c), but that it is unlikely that culling of active carriers alone will lead to complete control (Veling, 2004). Control efforts have to be implemented over months to years to effectively control and possibly eradicate *S.* Dublin from the herd (Jensen et al., 2004; Nielsen and Nielsen, 2011) due to both the survival of the bacteria in the environment and the carrier state of *S.* Dublin. This means that control of this infection can be costly, and it is therefore necessary to get an overview of the losses infection causes in the herd in order for farmers to decide on control options. Furthermore, it is in the interest of the cattle industry to know the losses of outbreaks and consecutive endemic infections, and potential benefits associated with control and eradication of this infection in the dairy sector in order to prioritise and plan future disease control strategies.

It is difficult to estimate the economic and production effects of *S.* Dublin under different production conditions based on observational data, mainly because it is almost impossible obtain good information about the infection stages of individual cattle over time. Instead simulation studies can be used to estimate these. Previous simulation studies of *Salmonella* have focused on transmission parameters within the herd as well as introduction and persistence of infection in the herd (Xiao et al., 2006; Nielsen et al., 2007; Lanzas et al., 2008; Chapagain et al., 2008). Bergevoet et al. (2009) investigated cost and cost-effectiveness compared to the reduction in herd *Salmonella* prevalence of different national control strategies for Dutch *Salmonella*-infected herds at national level. However, there were no estimations of losses associated with the disease at herd level in these studies.

The objective of this study was to estimate the gross margin (GM) losses of introduction and spread of *S.* Dublin in dairy herds up to 10 years after introduction of the infection. Results can be used to inform farmers and farmers' organisations of the potential benefits of preventing and controlling *S.* Dublin infection in dairy herds.

2. Materials and methods

2.1. Structure of the Dublin-Simherd model

The "Dublin-Simherd" model used in this study is a further development of the Simherd model, which is a stochastic, mechanistic and dynamic simulation model (Østergaard et al., 2000). The Simherd model has been developed to simulate the real situation in Danish dairy herds and incorporates the complex feedback mechanism between feeding, reproduction and culling. It is used to simulate the production and state changes of animals, including young stock, in dairy herds in discrete weekly time steps. Individual

discrete events (e.g. death, disease, heat detection, conception etc.) are triggered stochastically using random numbers from relevant distributions. Variables describing general management are specified to represent a typical management of a dual-purpose (milk and meat) dairy cattle herd of large breed. These are described in Østergaard et al. (2003). Simherd is used commercially for herd health consultancy and more information about the model is available at: www.Simherd.com (accessed January 4th 2012).

2.2. Simulation

2.2.1. S. Dublin infection

The basic Dublin-Simherd model which models the within-herd epidemiology of *S.* Dublin infection is described in detail elsewhere (Nielsen et al., 2012a). Briefly, the population dynamics are mimicked by simulation of individual objects (animals) stored in computer memory in one of six age groups in each weekly time step: Neonatal (0 to 7 days), pre-weaned calves (1-7 weeks old), weaned calves (8-22 weeks old) growing heifers (23 to 59 weeks old), breeding heifers (60 weeks old to first calving) and cows (from first calving until culling or death). Superimposed on this herd structure, animals are virtually allocated to one of five infection stages: Susceptible, acute infection, super shedder, carrier and resistant. The probability that susceptible animals become acutely infected depends on contact structures, age-dependent susceptibility of the individual and number of infectious animals in the barn section and in the whole herd. The duration of each infection stage are determined by distributions, and the duration of the resistant stage increases each time the animal is infected. The number of infectious contacts is determined by four hygiene levels, and four herd susceptibility levels indicating different susceptibility parameters for the individual animals in each of the six age groups in the herd. Animals in the younger age groups in the model are assumed to have higher susceptibility to *S.* Dublin than older age groups. The model keeps track of the infection stage of every animal in each weekly time step. Number of deaths and abortions as well as infected and clinically ill animals during a 10-year period after introduction of the infection were reported for each relevant age group in 48 scenarios representing all combinations of three herd sizes, four hygiene and four susceptibility levels in Nielsen et al. (2012a).

2.2.2. S. Dublin effect on milk yield

The effects of *S.* Dublin on milk yield of individual cows in the Dublin-Simherd model were calibrated to obtain the same herd level pattern for yield loss in parities 1, 2, and higher parity cows found by Nielsen et al. (2012b). They modelled milk yield for 18 months after estimated herd infection. However, there were indications that the herd infection date might have been set too late in that study, and that the milk yield losses possibly started earlier after the alternative herd infection date. This would make sense, because the milk yield would most likely be highest in acutely infected cows in the beginning of the outbreak. Hence, milk yield losses for two years after the alternatively estimated herd infection from that study were used to calibrate the milk yield losses associated with each infection stage in the Dublin-Simherd model. Milk yield losses were calibrated for 85 cow herds, the average herd size in the study by Nielsen et al. (20012b), and herds with hygiene and susceptibility levels corresponding to poor

management (see below).This way the best estimate of losses in ECM in the Dublin-Simherd (Table 1) was calibrated, so that the losses for each of the three parity-groups first, second and third or older cows had similar pattern and size to the loss for the cows in the respective parities reported in the study by Nielsen et al. (2012b). In data from Nielsen et al (2012b), it was found that second parity cows on average lost 3% more ECM and parity 3+ cows on average lost 11% more ECM in the two-year period than first parity cows. When modelling the milk yield losses, acutely infected cows were divided into acutely infected with clinical signs and acutely infected without clinical signs (Table 1). Super shedders were modelled with the same milk yield losses as acutely infected without clinical signs, and carriers were modelled with the same milk yield losses as resistant cows. Susceptible cows were assumed not affected by *S.* Dublin being present in the herd.

Table 1 Percentage lost energy corrected milk yield (ECM) compared to that of cows in non-infected herds used to model the production effects of *S.* Dublin in the Dublin-Simherd model simulations for parity 1, 2 and 3+ cows. The table includes losses used as default (best estimate obtained through calibration of model settings to fit observations from 28 real life case herds) and for sensitivity analysis (minimum and maximum).

		Parity 1	Parity 2	Parity 3+[1]
Acutely infected (clinically ill)				
	Minimum	30%	30%	30%
	Best estimate	70%	71%	73%
	Maximum	90%	90%	90%
Acutely infected (not clinically ill), or super shedder				
	Minimum	10%	10%	10%
	Best estimate	30%	31%	33%
	Maximum	50%	50%	50%
Resistant or carrier				
	Minimum	0%	0%	0%
	Best estimate	7%	8%	10%
	Maximum	20%	20%	20%

[1]parity 3 and higher

2.2.3. Simulation

The effects of *S.* Dublin introduction into dairy herds were modelled in the following way: One infectious heifer without clinical signs was introduced into the herd four weeks before calving. Due to stochasticity and depending on specified management, infection could then spread to one or more animals (including its own calf in the week it was born), or not spread at all. We simulated three herd sizes: 85 (mean herd size in the 28 case

herds in Nielsen et al. (2012b)), 200 (medium sized Danish dairy herd) and 400 cows (large Danish dairy herd). From the original 16 combinations of herd hygiene and susceptibility levels simulated in Nielsen et al. (2012a), four were used for simulations in this study. These were classified as very good, good, poor and very poor management level, corresponding to the best, two intermediate and worst management levels from Nielsen et al. (2012a). This resulted in 12 scenarios (one for each herd size and each of the four management levels) and 1000 iterations were performed for each scenario. These management levels were based on herd susceptibility and hygiene levels. General management variables were kept equal across all simulations. Management levels in this study are therefore only concerning the herds' and animals' risk of becoming infected with *S.* Dublin and not general management as such. Only iterations in which infection spread from the introduced heifer were used in further analyses of GM losses, and estimates were summarised per year. No specific control efforts directed against *S.* Dublin were included in the simulations.

GM was in this study defined as income minus variable costs. To estimate the GM losses attributed to *S.* Dublin, discounted GM in Euros and ECM were compared to 1000 simulations with the same management settings and herd sizes, but where no infectious heifer was introduced to the herd (non-infected herds). GM per cow stall for the non-infected herds was calibrated to be similar to GM per cow stall in Danish large breed herds in December 2011. The following effects of *S.* Dublin were included in the model: risk of animal becoming infected and risk of becoming clinically ill if infected (specified for each of the six age groups), mortality of clinically ill animals (specified for each of the six age groups), milk yield losses (for acutely infected clinically ill, acutely infected not clinically ill/super shedders and resistant/carriers), abortions and treatment costs. Risk of infection, risk of becoming clinically ill and mortality of clinically ill animals were all assumed highest for the youngest calves and lowest for the adult cows (Nielsen et al., 2012a). Mortality was dependent on whether the animal was treated or not; it was assumed that the farmer would recognise 75% of the clinically ill animals and that these would be treated. The price for treatment was obtained from the Knowledge Centre for Agriculture, Cattle, and was set to 36 Euros for a calf <50 days, 30 Euros for a calf aged 50 to 149 days and 70 Euros for animals >154 days. GM losses were summarised per cow stall rather than per cow, because herd size varied the first years after herd infection due to increased slaughter of low yielding salmonella-infected cows.

2.2.4. Sensitivity analysis

Sensitivity analyses were performed in order to evaluate which input parameters were most influential on the results of the simulations. In the sensitivity analyses, we used the herd size 200 and included all four management levels. For each management level, 10 different settings were used resulting in 40 scenarios that were compared to the non-infected herd. Firstly, three scenarios were simulated to assess the effects of changed assumptions regarding milk yield losses on the GM. These included 1) assuming no yield loss in resistant and carrier cows, 2) assuming no yield loss in acutely infected cows without clinical signs and super shedders and 3) assuming no yield loss in acutely infected cows with clinical signs. Next, four scenarios were modelled in which disease effects associated with *S.* Dublin were excluded: 4) no *S.* Dublin-associated abortions, 5) no *S.* Dublin-associated calf mortality, 6) no *S.* Dublin-associated mortality in adult cows

and 7) no clinical disease effects of *S*. Dublin including associated treatment costs and mortality. Best estimate milk yield losses were still included in this scenario. Lastly, GM was estimated by using what was presumed to be 8) minimum realistic estimates from literature of all effects, 9) maximum realistic estimates and 10) assumed best estimates, except that milk yield effects were set to minimum realistic estimates. The estimates for input parameters in the model used in sensitivity analysis scenarios 8 to 10 can be seen in Table 1 and 2.

Table 2 Minimum and maximum parameter estimates concerning probability of disease, mortality and abortions used in sensitivity analyses of model assumptions in the Dublin-Simherd model.

Parameter	Neonatal calves	Pre-weaned calves	Weaned calves	Growing heifers	Breeding heifers	Adult cows
Probability of clinical disease in acutely infected						
Minimum	0.10	0.10	0.05	0.05	0.05	0.05
Maximum	0.80	0.80	0.50	0.30	0.30	0.30
Probability of dying if clinically ill from S. Dublin and not treated						
Minimum	0.50	0.40	0.10	0.05	0.05	0.02
Maximum	0.95	0.85	0.60	0.30	0.30	0.30
Probability of dying if clinically ill from S. Dublin and treated						
Minimum	0.50	0.30	0.10	0.02	0.02	0.01
Maximum	0.95	0.80	0.50	0.30	0.30	0.30
Probability of abortion if acutely infected						
Minimum	NA	NA	NA	NA	0.02	0.02
Maximum					0.15	0.15

3. Results

3.1. Simulation results

The simulated annual mean GM per stall averaged over 10 years for the reference herds with no infectious heifer introduced were 1319 (5^{th} to 95^{th} percentiles: 1170 to 1460), 1370 (1254 to 1477) and 1344 (1266 to 1417) Euros per stall for 85, 200 and 400 cow herd, respectively. There was no difference between management levels in the reference herds. The mean annual milk yield averaged over 10 years was 9482 (9233 to 9727), 9647 (9483 to 9809) and 9589 (9472 to 9707) Kg ECM per cow per year for 85, 200 and 400 cow herds, respectively, again with no difference between management levels.

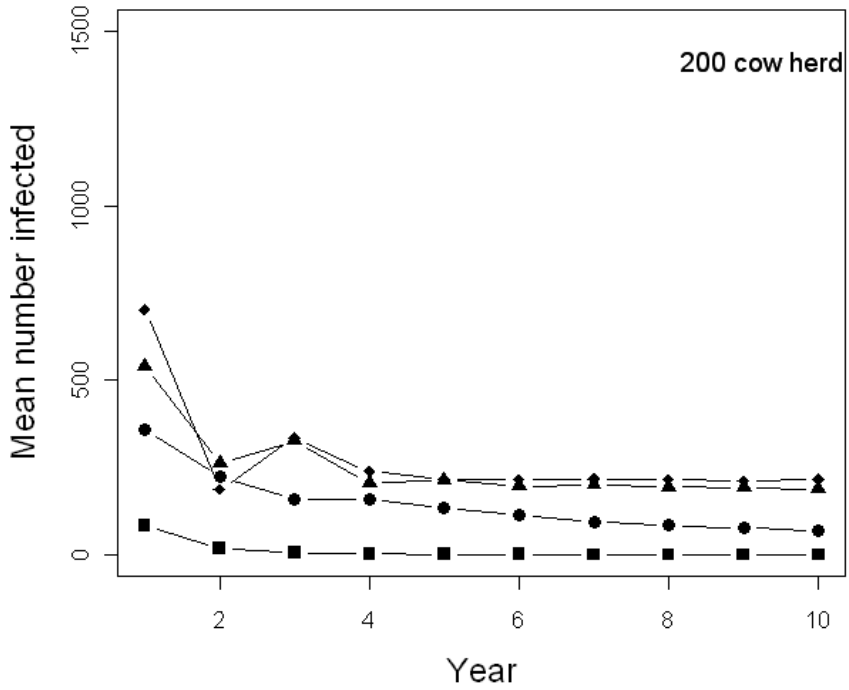

Figure 1 Model predicted mean annual number of *S.* Dublin infections in a 200 cow stall dairy herd (multiple infections occurred in some animals). Estimates were derived from 1000 iterations of the 10 years following introduction of one infectious heifer and only iterations in which spread of *S.* Dublin occurred were used. ■ corresponds to very good, ● good, ▲ poor, and ♦ very poor management.

Estimated number of infected animals and duration of herd infection were reported by Nielsen et al. (2012a). The simulated annual mean number of infections in the 200 cow herd can be seen in Fig. 1. The number of annual infections can be higher than the herd size, because animals can become infected more than once per year, if infection is still present in the herd after they return to the susceptible state. The management level influenced how long the infection persisted in the herd with median number of infected animals reaching 0 in year four after introduction of *S.* Dublin in the very good management level. For poor and very poor management levels, the mean annual number of infections appeared to be stabilising at around 260 (Fig. 1). This indicated presence of active spread in some of the iterations in all 10 years in these scenarios, although the infection disappeared in some iterations after the fourth year (results not shown). The estimated losses in ECM were correlated to the number of infections, and the poorer management levels were estimated to have the largest and most prolonged losses (Fig. 2).

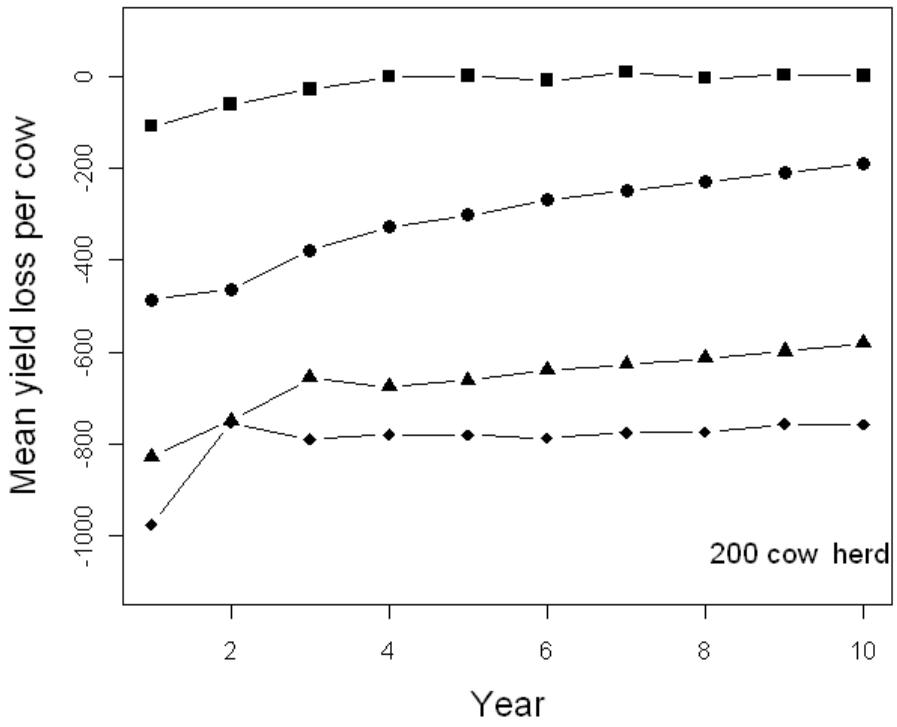

Figure 2 Model predicted difference in mean annual energy corrected milk yield per cow between *S.* Dublin infected and non-infected herds in a 200 cow stall dairy herd (mean yield losses per cow). Estimates were derived from 1000 iterations of the simulated 10 year period. ■ corresponds to very good, ● good, ▲ poor, and ♦ very poor management.

Estimated annual mean GM per stall was 1361 (1252 to 11474), 1271 (1145 to1404), 1171 (1054 to 1307) and 1141 (1014 to 1288) Euros for very good, good, poor and very poor management, respectively, in the 200 cow stall herd averaged over the 10 years after introduction of infection. Similarly, differences in annual mean GM per stall averaged over the 10 years were estimated. For very good management, GM differences reached 3 (-41 to 35), 9 (-35 to 16) and 12 (-43 to 11) Euros between infected and non-infected herds for 85, 200 and 400 cow stall herd, respectively (Fig. 3). For very poor management the differences per stall were lower for the 85 cow stall herd, i.e. -164 (-238 to -52) Euros compared to the larger herds, -230 (-272 to -197) and -232 (-255 to -207) Euros for 200 and 400 cow stall herds, respectively.

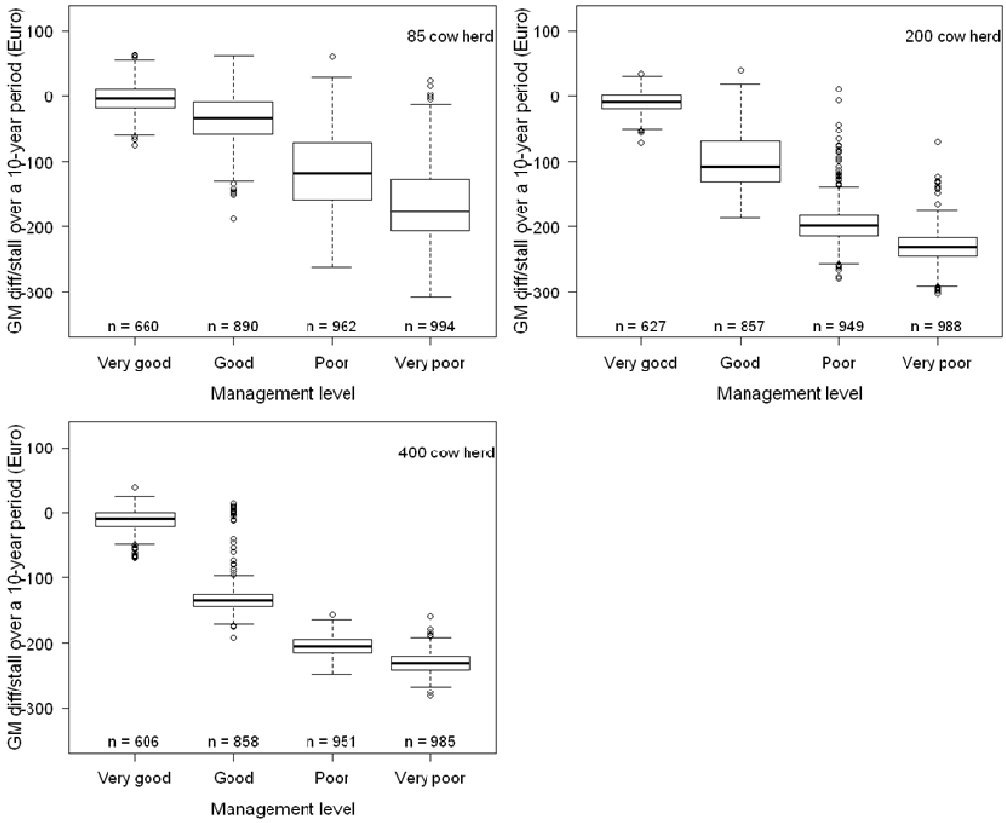

Figure 3 Model predicted difference in annual mean gross margin (GM) per stall in Euro averaged over the 10 years after *S.* Dublin herd infection between infected herds and non-infected herds for three herd sizes (i.e. 85, 200 and 400 cows) and four management levels (i.e. Very good, Good, Poor and Very poor). Estimates were derived from 1000 iterations, and n denotes the number of iterations in which spread of *S.* Dublin occurred. These were the iterations used to calculate the loss in gross margin.

Fig. 4 illustrates the GM losses per year over the simulated 10-year period. For very good management GM losses mainly occurred in the first year after herd infection. GM losses increased from year three to four after herd infection for good, poor and very poor management in the 200 and 400 cow stall herds. This is likely to be due to the increase in number of infected animals in year three (Fig. 1).

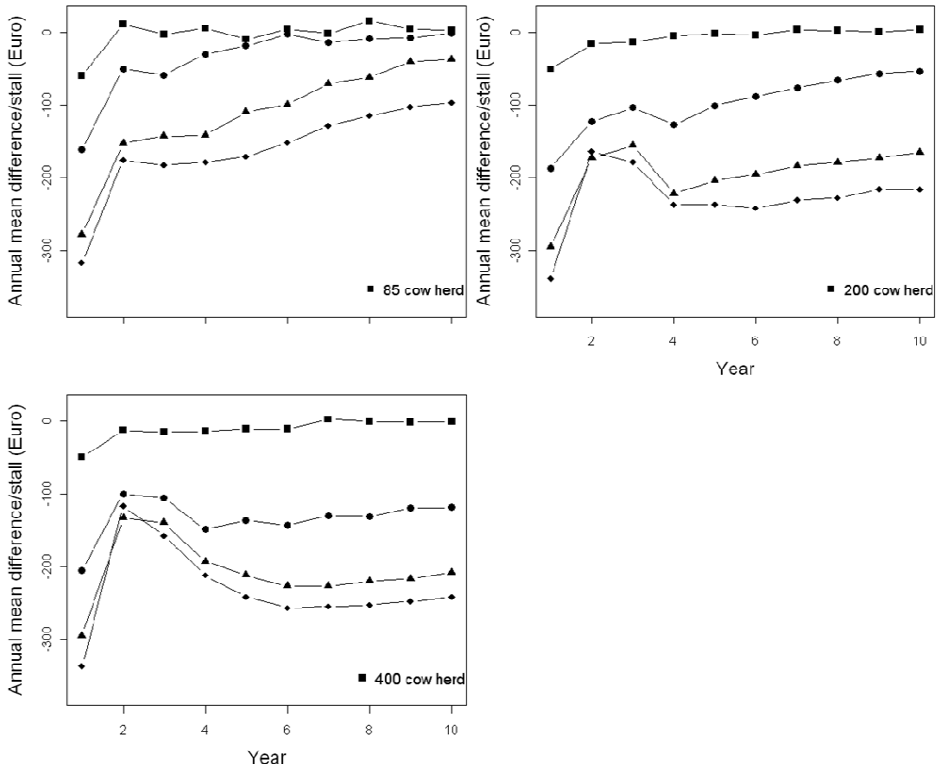

Figure 4 Model predicted difference in annual mean gross margin (GM) per stall in Euro for the 10 years after *S.* Dublin herd infection between infected herds and non-infected herds for three herd sizes (i.e. 85, 200 and 400 cows) and four management levels (i.e. Very good, Good, Poor and Very poor). Estimates were derived from 1000 iterations. These were the iterations used to calculate the loss in gross margin.

3.2. Sensitivity analysis results

Table 3 provides sensitivity analysis estimates of GM losses per stall in the first year after *S.* Dublin herd infection and averaged annual GM losses per stall over the 10 years after herd infection in a 200 cow stall herd. It can be seen that GM losses per stall in the first year for the best estimate scenario were 57 Euros for very good management. Likewise, mean GM losses per stall were on average 9 Euros per year in the 10 years after herd infection for this scenario. The annual mean GM losses per stall averaged over 10 years were lower than losses in the first year after herd infection independent on management levels and magnitude of the effects simulated in the sensitivity analyses. Increasing all *S.* Dublin effects to assumed maximum realistic estimates increased GM losses per stall more the poorer the management level was. Reducing all milk yield effects of *S.* Dublin by 50% reduced GM losses per stall more for very poor management than for very good management. This followed the pattern of simulating no milk yield

losses in resistant or carrier cows which reduced the GM losses more, the poorer the management.

Mean GMs per stall and the 5th and 95th percentiles for the sensitivity scenarios, best estimate and non-infected herd in absolute values are shown in Fig. 5 averaged for the 10 years after herd infection. Simulating no yield losses in resistant or carrier cows, no yield effects at all or reduction of all effects resulted in relatively small averaged GM losses per stall over 10 years compared to the non-infected herd for good, poor and very poor management (Fig. 5). All simulated sensitivity scenarios resulted in relatively small averaged GM losses per stall for very good management for the 10 years after herd infection. Only when all effects were increased did it result in much higher losses than any other scenario for very good management level.

Table 3 Model predicted differences in mean annual gross margin (GM) per stall between infected and non-infected under the assumptions used in the sensitivity scenarios specified in Table 2, and for the best estimate scenario. Estimates are given in Euros for the first year and averaged over 10 years in a 200 cow stall. Estimates were derived from 1000 iterations simulating *S.* Dublin introduction into Danish dairy herds in the Dublin-Simherd model and compared to 1000 simulations of non-infected herd over one and 10 years, respectively.

	Change in GM per stall (Euros) from non-infected herd							
Management level	Very good		Good		Poor		Very poor	
Assumptions	1st year	10 years	1st year	10 years	1st year	10 years	1st year	10 years
Best estimate	-57	-9	-201	-99	-315	-196	-357	-230
No milk loss acute infected and diseased	-56	-14	-187	-105	-283	-198	-324	-232
No milk loss acute infected not diseased/ supershedders	-54	-13	-182	-99	-282	-190	-310	-224
No milk loss resistant/carriers	-45	-10	-127	-40	-187	-60	-215	-70
No abortions	-26	-11	-130	-95	-208	-183	-240	-217
No dead calves/heifers	-58	-13	-205	-113	-319	-192	-369	-216
No dead cows	-59	-13	-195	-104	-302	-199	-345	-233
No clinical symptoms of infection[a]	-45	-9	-154	-43	-262	-108	-295	-150
All *S.* Dublin effects reduced[b]	-7	-4	-22	-9	-31	-13	-37	-13
All *S.* Dublin effects increased[b]	-114	-41	-489	-272	-710	-469	-784	-544
All milk yield effects reduced by 50%	-42	-9	-97	-33	-137	-50	-154	-60

[a]No clinical symptoms and no deaths, hence no treatment costs either. Milk yield losses still present

[b]Parameter estimates displayed in Table 2

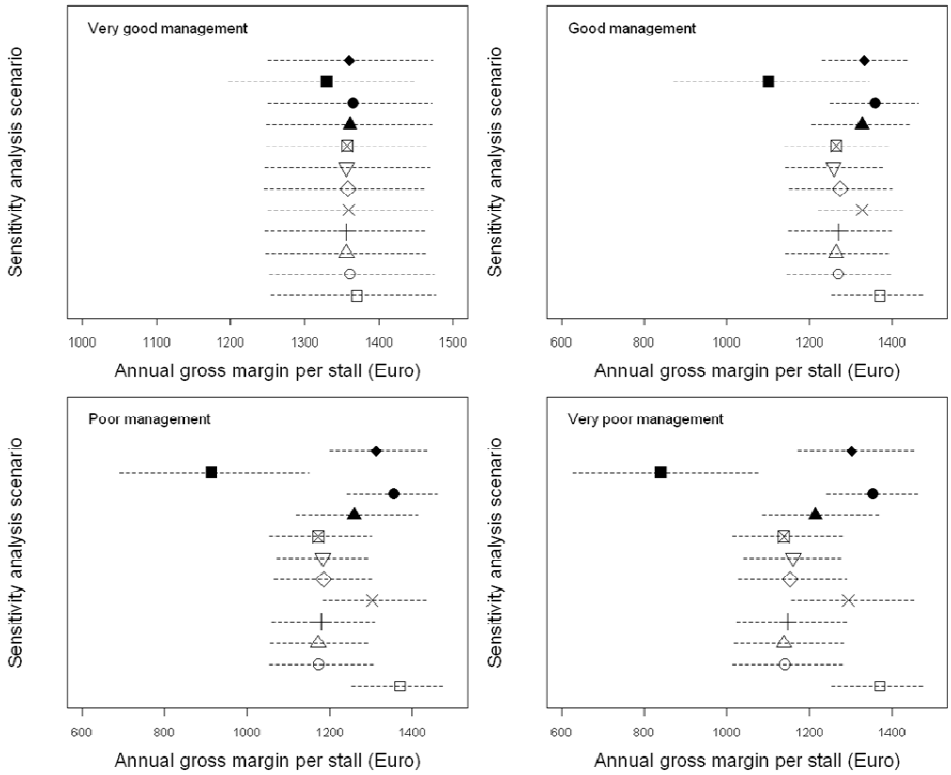

Figure 5 Model predicted differences in mean annual gross margin (GM) per stall between infected and non-infected under the assumptions used in the sensitivity scenarios specified in Table 2, and the best estimate scenario. Estimates are averaged over 10 years in a 200 cow stall herd in Euros. Estimates were derived from 1000 iterations simulating *S.* Dublin introduction into Danish dairy herds in the Dublin-Simherd model and compared to 1000 simulations of uninfected herd over 10 years. □ is GM for non-infected reference herd, ○ for best estimate, Δ No milk loss acute infected and diseased, + No milk loss acute infected not diseased/supershedders, x No milk loss resistant/carriers ◊ No abortions v No dead calves/heifers ☒No dead cows ▲No clinical effects of infection ●All effects reduced ■All effects increased ♦All yield effects reduced by 50%. The dashed lines show the 5th and the 95th percentiles from the simulations.

4. Discussion

To our knowledge this is the first study quantifying all direct and indirect economic losses of *S.* Dublin in dairy herds. GM losses following *S.* Dublin infection were quantified by simulation modelling. Results in this study estimated higher losses than previous studies. The Dublin-Simherd model was calibrated to field data estimating that milk yield was

affected for at least 18 months after herd infection and simulations estimated that milk yield was decreased even longer than this for many of the scenarios. Hence, milk yield was affected much longer than the two months that Bazeley (2006b) used for estimating losses. Visser et al. (1997) included herds after isolating *S.* Dublin from samples, which means that they did not necessarily include newly infected herds like we simulated in this study. This would result in expected lower losses than what was found in this study where the newly infected herd phase was included.

4.1. Results

GM losses per stall increased with increasing herd size and with decreasing quality of management. This indicates that it is even more important to control *S.* Dublin in large herds, and that more resources can be spent on control efforts than in smaller herds. The increased effects in large herds were partly due to the infection persisting in the herds and partly due to a higher number and proportion of the animals in the herds becoming infected.

In order to achieve the milk yield reduction following *S.* Dublin herd infection that was observed in data used by Nielsen et al. (2012b), it was necessary to model milk yield losses into the resistant stage of the infection cycle in the individual animals. Nielsen et al. (2012b) reported that milk yield at herd level appeared to be returning to pre-infection levels approximately 15 months after estimated time of herd infection. In contrast to this, Bazeley (2006a) reported milk yield losses for a period of approximately two months. Other types of *Salmonella* have been reported to affect milk yield for shorter periods of time, e.g. *S.* Anatum for four months (Glickman et al., 1981) and six months for *S.* Typhimurium (Kahrs et al., 1972). The effects in our study appear to be lasting longer even for the 85 cow herd. This might be explained by the fact that we assumed that no control efforts were implemented in the infected herds and the management level was kept constant during all 10 years. This was done in order to separate the effects of the infection from the effects of control efforts. In real life, some control efforts were most likely implemented in herds experiencing an outbreak of *S*. Dublin in the study by Nielsen et al. (2012b) and other studies. These could shorten the period with active *Salmonella* infection in the herds by management changes, and potentially lead to less yield loss in infected animals through intensified treatment or isolation of sick animals. Finally, culling of sick, affected or suspected carrier animals may have been used in some herds in relation to *Salmonella* outbreaks described in the literature, decreasing the period where *Salmonella* affected milk yield (Bergevoet et al., 2009; Nielsen and Nielsen, 2011). The Dublin-Simherd model also includes culling of animals, but only related to production performance and age, not related to the *S*. Dublin infection stages.

GM losses estimated in the sensitivity analyses indicated that no single effect of *S.* Dublin (e.g. abortion or milk yield losses in resistant or carrier cows) determined the GM losses averaged over the 10 years when management was very good, but for the poorer management scenarios, the assumptions regarding milk yield losses in resistant or carrier cows influenced results markedly. The infection died out within a relatively short time period in the very good management scenarios and this reduced the overall number of resistant cows in the herd over the 10 years. This group of animals was large in the poorer management scenarios, where the herd infection persisted longer, resulting in

higher GM losses per stall. However, the sensitivity analyses showed that even if we overestimated the milk yield losses in cows, there were still substantial economic losses associated with introduction and spread of *S.* Dublin in dairy herds.

4.2. Method

The only cost of *S.* Dublin herd infection included in this study was treatment of clinically ill animals. Other costs such as extra labour and disease control procedures were not included. Hence, effects of *S.* Dublin on GM per stall were reported as losses, even though it could be defined as costs of infection (McInerney et al., 1992).

GM and milk yield for all non-infected herds were identical independent of management level. This was due to the definition of the management levels in this study, which were based exclusively on the risk of infection with *S.* Dublin. However, this is unlikely to reflect the real situation, where poorer management might lead to lower milk yield and lower GM due to other diseases not being controlled, such as mastitis and paratuberculosis (Gröhn et al., 2004; Lombard et al., 2005). Hence, results in this study could be biased, but it is not known, if we overestimated the GM losses under poor management conditions or underestimated GM losses under good management conditions.

For very good management, it appeared that GM per stall decreased when omitting the single effects in the sensitivity analysis. This was due to feedback mechanisms in the model. For example, if no or few infected cows died, they would stay in the herd and contribute with less milk than a healthy replacement animal and lower the GM in the actual scenario. This illustrates the advantage of using a simulation model that mimics natural feedback mechanisms in dairy herds. Next step is to use the model to simulate actual control scenarios and decide on cost effective ways of controlling *S.* Dublin in herds depending on herd size.

The effect of introduction of *S.* Dublin on milk yield was based on Nielsen et al. (2012b). In that study, the milk yield was modelled for 18 months after estimated herd infection. There were indications in data that milk yield decreased earlier than estimated Nielsen et al. (2012b), which indicate that the infection date might have been estimated to be later than what actually was the case in that study. Hence, we used yield losses over two years to calibrate milk yield effects in this study. However, it is possible that this has over- or underestimated the milk yield effects of infection and thereby the estimated losses in GM. The sensitivity analyses showed that the assumptions regarding milk yield losses were important for the estimates of GM losses associated with *S.* Dublin, and further studies are needed to quantify the effect on milk yield in individual cows in different infection stages to validate the findings of this study.

Estimated milk yield losses were calibrated at poor management level settings in the model. It is not known how management in the infected herds in the study by Nielsen et al. (2012b) corresponded to management in this study, since the previous study was register based. Furthermore, the management definitions in this study are created based on hygiene levels and herd susceptibility levels, which can be difficult to translate into an actual management level. However, the herds studied by Nielsen et al. (2012b) were selected due to very high and relatively sudden increase in antibody levels in bulk tank

milk indicating that they were heavily infected and therefore a poor management was assumed.

Only one infectious heifer was introduced in the infected scenarios in this study. It is possible that farmers purchasing animals will introduce more than one infectious animal at once, or will introduce infectious animals to the herd repeatedly. Particularly in herds with very good management, this could lead to higher GM losses due to more infected animals. Furthermore, the animal could be introduced to other age groups than heifers just before calving. This could lead to different infection dynamics in the herd than simulated in this study, depending on the age of the animal since younger animals are more susceptible to *Salmonella* (Hall and Jones, 1979; Segall and Lindberg, 1991) and group sizes and dynamics differ.

No labour costs were included in this study. These would probably further decrease GM per stall. It is likely that diseased animals would need extra attention and that this would increase labour costs. These would need to be included in control simulations, where extra labour could be required to control the infection. Treatment costs would be dependent on the farmer's ability to discover diseased animals and threshold for when he would contact the veterinarian. These were held constant throughout the different managements in this study, and could have been included in the sensitivity analyses. They were left out of the sensitivity analyses to reduce complexity in the presentation of the study.

The simulations in this study estimated potentially high losses in GM per stall following introduction and spread of *S.* Dublin in dairy herds. The GM losses were highest in the first year after herd infection and large herds experienced higher losses than small herds. Furthermore, poorer management resulted in higher GM losses per stall. Milk yield losses appeared to be the effect of *S.* Dublin that had the highest impact on GM losses, and therefore these need to be parameterised with care in the simulation model. Further studies are needed to quantify effects of *S.* Dublin infection in cattle such as milk yield losses and probability of abortions in different *S.* Dublin infection stages of dairy cows.

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10 Appendix 1

10.1 Questionnaire for Manuscript 3 (Danish)

Besætningskarakteristika

1. **Hvilken funktion har du i besætningen?**
   Ejer Driftsleder Ejer og driftsleder

   Familie til ejer Anden medarbejder

2. **Hvor mange personer arbejder i besætningen i alt?**
   1-2 3-4 5-6 >6

3. **Staldsystem hos lakterende køer:**
   Løsdrift Bindestald
   ↓
   Dybstrøelse Sengebåse

4. **Staldsystem hos goldkøer:**
   Løsdrift Bindestald
   ↓
   Dybstrøelse Sengebåse

5. **Staldsystem hos opdræt:**
   Løsdrift Bindestald
   ↓
   Dybstrøelse Sengebåse

6. **Er nogle af dine nærmeste nabobesætninger smittede med Salmonella?**
   Ja, der er smittede nabobesætninger

   Nej, der er ikke smittede nabobesætninger

   Ved ikke

7. **Har du/I fået resultatet af 2. hold blodprøver fra 3-6 måneders kalve fra dette efterår?**
   Ja Nej Ved ikke
   ↓
   a. **Har du/I en forventning om flere negative blodprøver hos kalvene i blodprøverunden her i efteråret?**
   Ja Nej Ved ikke

   b. **Hvis ja, (nr. 7) var der nogle positive prøver imellem?**
   (Registerdata indtastes. Positive prøver er dem der er over 50)

   Ja Nej

8. Har du/I set en ændring i sundheden hos kalvene under 6 mdr. i løbet af det seneste år?

Ja Nej Ved ikke

↓

a. Hvilke ændringer?

Lavere sygdomsforekomst	Højere sygdomsforekomst
Færre behandlinger	Flere behandlinger
Lavere kalvedødelighed	Højere kalvedødelighed

9. Er der udført en risikovurdering i besætningen?
(Dvs. har de brugt manual eller registreringsskemaer til at udpege smitteveje i besætningen?)

Ja Nej Ved ikke

↓

a. Er der udarbejdet en handlingsplan?

Ja Nej Ved ikke

↓

b. Har du/I talt med en rådgiver angående handlingsplan?

Ja Nej Ved ikke

↓

i. Hvilken rådgiver

Dyrlæge Kvægbrugskonsulent

Anden: (skrivefelt) _____

ii. Har du/I aftalt opfølgning med rådgiveren?

Ja Nej Ved ikke

10. Kommentarer ang. besætningskarakteristika:
(skrivefelt)_____

Kælvningsområde

11. Hvem har det primære ansvar for kælvningerne og råmælkstildelingen? *(Et kryds pr. person)*

Ejer	Respondenten selv
Familie til ejer hankøn	Familie til ejer hunkøn
Ansat hankøn, dansk	Ansat hunkøn, dansk
Ansat hankøn, udenlandsk	Ansat hunkøn, udenlandsk

12. Hvor mange køer opholder sig i kælvningsboksene på samme tid?

Ingen kælvningsboks 1 2-4 >4

13. Har nogle køer, inden for det seneste år, kælvet inden de blev flyttet til en kælvningsboks? *(Hyppigst)*

Ja Nej Ved ikke

↓

a. Hvor mange køer?

1-2 3-5 >5

14. Bruges kælvningsbokse som sygebokse?

Ja Nej

↓

a. Rengøres boksen mellem syge køer og nykælvere?

Altid Nogle gange Aldrig

15. Hvor ofte udmuges og udtørres kælvningsboksen?

(Hvis respondenten svarer "efter behov" bør der spørges ind til ca. hvor ofte det er)

Efter hver kælvning 1-2 gange om ugen

1-2 gange om måneden 1-4 gange om året Aldrig

16. Hvor ofte strøs kælvningsboksen?

Efter hver kælvning Dagligt

Oftere end 1 gang om ugen 1 gang om ugen

Sjældnere end 1 gang om ugen

17. Er der lavet tiltag i kælvningsområdet i forbindelsen med saneringen siden september 2008?

Ja Nej Ved ikke

↓

Hvilke tiltag er udført? (*Notér måned og år for påbegyndelse i formatet: jan 2009)*

Faste skillevægge Påbegyndt: _____ _____

Flere kælvningsbokse Påbegyndt: _____ _____

Færre køer i kælvningsområdet Påbegyndt: _____ _____

Øget rengøring/strøelse Påbegyndt: _____ _____

Andet: (skrivefelt) _____ Påbegyndt: _____ _____

a. Udføres tiltagene konsekvent?

Ja Nej Ved ikke

b. Hvordan har tidsforbruget pr. dag ændret sig i kælvningsområdet efter at tiltagene er påbegyndt?

___timer mindre Uændret ___timer mere Ved ikke

c. Anslået udgift til materialer i forbindelse med saneringstiltag siden september 2008:

(F.eks. indkøb af skillevægge/inventar, bygning af nye kælvningsbokse/nyt staldområde, indkøb af udtørringsprodukter eks. Stalosan, hydratkalk, måtter el. lign.)

_____ kr. Ved ikke

18. Kommentarer ang. kælvningsområde: (skrivefelt)

_____

Råmælk

19. Hvor hurtigt fjernes kalven fra koen?

Straks efter kælvning | Så snart kalven opdages | Ved først kommende malkning | Indenfor det første døgn efter kælvning | Mere end 1 døgn efter kælvning | Ved ikke

20. Hvor lang tid efter kælvning tildeles kalvene råmælk?

Indenfor 6 timer | Efter 6 timer | Varierende | Kalven tildeles ikke råmælk | Ved ikke

a. Hvordan tildeles kalven råmælk?

Sonde | Sutteflaske | Trug/Spand | Patter ved koen | Ved ikke

↓ ↓

i. Rengøres sutteflaske/sonde/trug/spand mellem hver kalv?

Altid | Nogle gange | Aldrig | Ved ikke

21. Fodres kalvene med råmælk blandet fra flere køer?

Altid | Nogle gange | Aldrig | Ved ikke

22. Anvendes colostrometer til måling af immunoglobulin i råmælk?

Altid | Nogle gange | Aldrig | Ved ikke

23. Har besætningen en råmælksbank?

Ja | Nej | Ved ikke

24. Gælder det for både tyre- og kviekalve med de ting, vi har snakket om vedr. fjernelse af kalven efter kælvning og råmælkstildeling?

Ja | Nej | Ved ikke

↓

a. Hvori består forskellen?

(skrivefelt)_____

25. Er der ændret noget omkring råmælkshåndtering og fjernelse af kalven efter kælvning i forbindelsen med saneringen?

Ja Nej

↓

a. Hvilke tiltag er udført? (*Notér måned og år for påbegyndelse i formatet: jan 2009)*

Hurtigere fjernelse af kalven fra koen Påbegyndt: _____ _____

Hurtigere råmælkstildeling Påbegyndt: _____ _____

Oprettelse af råmælksbank Påbegyndt: _____ _____

Måling af immunoglobulin i råmælk Påbegyndt: _____ _____

Øget fokus på hygiejne ved håndtering af råmælk
Påbegyndt: _____ _____

Andet: (skrivefelt) _____ Påbegyndt: _____ _____

b. Udføres tiltagene konsekvent?

Ja Nej Ved ikke

c. Hvordan har tidsforbruget pr. dag ændret sig vedr. råmælkshåndtering efter at tiltagene er påbegyndt?

___timer mindre Uændret ___timer mere Ved ikke

d. Anslået udgift til materialer i forbindelse med saneringstiltag siden september 2008:

(F.eks. indkøb af sonder, køleskab, sutteflasker, rengøringsmidler, oprettelse af kalvekøkken mm)

_____ kr. Ved ikke

26. Kommentarer ang. råmælk:

(skrivefelt)_____

Spædekalve (første afsnit efter kælvningsområde)

27. Hvem har det primære ansvar for pasning af spædekalvene? *(Et kryds pr. person)*

Ejer Respondenten selv

Familie til ejer hankøn Familie til ejer hunkøn

Ansat hankøn, dansk Ansat hunkøn, dansk

Ansat hankøn, udenlandsk Ansat hunkøn, udenlandsk

28. Anvendes mælk fra syge / behandlede køer til fodring af spædekalvene?

Altid Nogle gange Aldrig Ved ikke

29. Fodres spædekalvene med mælk fra køer med Salmonellapositive tests?

Ja Nej Ved ikke, da status ikke kendes på enkeltdyrs-niveau

Ved ikke

↓

Behandles mælken?

Nej Mælken syrnes Mælken pasteuriseres

30. Behandles spædekalvene med Salmonella Dublin anti-serum?

Ja, 1 gang umiddelbart efter fødslen

Ja, 2 gange *(typisk umiddelbart efter fødslen og igen ca. 17 dage senere)*

Nej Ved ikke

31. Er der et separat staldafsnit til spædekalvene?
(Forstået som, at kalvene ikke har nem kontakt til andre aldersgrupper, og der er total adskillelse enten som afstand eller vægadskillelse)

Ja Nej

32. Hvor mange spædekalve er opstaldet i hver boks/hytte? *(Hyppigst)*

1 2 >2

33. Hvor længe bliver kalvene i første opstaldningssystem? *(Boks/hytte)*

_____ uger Ved ikke

34. Hvornår fravænnes kalvene?

_____ uger Ved ikke

35. Hvordan er adskillelsen mellem boksene/hytterne? *(Op til 3 krydser)*

Total (Der er afstand mellem boksene) Fast skillevæg

Tremmer

36. Hvor mange andre spædekalve har én spædekalv kontakt med i mælkefodringsperioden?
(Respondenten kan svare 0 uden det får konsekvenser. Det bliver ikke brugt imod dem, og vi er helt klar over, at det forekommer.)

0 1 2 >2

37. Fjernes al gødning fra bokse/hytter før indsætning af spædekalv?

Ja, der rengøres grundigt og boks/hytte udtørres altid mellem kalve

Ja, al gødning fjernes hver gang, men der vaskes ikke og udtørres heller ikke nødvendigvis

Nogle gange fjernes gødning el. rengøres boksen/hytten

Meget sjældent

Ved ikke

38. Gælder de ting vi har snakket om vedr. opstaldning og behandling af spædekalvene for både tyre og kvier?

Ja Nej Ved ikke

↓

a. Hvori består forskellen?
(skrivefelt) _____

39. Hvor ofte rengøres spædekalvens trug/sutteautomat/suttespand? *(Sæt op til 3 krydser)*

Ved indsættelse af ny kalv Ved gødningsforurening Dagligt

Ugentligt Hver anden uge Månedligt

Aldrig

40. Er der lavet tiltag i forbindelsen med sanering for Salmonella hos spædekalvene?
Ja Nej Ved ikke
↓

a. Hvilke tiltag er udført? (*Notér måned og år for påbegyndelse i formatet: jan 2009)*
Ingen fodring af kalve med mælk fra køer med Salmonellapositive tests

Påbegyndt: _____ _____

Ændring af behandling af mælk Påbegyndt: _____ _____

Ændring af længden af mælkefodringsperioden
Påbegyndt: _____ _____

Hyppigere eller grundigere rengøring af trug/sutteautomat/suttespand

Påbegyndt: _____ _____

Serumbehandling Påbegyndt: _____ _____

Hyppigere eller grundigere rengøring af hytter
Påbegyndt: _____ _____

Ændring af behandlingsstrategi Påbegyndt: _____ _____

Nyt staldafsnit eller ombygning Påbegyndt: _____ _____

Andet: *(*skrivefelt) _____ Påbegyndt: _____ _____

b. Udføres tiltagene konsekvent?
Ja Nej Ved ikke

c. Hvordan har tidsforbruget pr. dag ændret sig i spædekalveafsnittet efter at tiltagene er påbegyndt?
___timer mindre Uændre ___timer mere Ved ikke

d. Anslået udgift til materialer i forbindelse med saneringstiltag siden september 2008:
(F.eks. til indkøb af hytter, rengøringsmaterialer, serum-behandling, pasteuriseringsanlæg mm.)
_____ kr. Ved ikke

41. Kommentarer ang. spædekalve: (skrivefelt)
_____

<u>Kalve i fællesbokse op til ca. 6 mdr</u>. (ofte det andet afsnit kalvene sættes i. Kan være både mælkefodrede og fravænnede kalve.)

42. Anvendes holddrift hos kalvene? *(alt-ind alt-ud)*
Altid Nogle gange Aldrig Ved ikke

43. Hvor mange dyr er der gennemsnitligt i fællesboksene?
2-4 5-7 ≥ 8

44. Sættes kalvene på græs?
Ja Nej
↓
a. Spredes kvæggødning /-gylle på afgræsningsområder i samme sæson?
Ja Nej

45. Er der lavet tiltag hos kalvene i fællesbokse i forbindelse med saneringen?
Ja Nej Ved ikke
↓
a. Hvilke tiltag er udført? (*Notér måned og år for påbegyndelse i formatet: jan 2009)*
Strikt holdrift *(alt-ind alt-ud)* Påbegyndt: _____ _____

Faste skillevægge Påbegyndt: _____ _____

Færre dyr pr. hold Påbegyndt: _____ _____

Øget fokus på hygiejne *(strøelse/udmugning)* Påbegyndt: _____ _____

Andet: (skrivefelt) _____ Påbegyndt: _____ _____

b. Udføres tiltagene konsekvent?
Ja Nej Ved ikke

c. Hvordan har tidsforbruget pr. dag ændret sig efter at tiltagene hos de fravænnede kalve er påbegyndt?
___timer mindre Uændret ___timer mere Ved ikke

d. Anslået udgift til materialer i forbindelse med saneringstiltag siden september 2008:
(F.eks. indkøb af skillevægge, mere strøelse, rengøringsmidler, nybyggeri mm.)
_____ kr. Ved ikke

46. Kommentarer ang. kalve i fællesbokse op til ca. 6 mdr.:(skrivefelt)
_____

Kvieopdræt (fra ca. 6 mdr.)

47. Anvendes systematisk/strikt holddrift hos kvierne? *(alt-ind alt-ud)*
Altid Nogle gange Aldrig Ved ikke

48. Sættes kvierne på græs?
Ja Nej Ved ikke
↓
a. Spredes der kvæggødning /-gylle på afgræsningsområder i samme sæson?
Ja Nej

49. Er der lavet tiltag hos kvieopdrættet i forbindelse med saneringen?
Ja Nej Ved ikke
↓

a. Hvilke tiltag er udført? (*Notér måned og år for påbegyndelse i formatet: jan 2009)*
Strikt holdrift *(alt-ind alt-ud)* Påbegyndt: _____ _____

Faste skillevægge Påbegyndt: _____ _____

Færre dyr pr. hold Påbegyndt: _____ _____

Øget fokus på hygiejne *(strøelse/udmugning)* Påbegyndt: _____ _____

Andet: (skrivefelt) _____ Påbegyndt: _____ _____

b. Udføres tiltagene konsekvent?
Ja Nej Ved ikke

c. Hvordan har tidsforbruget pr. dag ændret sig efter at tiltagene er påbegyndt?
___timer mindre Uændret ___timer mere Ved ikke

d. Anslået udgift til materialer i forbindelse med saneringstiltag siden september 2008:
(F.eks. indkøb af skillevægge, mere strøelse, rengøringsmidler, nybyggeri mm.)
_____ kr. Ved ikke

50. Kommentarer ang. kvieopdræt:
(skrivefelt)_____

Generel smittehåndtering

51. Er der tiltag som forhindrer, at køerne kommer i kontakt med kvæggødning f.eks. på foderbord og ved vandtrug?
Ja Nej
↓
a. Hvilke tiltag? *(F.eks.: støvlevask ved foderbord, rengøring af vandtrug, ingen køer på foderbordet, kørsel på/over foderbordet)*
(skrivefelt) _____

52. Forekommer det, at opbevaret foder (f.eks. ensilage, korn) bliver forurenet med kvæggødning?
Ja Nej

53. Er der bestemte rutiner ved bevægelse mellem afsnit i stalden? *(Ejer og ansatte)*
Ja Nej Ved ikke
↓

a. Støvlevask

Altid Nogle gange Aldrig
↓ ↓

i. Påbegyndt senere end september 2008
Ja Nej
↓
Investering: _____kr.

b. Støvleskift

Altid Nogle gange Aldrig
↓ ↓

i. Påbegyndt senere end september 2008

Ja Nej
↓

Investering: _____kr.

c. Vask af hænder

Altid Nogle gange Aldrig
↓ ↓

i. Påbegyndt senere end september 2008

Ja Nej
↓

Investering: _____kr.

d. Tøjskift/ overtrækstøj

Altid Nogle gange Aldrig
↓ ↓

i. Påbegyndt senere end september 2008

Ja Nej
↓

Investering: _____kr.

e. Andre tiltag:

(skrivefelt) _____
↓

i. Påbegyndt senere end september 2008

Ja Nej
↓

Investering: _____kr.

54. Er der hygiejneforanstaltninger for besøgende? (*dyrlæge, klovbeskærer, landmand, inseminør m.fl.)*

Ja Nej Ved ikke
↓

a. Støvlevask

Altid Nogle gange Aldrig
↓ ↓

i. Påbegyndt senere end september 2008

Ja Nej
↓

Investering: _____kr.

b. Desinfektion af støvler

Altid Nogle gange Aldrig
↓ ↓

i. Påbegyndt senere end september 2008

Ja Nej
↓

Investering: _____kr.

c. Støvleskift

Altid Nogle gange Aldrig
↓ ↓

i. Påbegyndt senere end september 2008

Ja Nej
↓

Investering: _____kr.

d. Overtrækstøj

Altid Nogle gange Aldrig

↓ ↓

i. Påbegyndt senere end september 2008

Ja Nej

↓

Investering: _____kr.

e. Vask af hænder

Altid Nogle gange Aldrig

↓ ↓

i. Påbegyndt senere end september 2008

Ja Nej

↓

Investering: _____ kr.

f. Andre tiltag:

(skrivefelt) _____

↓

i. Påbegyndt senere end september 2008

Ja Nej

↓

Investering: _____kr.

55. Bruges redskaber og maskiner i flere staldafsnit?

Altid Nogle gange Aldrig Ved ikke

↓ ↓

a. Rengøres de mellem hvert afsnit?

Altid Nogle gange Aldrig Ved ikke

56. Hvordan er belægningsgraden hos de forskellige dyregrupper i forhold til før projektets start? (September 2008)

a. Kalve

Lavere Uændret Højere

Ved ikke

b. Kvier

Lavere Uændret Højere

Ved ikke

c. Køer

Lavere Uændret Højere

Ved ikke

57.

58. Indkøbes dyr til besætningen?

Ja Nej

↓

a. Kendes Salmonella status på indkøbte dyr?

Ja Nej Ikke altid Ved ikke

↓

i. Indkøbes kun dyr fra niveau 1 besætninger?

Ja Nej Ved ikke

59. Testes der for Salmonella på enkeltdyrs-niveau udover projektprøver? *(Registerdata indtastes)*

Ja Nej

↓

a. Hvilke dyregrupper testes?

Køer Kvier

Hvis ja, bruges status på enkelt dyr til beslutninger i det daglige arbejde?

Ja Nej Ved ikke

↓

i. Hvilke beslutninger:

Separat kælvningsområde til Salmonella positive dyr

Én ko/kvie pr. kælvningsboks for testpositive dyr

Rengøring af yver før kælvning

Kalven fjernes straks efter kælvning

Råmælk kasseres

Mælken anvendes ikke til fodring af kalve

Udsætningsstrategi

Gruppering af test-positive dyr

Andet: (skrivefelt) _____

60. Er der smitteforebyggende foranstaltninger ved afhentning eller levering af dyr?

Ja Nej Ved ikke

↓

a. Hvilke foranstaltninger

Særskilt staldafsnit Separat indkørsel til særskilt staldafsnit Andet: (skrivefelt) _____

61. Bruges maskinstationen til gylleudkørsel eller er der maskinfællesskab om gyllevogn?

Ja Nej

62. Har besætningen maskinfællesskab med andre besætninger udover gyllevogn?

Ja Nej

63. Kommentarer ang. generel smittehåndtering:

(skrivefelt)_____

64. Har du/I haft planer om saneringstiltag som ikke har været muligt at udføre?

Ja Nej

↓

Hvilke tiltag: (skrivefelt)_____

Hvorfor har det ikke været muligt: (skrivefelt)

_____

10.2 Questionnaire for Manuscript 3 (English)

Herd demographics

1. Your function in the herd?

(1) ❑ Owner
(2) ❑ Daily manager
(3) ❑ Owner and daily manager
(4) ❑ Family of owner
(5) ❑ Other staff

2. How many employees in total are working with the herd?

(1) ❑ 1-2
(2) ❑ 3-4
(3) ❑ 5-6
(4) ❑ >6

3. Barn type lactating cows:

(1) ❑ Free stall, go to 3a
(2) ❑ Tie stall
(3) ❑ Other_____

3.aFree stall type:

(1) ❑ Deep Bedding
(2) ❑ Free stall

4. Barn type dry cows:

(1) ❑ Free stall, go to 4a
(2) ❑ Tie stall
(3) ❑ Other:_____

4.a Free stall type:

(1) ❑ Deep Bedding
(2) ❑ Free stall

5. Barn type heifers:

(1) ❑ Free stall, go to 5a
(2) ❑ Tie stall
(3) ❑ Other:_____

5.a Free stall type:

(1) ❑ Deep Bedding
(2) ❑ Free stall

6. Any Salmonella positive neighbour herds?

(1) ❑ Yes
(2) ❑ No
(3) ❑ Unknown

7. Have you noticed changes in calf health during the past year?

(1) ❑ Yes
(2) ❑ No
(3) ❑ Unknown

7a. Which changes?

(1) ❑ Lower disease level
(2) ❑ Higher disease level
(3) ❑ Fewer treatments
(4) ❑ More treatments
(5) ❑ Lower calf mortality
(6) ❑ Higher calf mortality

8. Have you received results from the second round of blood tests from 3-6 months old calves this autumn?

(1) ❑ Yes, go to 8b
(2) ❑ No, go to 8a
(3) ❑ Unknown

8a. Do you expect more negative blood tests from the calves this autumn?

(1) ❑ Yes
(2) ❑ No
(3) ❑ Unknown
(4) ❑ Other_____

8b. If yes, was there any positive blood test from the calves? (Results will be collected from registry data)

(1) ❑ Yes
(2) ❑ No

9. Has risk assessment for *Salmonella* transmission in herd been performed together with herd health advisor?

(I.e. have the herd health advisor used a manual or recording schemes to point out transmission routes in the herd?)

(1) ❑ Yes, go to 9a
(2) ❑ No
(3) ❑ Unknown

9a. Is there an action plan?

(1) ❑ Yes, go to 9b
(2) ❑ No
(3) ❑ Unknown

9b. Have you discussed the action plan with a heard health advisor?

(1) ❑ Yes, go to 9c and 9d
(2) ❑ No
(3) ❑ Unknown

9c. Which advisor?

(1) ❑ Veterinary advisor
(2) ❑ Herd health consultant?
(3) ❑ Other_____

9d. Have you agreed on follow up action on action plan?

(1) ❑ Yes
(2) ❑ No
(3) ❑ Unknown

10. Comments herd demographics

_____

Calving area

11. Who is primarily responsible for calvings and colostrum feeding?

(1) ❑ Owner
(2) ❑ Respondent
(3) ❑ Family to owner, male
(4) ❑ Family to owner, female
(5) ❑ Employee, male, Danish nationality
(6) ❑ Employee, female, Danish nationality
(7) ❑ Employee, male, other nationality
(8) ❑ Employee, female, other nationality
(9) ❑ Other_____

12. Number of cows in calving pen at any time?

(In general)

(1) ❑ No calving pen
(2) ❑ 1
(3) ❑ 2-4
(4) ❑ > 4

13. Any cows calved before they were moved to the calving pen in the previous 12 months?

(1) ❑ Yes, go to 13a
(2) ❑ No
(3) ❑ Unknown

13a. How many cows?

(1) ❑ 1-2
(2) ❑ 3-5
(3) ❑ > 5

14. Is the calving pen used for sick animals?

(1) ❑ Yes, go to 14a
(2) ❑ No

14a. Does the calving pen get cleaned before next calving?

(1) ❑ Always
(2) ❑ Sometimes
(3) ❑ Never

15. How often does calving pen get cleaned and left to dry out? (If respondent answers 'when needed', please ask how often that approximately is)

(1) ❑ After each calving
(2) ❑ 1-2 times a week
(3) ❑ 1-2 a month
(4) ❑ 1-4 a year
(5) ❑ Never
(6) ❑ Other_____

16. How often is new bedding provided in calving pen?

(1) ❑ After each calving
(2) ❑ Daily
(3) ❑ More than once a week
(4) ❑ Once a week
(5) ❑ Less than once a week

17. Any new routines in calving area since September 2008 in connection with Salmonella control?

(1) ❑ Yes, go to 17a
(2) ❑ No
(3) ❑ Unknown

17a. Which new routines? (Note month and year for start in the format Jan 2009)

Solid walls	Initiated: _____ _____
More calving pens	Initiated: _____ _____
Fewer cows in the calving area	Initiated : _____ _____
Increased cleaning/bedding	Initiated: _____ _____
Other: (Please note) _____	Initiated: _____ _____

17b. Do you always use new routines?

(1) ❑ Yes
(2) ❑ No
(3) ❑ Unknown

17c. Has the time you spent per day in the calving area changed after the new routines?

(1) ❑ Hours less _____
(2) ❑ Unchanged
(3) ❑ Hours more _____
(4) ❑ Unknown

17d. Expense estimate for materials and man hours relating to changes in calving area since September 2008 in connection with Salmonella control: (E.g. Purchase of inventory, building calving boxes/new barn, purchase disinfection agents etc.)

(1) ❑ DKK. _____
(2) ❑ Unknown

18. Time from calving to calf removed from cow?

(1) ❑ Immediately after calving
(2) ❑ As soon as calf noticed
(3) ❑ First milking after calf noticed
(4) ❑ Within 24 hours after calving
(5) ❑ More than 24 hours after calving
(6) ❑ Unknown

19. Comments regarding calving area:

_____

Colostrum

20. How soon after calving is calf fed colostrum?

(1) ❑ Within 4 hours, go to 20a
(2) ❑ Within 6 hours, go to 20a
(3) ❑ Later than 6 hours, go to 20a
(4) ❑ Varies, go to 20a
(5) ❑ Calf is not fed colostrum
(6) ❑ Unknown

20a. How is colostrum fed?

(1) ❑ Naso-gastric tube, go to 20b
(2) ❑ Bottle, go to 20b
(3) ❑ Trough/bucket, go to 20b
(4) ❑ Feeding from cow
(5) ❑ Unknown

20b. Is bottle/naso-gastric tube/trough/bucket cleaned between each calf?

(1) ❑ Always
(2) ❑ Sometimes
(3) ❑ Never
(4) ❑ Unknown

21. Are calves fed with pooled colostrum?

(1) ❑ Always
(2) ❑ Sometimes
(3) ❑ Never
(4) ❑ Unknown

22. Use of colostrometer to measure immunoglobulin in colostrum?

(1) ❑ Always
(2) ❑ Sometimes
(3) ❑ Never
(4) ❑ Unknown

23. Is there a colostrum bank on the farm?

(1) ❑ Yes
(2) ❑ No
(3) ❑ Unknown

24.Same colostrum management routines for bull and heifer calves?

(1) ❑ Yes
(2) ❑ No, what is the difference?_____
(3) ❑ Unknown

25. Any changes in handling of colostrum since September 2008 in connection with Salmonella control?

(1) ❑ Yes, go to 25a
(2) ❑ No

25a. **Which new routines?** (Note month and year for start in the format Jan 2009)

Earlier separation of cow and calf	Initiated: _____ _____
Earlier colostrum feed	Initiated: _____ _____
Colostrum bank	Initiated: _____ _____
Measuring immunoglobulins in colostrum	Initiated: _____ _____
Increased attention to hygiene when handling colostrum	Initiated: _____ _____
Other: _____	Initiated: _____ _____

25b. Do you always use new routines?

(1) ❑ Yes
(2) ❑ No
(3) ❑ Unknown

25c. Has the time you spent per day handling colostrum changed after the new routines?

(1) ❑ Hours less _____
(2) ❑ Unchanged
(3) ❑ Hours more _____
(4) ❑ Unknown

25d. Expense estimate for materials and man hours with changes in colostrum area since September 2008 in connection with Salmonella control: (E.g. Purchase of feeding equipment, refrigerator, bottles, cleaning agents, installing 'calf kitchen' etc.)

(1) ❑ DKK. _____
(2) ❑ Unknown

26. Comments regarding colostrum?

_____

Pre-weaned calves

27. Who is primarily responsible for pre-weaned calves?

(1) ❑ Owner
(2) ❑ Respondent
(3) ❑ Family to owner, male
(4) ❑ Family to owner, female
(5) ❑ Employee, male, Danish nationality
(6) ❑ Employee, female, Danish nationality
(7) ❑ Employee, male, other nationality
(8) ❑ Employee, female, other nationality
(9) ❑ Other_____

28. Weeks in pre-weaned area? (Pen/calf hut)

(1) ❑ Weeks_____
(2) ❑ Unknown

29. Number calves in each pen/calf hut? (Most commonly)

(1) ❑ 1
(2) ❑ 2
(3) ❑ > 2

30. How are pens/calf huts separated? (Can tick all)

(1) ❑ Free area between pens
(2) ❑ Solid wall

(3) ❑ Bars

31. Number of contact calves in pre-weaned area? (Respondent can tick 0 without consequences, it will not be reported to the authorities and we do know that this happens)
(1) ❑ 0
(2) ❑ 1
(3) ❑ 2
(4) ❑ > 2

32. Separate barn area for pre-weaned calves? (There is no easy contact with other age groups and the pre-weaned calves are separated from other age groups by some distance or walls)
(1) ❑ Yes
(2) ❑ No

33. Use of waste milk to feed pre-weaned calves?
(1) ❑ Always
(2) ❑ Sometimes
(3) ❑ Never
(4) ❑ Unknown

34. Use of milk from *Salmonella* antibody positive cows to feed pre-weaned calves?
(1) ❑ Yes, go to 34a
(2) ❑ No
(3) ❑ Unknown, do not test cows
(4) ❑ Unknown

34a. Is milk treated?
(1) ❑ No
(2) ❑ Milk acidified
(3) ❑ Milk pasteurised

35. At what age are calves weaned?
(1) ❑ Age in weeks_____
(2) ❑ Unknown

36. Are the pre-weaned calves treated with Salmonella Dublin anti-serum?
(1) ❑ Yes, once immediately after calving
(2) ❑ Yes, twice (typically immediately after calving and approximately 17 days later)
(3) ❑ No
(4) ❑ Unknown

37. Removal of all manure from pen between calves? (If respondent answers 'as necessary', please ask how often this approximately is)

(1) ❑ Yes, thorough cleaning/washing and pen left to dry out between calves
(2) ❑ Yes, all manure removed, but no washing or drying out of pen
(3) ❑ Manure removed or pen cleaned/washed occasionally
(4) ❑ Rarely
(5) ❑ Unknown
(6) ❑ Other_____

38. Same management routines regarding housing and feeding for pre-weaned bull and heifer calves?

(1) ❑ Yes
(2) ❑ No, what differences?_____
(3) ❑ Unknown

39. How often does feeding equipment get cleaned? (Tick a maximum of 3 answers)

(1) ❑ Between calves
(2) ❑ When manure is noticed in trough/bucket
(3) ❑ Daily
(4) ❑ Weekly
(5) ❑ Every other week
(6) ❑ Monthly
(7) ❑ Never

40. Any changes in management of pre-weaned calves since September 2008 in connection with Salmonella control?

(1) ❑ Yes, go to 40a
(2) ❑ No
(3) ❑ Unknown

40a. Which changes have been implemented? (Note month and year for start in the format Jan 2009)

No feeding of calves with milk from Salmonella test-positive cows Initiated: _____ _____

Changed milk handling routines Initiated: _____ _____

Shorter/longer milk fed period Initiated: _____ _____

More frequent or thorough cleaning of bucket or other feeding equipment Initiated: _____ _____

Salmonella vaccination Initiated: _____ _____

More frequent or thorough cleaning of calf huts Initiated: _____ _____

Changed treatment strategy of sick calves Initiated: _____ _____

New barn or renovation Initiated: _____ _____

Other:_____ Initiated: _____ _____

40b. Do you always use new routines?

(1) ❑ Yes
(2) ❑ No
(3) ❑ Unknown

40c. Has the time you spent per day for handling pre-weaned calves changed after the new routines?

(1) ❑ Hours less _____
(2) ❑ Unchanged
(3) ❑ Hours more _____
(4) ❑ Unknown

40d. Expense estimate for materials and man hours with changes in pre-weaned calf area since September 2008 in connection with Salmonella control. (E.g. purchase of calf huts, cleaning agents, serum treatment, pasteuriser for milk for calves*)*

(1) ❑ DKK. _____
(2) ❑ Unknown

41. Comments regarding pre-weaned calves

_____

Calves less than 6 months

(Usually second section that calves are moved to. This includes both milk-fed and weaned calves)

42. Use of sectioning of calves?

(All in all out)

(1) ❑ Always
(2) ❑ Sometimes
(3) ❑ Never
(4) ❑ Unknown

43. Number of calves in a section?

(1) ❑ 2-4
(2) ❑ 5-7
(3) ❑ > 7

44. Do the calves go on pasture?

(1) ❑ Yes, go to 44a
(2) ❑ No

44a. Spread of manure on pasture in the same season?

(1) ❑ Yes
(2) ❑ No

45. Any changes in management of calves since September 2008 in connection with Salmonella control?

(1) ❑ Yes, go to 45a
(2) ❑ No
(3) ❑ Unknown

45a. Which changes have been implemented? (Note month and year for start in the format Jan 2009)

Sectioning *(all in – all out)* Initiated: _____ _____

Walls between pens rather than bars Initiated: _____ _____

Fewer animals per section Initiated: _____ _____

Increased focus on hygiene (new bedding/cleaning) Initiated: _____ _____

Other: (Please note) _____ Initiated: _____ _____

45b. Do you always use new routines?

(1) ❑ Yes
(2) ❑ No
(3) ❑ Unknown

45c. Has the time you spent per day for handling calves changed after the new routines?

(1) ❑ Hours less _____
(2) ❑ Unchanged
(3) ❑ Hours more _____
(4) ❑ Unknown

45d. Expense estimate for materials and man hours with changes in calf area since September 2008 in connection with Salmonella control. (E.g. purchase of separation walls, increased bedding amount, cleaning agents, building new barns etc)

(1) ❑ DKK. _____
(2) ❑ Unknown

46. Comments about calves less than 6 months

_____

Heifers

47. Use of sectioning for heifers? (All in all out)

(1) ❑ Always
(2) ❑ Sometimes
(3) ❑ Never
(4) ❑ Unknown

48. Do the heifers go on pasture?

(1) ❑ Yes, go to 48a

(2) ❑ No

48a. Spread of manure on pasture in the same season?

(1) ❑ Yes
(2) ❑ No

49. Any changes in management of heifers since September 2008 in connection with Salmonella control?

(1) ❑ Yes, go to 49a
(2) ❑ No
(3) ❑ Unknown

49a. Which changes have been implemented? (Note month and year for start in the format Jan 2009)

Sectioning *(all in – all out)* Initiated: _____ _____

Walls between pens rather than bars Initiated: _____ _____

Fewer animals per section Initiated: _____ _____

Increased focus on hygiene (new bedding/cleaning) Initiated: _____ _____

Other: (Please note) _____ Initiated: _____ _____

49b. Do you always use new routines?

(1) ❑ Yes
(2) ❑ No
(3) ❑ Unknown

49c. Has the time you spent per day for handling calves changed after the new routines?

(1) ❑ Hours less _____
(2) ❑ Unchanged
(3) ❑ Hours more _____
(4) ❑ Unknown

49d. Expense estimate for materials and man hours with changes in calf area since September 2008 in connection with Salmonella control. (E.g. purchase of separation walls, increased bedding amount, cleaning agents, building of new barns etc)

(1) ❑ DKK. _____
(2) ❑ Unknown

50. Comments regarding heifers

General biosecurity

51. Are there biosecurity routines in place to prevent cows getting into contact with faeces at e.g. feed area and water troughs? (E.g. boot wash at feed area, cleaning of water troughs, not allowing possible faecal contaminated machinery in feeding area)

(1) ❑ Yes, which routines?_____

(2) ❑ No

52. Is it possible for stored feed to become contaminated with cow faeces?

(1) ❑ Yes

(2) ❑ No

53. Any routines between areas with-in the cow barn? (Owner and employees)

(1) ❑ Yes, go to 53a-i

(2) ❑ No

(3) ❑ Unknown

53a. Boot wash

(1) ❑ Always

(2) ❑ Sometimes

(3) ❑ Never

53a1. Initiated after September 2008

(1) ❑ Yes, investment DKK _____

(2) ❑ No

53b. Boot change

(1) ❑ Always

(2) ❑ Sometimes

(3) ❑ Never

53b1. Initiated after September 2008

(1) ❑ Yes, investment DKK _____

(2) ❑ No

53c. Hand wash

(1) ❑ Always, go to 53c1

(2) ❑ Sometimes, go to 53c1

(3) ❑ Never

53c1. Initiated after September 2008

(1) ❑ Yes, investment DKK _____

(2) ❑ No

53d. Change of clothes/coveralls

(1) ❑ Always, go to 53d1
(2) ❑ Sometimes, go to 53d1
(3) ❑ Never

53d1 Initiated after September 2008

(1) ❑ Yes, investment DKK _____
(2) ❑ No

53e. Other routines

(1) ❑ Yes _____

53e1. Initiated after September 2008

(1) ❑ Yes, investment DKK _____
(2) ❑ No

53f. Other actions

(1) ❑ Yes _____

53f1. Initiated after September 2008

(1) ❑ Yes, investment DKK _____
(2) ❑ No

53g. Other actions

(1) ❑ Yes _____

53g1. Initiated after September 2008

(1) ❑ Yes, investment DKK _____
(2) ❑ No

53h. Other actions

(1) ❑ Yes _____

53h1. Initiated after September 2008

(1) ❑ Yes, investment DKK _____
(2) ❑ No

53i. Other (I.e. actions Initiated before September 2008)

_____

54. Any biosecurity routines for visitors? (vet, hoof trimmer, farmers, AI-technicians and others)

(1) ❑ Yes, go to 54a-i
(2) ❑ No
(3) ❑ Unknown

54a. Boot wash

1) ❑ Always
(2) ❑ Sometimes
(3) ❑ Never

54a1. Initiated after September 2008

(1) ❑ Yes, investment DKK _____
(2) ❑ No

54b. Boot disinfection

1) ❑ Always
(2) ❑ Sometimes
(3) ❑ Never

54b1. Initiated after September 2008

(1) ❑ Yes, investment DKK _____
(2) ❑ No

54c. Boot change

1) ❑ Always
(2) ❑ Sometimes
(3) ❑ Never

54c1. Initiated after September 2008

(1) ❑ Yes, investment DKK _____
(2) ❑ No

54d. Coveralls

(1) ❑ Always
(2) ❑ Sometimes
(3) ❑ Never

54d1. Initiated after September 2008

(1) ❑ Yes, investment DKK _____
(2) ❑ No

54e. Hand wash

1) ❑ Always
(2) ❑ Sometimes
(3) ❑ Never

54e1. Initiated after September 2008

(1) ❑ Yes, investment DKK _____
(2) ❑ No

54f. Other routines

(1) ❑ Yes _____

54f1. Initiated after September 2008

(1) ❑ Yes, investment DKK _____

(2) ❑ No

54g Other actions

(1) ❑ Yes _____

54g1. Initiated after September 2008

(1) ❑ Yes, investment DKK _____

(2) ❑ No

54h. Other actions

(1) ❑ Yes _____

54h1 Initiated after September 2008

(1) ❑ Yes, investment DKK _____

(2) ❑ No

54i. Other actions

(1) ❑ Yes _____

54i1. Initiated after September 2008

(1) ❑ Yes, investment DKK _____

(2) ❑ No

54j . Other

(I.e. actions initiated before September 2008)

_____

55. Use of equipment and machinery in more than one barn?

(1) ❑ Always, go to 55a

(2) ❑ Sometimes, go to 55a

(3) ❑ Never

(4) ❑ Unknown

55a. Is the equipment cleaned between use in different barns?

(1) ❑ Always

(2) ❑ Sometimes

(3) ❑ Never

(4) ❑ Unknown

56. How is stocking rate in different groups compared to before beginning of the project? (September 2008)

	Lower	Same	Higher	Unknown
Calves	(1) ❑	(2) ❑	(3) ❑	(4) ❑
Heifers	(1) ❑	(2) ❑	(3) ❑	(4) ❑
Cows	(1) ❑	(2) ❑	(3) ❑	(4) ❑

57. Purchase of animals to the herd?

(1) ❑ Yes, go to 57a
(2) ❑ No

57a. Is Salmonella antibody level known for purchased animals?

(1) ❑ Yes, go to 57b
(2) ❑ No
(3) ❑ Sometimes
(4) ❑ Unknown

57b. Only purchase from level 1 herds?

(1) ❑ Always
(2) ❑ Sometimes
(3) ❑ Never

58. Any Salmonella tests for individual animals in addition to tests included in this project?

(Results will be collected from registry data)

(1) ❑ Yes, go to 58a
(2) ❑ No

58a. Which animals are tested?

(1) ❑ Cows
(2) ❑ Heifers

58b. If yes, does antibody test results get used in daily management?

(1) ❑ Yes, go to 58b
(2) ❑ Sometimes
(3) ❑ Never

58b1. Which decisions

(1) ❑ Separate calving area for Salmonella positive animals
(2) ❑ One cow/one heifer per calving pen for test-positive animals
(3) ❑ Cleaning udder before calving
(4) ❑ Removal of calf immediately after calving
(5) ❑ Colostrum not used
(6) ❑ Milk not used to feed calves
(7) ❑ Culling strategy
(8) ❑ Grouping of test-positive animals
(9) ❑ Other_____

59. Biosecurity routines when transporting animals to and off farm?

(1) ❑ Yes, go to 59a
(2) ❑ No
(3) ❑ Unknown

59a. Which routines?

(1) ❑ Separate barn section
(2) ❑ Separate entrance to separate barn section
(3) ❑ Other_____

60. Use of machine pool for spread of manure?

(1) ❑ Yes
(2) ❑ No

61. Do you share machines for manure spreading with others?

(1) ❑ Yes, go to 61a
(2) ❑ No

61a. Any other machines shared than manure spreader?
(I.e machinery for feeding, straw, transportation)

(1) ❑ Yes
(2) ❑ No

62. Comments regarding general biosecurity:

_____

63. Have you had plans of control actions which have not been possible to implement?

(1) ❑ Yes,
which?_____
(2) ❑ No,
why?_____

DEPARTMENT OF LARGE ANIMAL SCIENCES
PHD THESIS 2012 · ISBN 978-87-7611-489-3

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