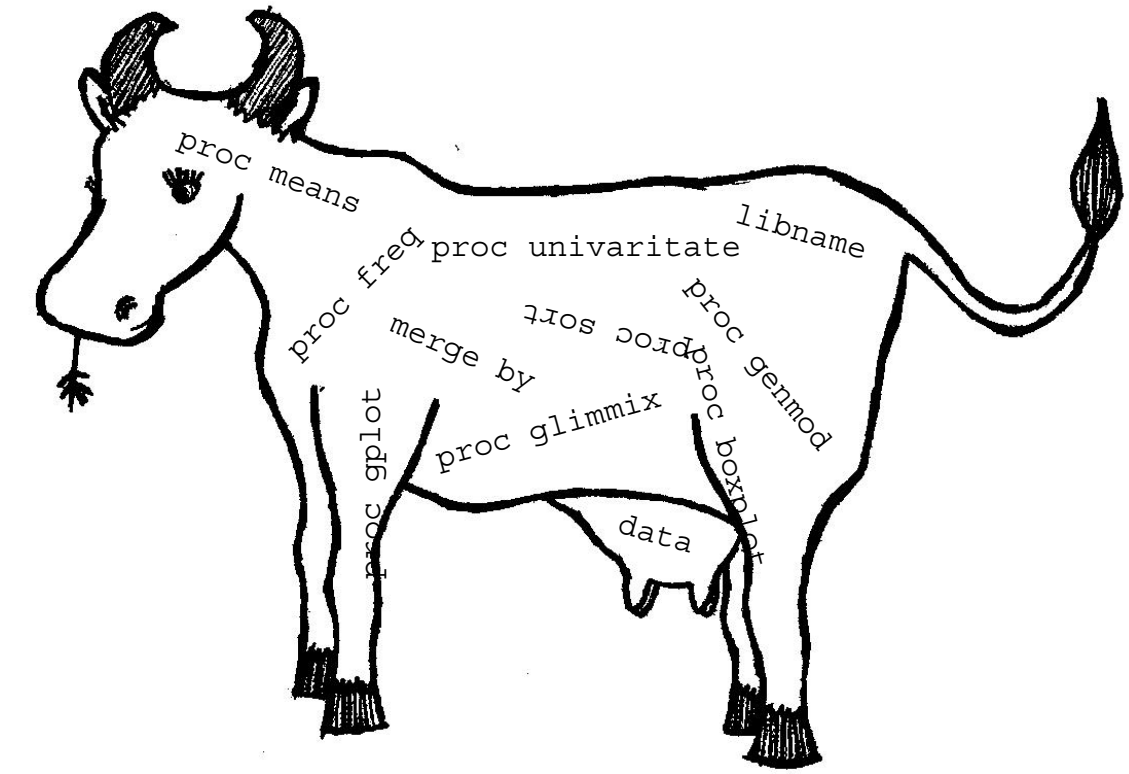
Association between *Salmonella* Dublin in cattle herds and recording of liver flukes and liver abscesses at slaughter



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PREFACE

This veterinary thesis was composed at the Department of Large Animal Sciences / Populationsbiology and Department of Veterinary Pathobiology / Section for Pathology at Faculty of Life Sciences, University of Copenhagen in the spring and summer of 2007. The objective of this thesis is to pass on the results of the studies to other persons with interest in the subjects. It primarily applies to veterinarians and other advisers in the cattle industry in Denmark. Also, the results are assumed to be of interest in an international perspective.

We would like to thank Jørgen Nielsen, the Danish Dairy Board for preparing data for the study and replying to clarifying questions. Also, we would like to thank Flemming Thune-Stephensen, Danish Meat Association for helping us getting started on the project. Also, thank you to Annette Kjær Ersbøll, Department of Large Animal Sciences / Populationsbiology, Faculty of Life Sciences for assistance with statistical procedures and composing of Maps. We would also like to thank Nina Monrad for proofreading the thesis.

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SUMMARY

This veterinary thesis deals with two different problems in relation to *Salmonella* Dublin (*S.* Dublin) in the Danish cattle industry. The studies were performed as observational studies based on register data from the Danish Cattle Database and the National Surveillance Programme for *S.* Dublin.

In one study, we assessed whether the *S.* Dublin classification status was a risk factor for liver abscesses in feedlot production sites. The study was divided into two parts, one part investigating risk factors for the individual animal having liver abscesses recorded at slaughter, and the other part investigating risk factors for the herd having a high prevalence of liver abscesses recorded at slaughter. In none of the two studies *Salmonella* test status of the herd was found to be associated with liver abscesses. The prevalence of liver abscesses between animals in this study was 12.5%, whereas the herd prevalence of liver abscesses among feedlot herds included in the study varied from 0 to 48%. Due to convergence problems, the model on animal level risk factors could not be performed the way we intended. Instead, the model was run ten times on five randomly selected animals from each herd. Because the model results were fairly different between runs, it was difficult to draw conclusions from that part of the study.

Production type (slaughter calf compared to young bull production) and herd size measured as the number of year-calves under 180 days were found to be associated with a high prevalence (>10%) of liver abscesses in the herd ($P < 0.0001$; $P = 0.001$ respectively) and there was a significant interaction between them ($P = 0.02$). For small herd size, herds with mainly slaughter calf production were more likely to have a high prevalence of liver abscesses detected at slaughter compared to herds with young bull production. As apposed to the large herd size, the young bull productions were more likely to have a high prevalence.

The second study investigated risk factors for *Salmonella* test status and changes in test status in the Danish surveillance program for *S.* Dublin. The main focus in this study was infection with *Fasciola hepatica* (*F. hepatica*; liver flukes) as a risk factor. In one model, herds with more than 2% liver flukes recorded at slaughter from January to May 2007 were more likely to be *Salmonella* test positive in the first quarter of 2007 than herds with 0-2% liver flukes (OR=2.1; 95% CI: 1.6-2.9, $P < 0.0001$). This corresponds to the results of two previous studies. Geographical region in Denmark

was included to control for regional cluster effects of *Salmonella* prevalence based on knowledge from previous work. Due to convergence problems, interactions with region could not be tested. In this model large herds had a higher risk of being *Salmonella* test positive than small herds (OR = 1.5 per 100 heads; 95%CI: 1.2-1.8, $P < 0.0001$).

In two alternative models, risk factors of changing *Salmonella* test status from negative to positive (indicating infection) and from positive to negative (indicating recovery) were also investigated. Again, geographical region of the herd was included to control for regional cluster effects and was a significant risk factor for changing *Salmonella* test status from negative to positive. Also, herd size was associated with changing *Salmonella* test status from negative to positive (OR = 1.7 per 100 heads; 95%CI: 1.2-2.2, $P = 0.0031$). For changing from *Salmonella* test positive to negative, odds were 1.7 (95%CI: 1-2.9) times higher among herds with 0-2% liver flukes compared to herds with more than 2% liver flukes ($P < 0.04$). This association has not previously been investigated in other studies. The result could explain why an association between liver flukes and *S.* Dublin exists, since herds with liver flukes are less likely to recover from *S.* Dublin. Organic status and purchase of animals was not found to be associated with neither *Salmonella* test status nor a change in test status in this study.

In conclusion, an association between *S.* Dublin and liver abscesses was not found in this study. This association has not previously been investigated, but recordings of other diseases at meat inspection, such as lung diseases, are known risk factors. An investigation of *S.* Dublin measured on animal level as risk factor for having liver abscesses would be of interest due to the lack of results and convergence problems in the model used in the present study. In the second study, prevalence of liver fluke was found to be associated with herd *Salmonella* test status. Furthermore, herds with 0-2% liver flukes were more likely to recover from *Salmonella* than herds with more than 2% liver flukes. This knowledge is beneficial in achieving the goal of eradicating *Salmonella* in the Danish cattle industry, since it could explain why some herds remain infected.

SAMMENDRAG

Dette veterinære speciale omhandler to forskellige problemstillinger i relation til *Salmonella* Dublin (*S.* Dublin) i den danske kvægproduktion. Undersøgelserne blev udført som observationsstudier på baggrund af registerdata fra den danske kvægdatabase og det nationale overvågningsprogram for *S.* Dublin.

I et studie undersøgte vi besætningens salmonellateststatus som en risikofaktor for leverabscesser i den danske fedekalveproduktion. Undersøgelsen blev inddelt i to dele, hvor sammenhængen blev undersøgt på henholdsvis enkeltdyrs- og besætningsniveau. Ingen af de to studier viste sammenhæng mellem *Salmonella* teststatus og leverabscesser. Prævalensen af leverabscesser blandt dyrene i denne undersøgelse var 12,5%, mens prævalensen af leverabscesser på besætningsniveau varierede fra 0 til 48%.

På grund af konvergensproblemer kunne undersøgelsen på enkeltdyrsniveau ikke køres med alle dyr i modellen på en gang. I stedet blev den kørt ti gange med fem forskellige, tilfældigt udvalgte dyr fra hver besætning. Da der var stor variation mellem resultaterne fra de enkelte runder kunne der ikke drages en endelig konklusion af denne del af undersøgelsen.

Produktionstype (slagtekalveproduktion overfor ungtyreproduktion) og besætningsstørrelse, målt som antal årsdyr under 180 dage, var associeret med en høj prævalens (>10%) af leverabscesser i besætningen i undersøgelsen på besætningsniveau (henholdsvis $P < 0,0001$; $P = 0,001$). Der var vekselvirkning mellem produktionstype og besætningsstørrelse ($P = 0,02$). For små besætningsstørrelser var der størst risiko for at besætninger med slagtekalveproduktion ville have en høj prævalens af leverabscesser ved slagtningen. Derimod var der større sandsynlighed for at store besætninger med ungtyrsproduktion havde en høj prævalens af leverabscesser end store besætninger med slagtekalveproduktion.

I det andet studie blev risikofaktorer for *Salmonella* teststatus og ændringer i teststatus i det danske overvågningsprogram for *S.* Dublin undersøgt for malkekvægsbesætninger. Fokus i denne undersøgelse var især rettet mod sammenhængen mellem *Fasciola hepatica* (*F. hepatica*; den store leverikte) og *S.* Dublin. En model viste at besætninger noteret for mere end 2% leverikter blandt dyr slagtet i perioden januar-maj havde en større sandsynlighed for at være *Salmonella* test positive i

første kvartal af 2007 end besætninger med 0-2% leverikter (OR=2,1;95% CI:1,6-2,9, $P < 0,0001$). Dette stemmer overens med resultaterne i to tidligere studier. Besætningernes placering i syv geografisk regioner i Danmark blev inddraget for at kontrollere for regionale cluster effekter for *Salmonella* prævalens på baggrund af tidligere studier. På grund af konvergensproblemer kunne vekselvirkning med region ikke undersøges. I denne model blev det desuden fundet at store besætninger havde en højere sandsynlighed for at være *Salmonella* test-positive end små besætninger (OR = 1,5 %; 95% CI: 1,2-1,8, $P < 0,0001$).

To alternative modeller undersøgte risikofaktorer for at besætningen ændrede *Salmonella* teststatus fra negativ til positiv (indikerer ny infektion) og fra positiv til negativ (indikerer helbredelse). Igen blev besætningens geografiske oprindelse inkluderet for at kontrollere for regionale cluster effekter. Region var en signifikant risikofaktor for at ændre *Salmonella* teststatus fra negativ til positiv (OR = 1,7%; 95%, CI:1,2-2,2, $P = 0,0031$). Sandsynligheden for at ændre *Salmonella* teststatus fra positiv til negativ var 1,7 gange højere for besætninger med 0-2% leverikter sammenlignet med besætninger med mere en 2% leverikter ($P < 0,04$) Denne sammenhæng er ikke tidligere blevet påvist. Resultatet kunne forklare hvorfor der er en sammenhæng mellem leverikter og *S.* Dublin, eftersom det tyder på at besætninger med leverikter har dårligere muligheder for at helbrede sig fra *Salmonella* end besætninger uden leverikter. Økologisk status og køb af dyr havde ingen indflydelse på hverken *Salmonella* teststatus eller en ændring i teststatus i dette studie.

Det kan konkluderes, at dette studie ikke fandt nogen sammenhæng mellem *S.* Dublin og leverabscesser. Denne sammenhæng er ikke tidligere blevet undersøgt, men tilstedeværelse af andre fund ved kødkontrollen, eksempelvis lungesygdomme, er kendte risikofaktorer for leverabscesser. Det kunne være interessant at foretage en undersøgelse af sammenhængen mellem *S.* Dublin og leverabscesser på enkeltdyrsniveau, hvor salmonellastatus blev målt på det enkelte dyr, ikke mindst på grund af manglende resultater og konvergensproblemer i modellen, der var opstillet i indeværende studie. Det andet studie fandt en sammenhæng mellem prævalensen af leverikter og besætningens *Salmonella* teststatus. Yderligere var der større chance for at besætninger med 0-2% leverikter ville komme sig af *Salmonella* end besætninger med mere end 2% leverikter. Denne viden er anvendelig i relation til målet om at udrydde *Salmonella* i den danske kvægproduktion, fordi det kan forklare hvorfor nogle besætninger forbliver inficerede.

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INTRODUCTION

In 2002 a National Surveillance Programme for *Salmonella* Dublin (*S.* Dublin) in the Danish cattle industry was introduced. Recently an objective of eradicating *S.* Dublin before 2014 was launched. In this context knowledge of association between *S.* Dublin and other diseases in cattle are of interest for one thing to motivate the farmer to reduce *S.* Dublin in the herd and for another to optimise the eradication. Currently 16.1% of milk-producing properties and 19.6% of non-milk producing properties are considered to be 'not free of *S.* Dublin'. This veterinary thesis looks into the association between *S.* Dublin and liver abscesses in the feedlot production and *S.* Dublin and *Fasciola hepatica* (liver flukes) in dairy herds.

Liver abscesses are widespread in the feedlot production with more than 10% of feedlot cattle in Denmark having liver abscesses at slaughter. Liver abscesses seldom cause clinical signs in the individual animal but are known to be of economic importance to the cattle industry both in terms of reduced weight gain as well as condemned livers at slaughter. It is also shown that animals with other finds at slaughter are at greater risk of having liver abscesses. As a motivating factor for the farmer in relation to eradicating *S.* Dublin it is of interest to investigate if *S.* Dublin is associated with liver abscesses.

Infection with liver flukes in cattle is usually sub-clinical and therefore its importance is mainly economic due to reduced performance. In Denmark the prevalence of liver flukes is expected to have increased in recent years due to an increased use of set aside land which often provides good conditions for the development of liver flukes. In 2003 2.7% and 8.3% of slaughtered dairy cattle and beef cattle respectively were recorded as having liver flukes. Foreign studies have found liver flukes to be associated with *S.* Dublin on individual animal as well as on herd level. In relation to the objective of eradicating *S.* Dublin it is of interest to investigate if this association applies to Denmark as well.

The main objectives in this veterinary thesis are to:

1. Investigate the association between *S.* Dublin and liver abscesses in the Danish feedlot production. Two statistical hypotheses will be tested.
   - H_{0A}: There is no significant association between the herd *Salmonella* status and the likelihood of the individual animal having liver abscesses at slaughter
   - H_{0B}: There is no significant association between the herd *Salmonella* status and the prevalence of liver abscesses
2. Investigate the association between liver flukes and *S.* Dublin in Danish dairy herds. In this part three statistical hypotheses will be tested.
   - H_{0A}: There is no significant association between the prevalence of liver fluke in the herd and the herd *Salmonella* status
   - H_{0B}: There is no significant association between the prevalence of liver flukes in the herd and the probability of getting infected with *Salmonella*
   - H_{0C}: There is no significant association between the prevalence of liver flukes in the herd and the probability of recovering from *Salmonella*

The studies were performed as observational studies based on register data from the Danish Cattle Database and the National Surveillance Programme for *S.* Dublin. An observational study was chosen in order to investigate the association under natural conditions. Register data was used in order to include as many herds as possible in the study and to give a realistic picture of the investigated associations in Danish herds.

The thesis consists of four chapters. The first chapter contains a short literature based presentation of basic knowledge of *S.* Dublin, liver abscesses and liver flukes in general and with particular focus on Denmark. Only theory, which is relevant for the present studies, is included. Chapter 2 contains a manuscript entitled “Association between *Salmonella* Dublin and liver abscesses in the Danish feedlot production”. Chapter 3 contains a manuscript entitled “Association between *Salmonella* Dublin and *Fasciola hepatica* in Danish dairy herds”. In the manuscripts all results of the studies are included in order to demonstrate the complete work of this project, but in the final manuscripts for publication only main results will be included. The last chapter contains the overall conclusions and perspectives of the veterinary thesis.

CHAPTER 1 - Theory

THEORY

Salmonella Dublin *in general and in Denmark*

Salmonella enterica subspecies *enterica* Dublin (*S.* Dublin) is a host-adapted salmonella serotype in cattle (Radostits *et al.*, 2000). Other species can also get clinically ill from the bacteria. It is a zoonotic infection with serious human cases each year causing high fever and septicaemia. In Denmark, 27 cases of human infections with *S.* Dublin were reported in 2006 (Anonymous, 2007c). It is considered one of the most pathogenic *Salmonella* bacteria known to infect humans from animals (Anonymous, 2007f) with a high mortality rate compared to other common zoonotic infections (Helms *et al.*, 2003). *S.* Dublin is not the only *Salmonella* serotype affecting cattle with *Salmonella enterica* subspecies *enterica* Typhimurium (*S.* Typhimurium) being the second most prevalent serotype in Danish cattle (Steffensen and Blom, 1999; Wray and Davies, 2000).

The most common route of infection is through ingestion of the bacteria by contaminated feed, milk and water (Nazer and Osborne, 1977; Wray and Davies, 2000), but other routes of infection have been detected experimentally (Nazer and Osborne, 1977; Spier *et al.*, 1991). The organism travels through the gastrointestinal tract and gains entry to the tissue by an invasive process, mainly in the lower small intestine (Wray and Davies, 2000). By invading M cells and enterocytes in the intestinal mucosa the bacteria comes in contact with macrophages which they enter. Surviving and replicating in the macrophages, the bacteria disseminates with the lymph fluid to other tissues and the circulating blood (Wray and Davies, 2000; Nielsen, 2003).

Infection with *S.* Dublin in cattle has a varied clinical appearance with peracute, acute or chronic courses (Wray and Davies, 2000). It is most often associated with clinical disease in calves (Steffensen and Blom, 1999) where clinical illness may show from approximately 2 weeks to 3 month of age (Wray and Davies, 2000). In calves, septicaemia resulting in fever and diarrhoea combined with pneumonia and varying degrees of arthritis are the most common clinical signs. The infection is also known to cause mortality in calves. Older cattle can also be affected by *S.* Dublin resulting in reduced yield, unthrifty animals and abortion (Wray and Davies, 2000). Cattle infected with *S.* Dublin may develop into passive, active or latent carrier animals, which do not show clinical signs, but excrete the bacteria continuously or intermittently (Richardson, 1973; Wray and Sojka, 1977). In carrier animals the bacteria are carried in lymph nodes or internal organs (Wray and Davies, 2000), for example in the gall bladder and intestines (Hoorfar *et al.*, 1996). Different studies

found the number of carrier animals with continuously high antibody titres in infected herds to vary from 3% to 7.7% (Hoorfar *et al.*, 1996; Nielsen *et al.*, 2007a). Stress at the time of calving may trick the infection so carrier animals will start to excrete bacteria, thereby increasing the risk of infecting the highly receptive calves (Wray and Davies, 2000; Radostits *et al.*, 2000). Carrier animals are an important reason why herds stay persistently infected after clinical symptoms have ceased, because they contaminate the environment and infect other animals from time to time (Wray and Davies, 2000; Radostits *et al.*, 2000).

Transmission of *S.* Dublin may occur within the herd as well as between herds. Management and hygiene in the herd are very important factors for the duration and severity of the infection (Wray *et al.*, 1989; Wray and Davies, 2000). *Salmonella* may persist in the environment for a long time. Ten months after animals had left the farm and disinfection was attempted, bacteria were still found in 25% of samples (Mclaren and Wray, 1991). After analysis of transmission of *Salmonella* among calves, Hardman *et al.* (1991) concluded that the indirect routes were more important than the direct transmission routes, and therefore their recommendations were cleaning and disinfection of utensils and other hygienic practices. Transmission between herds usually occurs when *Salmonella* free herds are introduced to the infection by purchase of infected animals (Wray and Davies, 2000; van Schaik *et al.*, 2002).

It is often assumed that *S.* Dublin is primarily a winter problem. A study by Steffensen & Blom (1999) disproved this theory. Instead, they found that temperatures deviating from the normal curve in spring and summer were decisive as to which month of the year would have the highest number of clinical cases. Low temperatures in the summer period resulted in little isolation of *S.* Dublin, whereas a warm summer resulted in an increased number of isolations of *S.* Dublin. The same conclusion was the result of a study of *Salmonella* spp. in general with most *Salmonella* positive faecal samples in the summer (Fossler *et al.*, 2004; Fossler *et al.*, 2005).

National Surveillance Programme for S. Dublin *in Denmark*

In 2002, the Danish Veterinary and Food Administration implemented a national surveillance programme for *S.* Dublin. The programme was developed in cooperation with the Danish Veterinary Institute and the Danish Cattle Federation. The objective of the programme is to keep non-infected herds free of infection. The programme is intended to describe the likelihood of *S.*

Dublin infection among the animals in the herd (Anonymous, 2006). In 2003, the Danish Cattle Federation took over the administration of the program.

In the programme, cattle properties are classified into three herd test classifications (Anonymous, 2006). Test classification 1 is least incriminating and test classification 3 is most incriminating. The programme is based on serological testing of blood and milk samples. All dairy herds are tested by antibodies in bulk tank milk (BTM) samples four times a year, while the number of blood samples from non-milk producing farms is determined on the basis of the herd size. In herds larger than 10 animals, the *S.* Dublin classification is calculated based on the last 8 samples from the herd. Independently of this, a herd is placed in test classification 3 if *S.* Dublin is isolated from the herd and there is clinical disease suspected as salmonellosis (Anonymous, 2006). Apart from the three test classifications a fourth classification of 'unknown' is defined. This classification only applies to non-milk producing properties and is used if the number of blood samples is insufficient and the threshold value has not been exceeded. Herds in test classification 1 are assumed to be 'free of *Salmonella'*. To be able to differentiate between the test methods used for classification, classification 1 is divided into 1a for BTM samples and 1b for blood samples. Herds placed in test classification 2 are assumed to be 'not free of *Salmonella'*. Herds are placed in test classification 3 if they are under public supervision due to salmonellosis caused by *S.* Dublin. The purpose of the surveillance program is that *S.* Dublin free herds (test classification 1a and 1b) may avoid getting infected by avoiding contact with herds in other classifications, this being in terms of purchase, physical contact etc.

Antibodies in BTM and blood are detected with an enzyme linked immunosorbent assay (ELISA) test of lipopolysaccharid from the *Salmonella* bacteria (Hoorfar and Bitsch, 1995). The value is measured as ODC%, which is the optical density in the sample in relation to a known control (Nielsen, 2003). There are three criteria which the herd should meet to be classified as 'free of *Salmonella'*. To be classified as a classification 1 herd, dairy herds need to have four valid BTM samples. These must show a mean *S.* Dublin ODC% value of less than 25, and an increase of more than 20 in the last sample compared to a mean of the three previous samples is not allowed. For non-milk producing herds the criteria are the following: Four blood samples in a row may not exceed an average antibody level of 50% ODC. The latest test may not exceed the previous three samples with more than 20 ODC% and *S.* Dublin may not be isolated from the herd during the

previous six months (Anonymous, 2006). Other serotypes can cross-react with the *S.* Dublin antigen used in the ELISA (Konrad *et al.*, 1994) and it is therefore possible that some herds infected with other serotypes are pointed out as 'not free of *S.* Dublin' even though they are actually free of *S.* Dublin. Since it is a surveillance programme false positive test results are considered acceptable. A study evaluating the use of multiple BTM samples to test for *S.* Dublin concluded that it was a usable method for screening purposes (Wedderkopp *et al.*, 2001a). A study by Warnick *et al.* (2006) evaluated the methods used to classify dairy herds in the surveillance program. They concluded that the method was consistent with the primary goal of the programme in that herds classified free were truly free of *Salmonella* infection.

In 2006 the Danish government decided that Denmark should aim at getting special salmonella status within the European Union (Finansministeriet, 2006). A potential effect of this could be that Denmark may put a restriction on the import of animal products from countries which have salmonella in their food industry, thereby improving the food safety for Danish consumers.

On July 11th 2007 16.1% (788/4.908) of Danish milk-producing properties and 19.6% (3.680/18.784) of non-milk producing properties were classified as 'not *Salmonella* free' (Anonymous, 2007d). In this calculation properties in classification 2 and 3 as well as classification 'unknown' in the surveillance programme are included in the definition of classification 'not *Salmonella* free'. Out of the 18.784 non-milk producing properties, 2.068 (11.0%) were classified as 'unknown status'. The Danish cattle industry has decided that *S.* Dublin should be eradicated in Danish cattle before 2014 (Nielsen, 2007). The eradication program is based on the surveillance program and the basis of the programme to break the transmission routes both between and within herds primarily by optimizing hygiene and management, but also by culling clinically healthy, but infected animals. Information about the salmonella level in each herd is available to the public on the Internet (www.glr-chr.dk). The farmers can use the information to decide which herds they want to trade with, thereby reducing the probability of spreading the infection.

Liver Abscesses

Liver abscesses are bacterial infections of the liver, the route of infection occurring via blood, local spread, migrating parasites and ascending through the biliary system (Cullen and MacLachlan,

2001). All types of cattle may develop liver abscesses, but it is especially seen in cattle raised for meat production in the USA, Canada, Europe, Japan and South Africa (Nagaraja *et al.*, 1996).

As early as 1944, an association between ulcerative lesions in the rumen and liver abscesses were observed (Smith, 1998), and ten years later, Jensen *et al.* (1954a) propounded a ruminitis-liver abscess complex because of high statistical correlation between occurrence of liver abscesses and ruminal pathology. Although the precise pathogenic mechanism is not recognized, it is accepted that ruminal lesions resulting from acidosis are the predisposing factor. A feeding strategy with large amounts of starch and glucose may initiate the ruminitis-liver abscess complex. Starch is fermented rapidly by ruminal microbes resulting in accumulation of organic acids (Nagaraja *et al.*, 1996). Too much free glucose in the rumen may induce acidosis in cattle, ranging from acute disease with clinical signs (Dunlop, 1972; Huber, 1976) to sub-acute and chronic, in which cattle exhibit no clinical signs but have reduced feed intake and performance (Stock *et al.*, 1990). When acidosis occurs, this will inhibit the absorption of volatile fatty acids (VFA) and lead to an accumulation of acid in the rumen, resulting in a decline in the ruminal pH (Mackie *et al.*, 1978; Goad *et al.*, 1998). The total ruminal lactate increases and the proportions between the ruminal bacteria changes (Goad *et al.*, 1998). Some bacteria use lactate as their source of energy, therefore the concentration of these bacteria increases (Mackie and Heath, 1979). The accumulation of total organic acids (VFA and lactate) dictates whether the rumen is acidotic (Owens *et al.*, 1998). Ruminal acidosis damages the rumen epithelium and leaves it more susceptible to invasion and colonisation of bacteria. The ruminal damage is often aggravated by foreign objects in the feed or sharp feed particles (Jensen *et al.*, 1954a). After colonisation the bacteria can access the circulation. Via Vena Porta the bacteria is transported to the liver where it is filtered from the blood. Thereby the spread remains local. In the liver, the bacteria proliferate and produce focal areas of hepatocellular necrosis and hepatitis, potentially developing into liver abscesses (Cullen and MacLachlan, 2001). Several studies concluded that *Fusobacterium necrophorum* is the primary etiologic agent (Scanlan and Hathcock, 1983; Lechtenberg *et al.*, 1988). In some instances the organism has been involved as a single pathogen, but it is often associated with a variety of other anaerobic and facultative bacteria (Scanlan and Hathcock, 1983). Abscesses eventually become sterile; they are replaced by fibrous tissue and will in time be resorbed (Nagaraja and Chengappa, 1998). The pathogenesis of cattle fed a high-grain diet is illustrated in Figure 1.

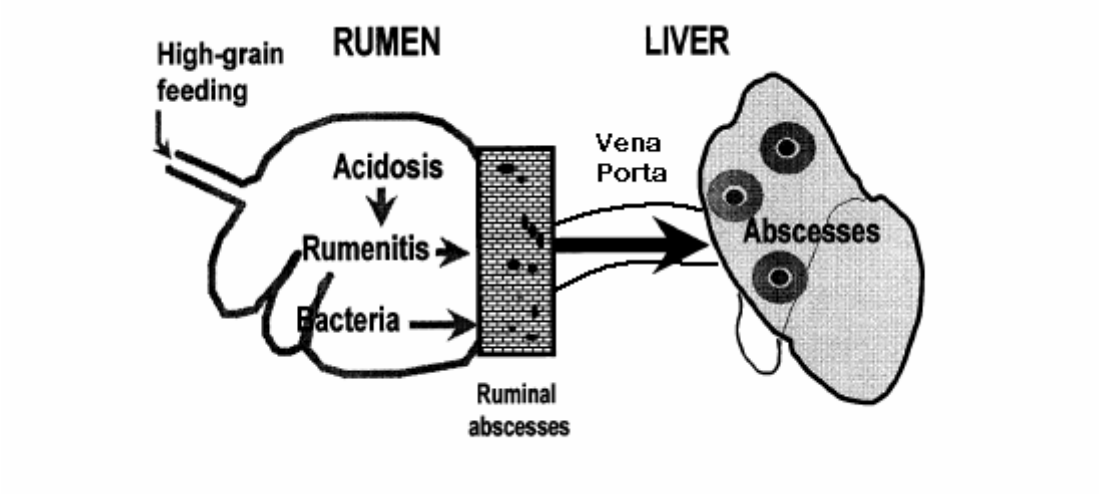

Figure 1. Pathogenesis of liver abscesses in feedlot cattle

Clinical symptoms are rare, but affected animals may have decreased growth rate (Brink *et al.*, 1990). The diagnosis is made by the veterinary meat inspection at the post mortem examination (Anonymous, 1987). Because of portal streaming, the abscesses are not evenly dispersed over the liver (Cullen and MacLachlan, 2001).

An aggressive feeding strategy in the feedlot cattle is often used to reach a high daily weight gain. Besides a high daily weight gain, the concentrate diet is favoured both by the cost per calorie and the operational efficiency in feedlot mills (Brown *et al.*, 2006b). In Denmark, the feedlot production is based on bull calves from the dairy breeds Black Holstein (SDM), Red Holstein (DRH), Danish Red (RDM) and Jersey (Anonymous, 2003). The specialised calf production in Denmark is around 50.000 heads per year. If the calf has reached 185 kg before slaughter, a financial advantage is achieved because of the special male premium (Anonymous, 2007b). To acquire special status as Danish veal, the calf must be less than 310 days of age at slaughter (Anonymous, 2007a).

Recordings in Denmark from September 2001 to August 2002 showed the prevalence of liver abscesses in cows, young stock (weight over 200 kg) and calves (weight under 200 kg) to be 4.6 %, 5.3 % and 10.6 %, respectively (Kjeldsen and Fisker, 2002). In another investigation in 2002 including 25.000 calves, the average prevalence of liver abscesses was 11.3% (Kjeldsen *et al.*, 2002). In Canada, the prevalence of liver abscesses in slaughtered steers was 17% (Van Donkersgoed *et al.*, 2001) and Harman *et al.* (1989) reported a prevalence of liver abscesses in feedlot cattle between 7 and 39% in North America, the variation was seen over the year and

depending on type of housing. In a survey conducted in Australia comparing grain-fed and grass-fed steers the prevalence of liver abscesses at slaughter were 7.2% and 0.5%, respectively (Roberts, 1982). Based on condemned livers at slaughter, Smith (1998) estimated a total loss of $36 million annually for the U.S cattle production. The condemned livers were not only because of abscesses, but also other diseases or losses from contamination. The total loss to the Canadian beef industry was calculated to $8.8 million annually (Van Donkersgoed *et al.*, 2001). No analyses of economic losses due to liver abscesses are available in Denmark. Economic loss may not only occur because of condemned livers at slaughter. A study of 566 cattle concluded that severe liver abscesses at slaughter were related to reduced carcass gain and dressing percentage (Brink *et al.*, 1990).

Fasciola hepatica in general and in Denmark

Infection with *Fasciola hepatica* (the large liver fluke) in cattle is most often asymptomatic and sub-clinical (Radostits *et al.*, 2000). Cattle get infected when they ingest the infective stadium, the metacercaries, along with the feed such as grass and vegetation (Andrews, 1999). The metacercaries are excysted in the intestine, each releasing one juvenile fluke larvae (Andrews, 1999). The young larvae fasten to and penetrate the intestinal wall, whereupon they wander in the peritoneal cavity eventually reaching the liver surface. Here they penetrate the liver capsule and wander in the liver parenchyma for about 4-5 weeks, foraging on hepatocytes (Behm and Sangster, 1999). They then move into the bile ducts, where they develop into adult, sexually mature flukes. The time from the cattle ingest the metacercaries to the flukes start laying eggs is about 10-12 weeks (Radostits *et al.*, 2000). This is called the pre-patent period. Cattle develop resistance against liver flukes. The acquired resistance is a combination of resistance against high primary doses of metacercaries, resistance against re-infection and spontaneous self-cure (Monrad and Nansen, 1994; Torgerson and Claxton, 2007). Ross (1968) found that the majority of liver flukes would have disappeared within 8-10 months after the infection, but that adult flukes could persist for at least 26 months in cattle.

Acute fasciolosis rarely occur in cattle, but heavy infestations in calves may result in severe illness with anaemia, hypoproteinaemia and death (Behm and Sangster, 1999). As mentioned previously infestation of adult cattle with liver flukes are usually sub-clinical (Radostits *et al.*, 2000). Common clinical signs of chronic fasciolosis are submandibular oedema and pale mucosal membranes caused by anaemia. Due to the chronic course of disease the significance of the infection is primarily impaired performance and economic losses. Reduced weight gain, reduced milk production

especially in young animals, and influence on reproduction performance are examples of reported effects of liver fluke on cattle production (Ross, 1970; Oakley *et al.*, 1979; Loyacano *et al.*, 2002; Charlier *et al.*, 2007).

The liver fluke is dependent on various factors in order to infect cattle. First, the parasite needs to pass through the intermediate host, a snail, in order to develop into the infective stadium on the vegetation (Radostits *et al.*, 2000). Under Danish circumstances the intermediate host is the lymnaeid snail, *Lymnaea trunculata* (Monrad and Nansen, 1994). The snail is restricted to damp or wet environments and prefer non-acidic, low-lying swampy areas with slow-moving water (Radostits *et al.*, 2000). Snail habitats may by temporary or permanent. Secondly, the development in the environment is influenced by the temperature (Boray, 1969; Andrews, 1999). Neither development in the intermediate host nor hatching of the egg takes place if the temperature is below 10°C, the ecological zero of the liver fluke. As a result hatching of eggs and therefore infection of cattle does not occur all year around in Denmark (Monrad and Nansen, 1994). Another factor affecting the liver fluke is the ambient humidity, because the intermediate host, as well as hatching of the egg, is dependent on humidity (Andrews, 1999).

The classical epidemiology on fascioliasis described two annual cycles of infection, the winter infection of the snail, with metacercaries on pasture in the spring and early summer and the summer infection of the snail, resulting in metacercaries on the pasture from midsummer and onwards (Torgerson and Claxton, 2007). The previously described epidemiology applies to Denmark as well (Monrad and Nansen, 1994). The infection pressure on pasture is variable throughout the year in Denmark with the summer infection in the snail being the most important under Danish conditions, but still with winter infection in the snail occurring (Nielsen *et al.*, 1973; Shaka and Nansen, 1979). An experiment with tracer lambs found the highest availability of metacercaries on the pastures in the summer and early autumn, from July to October (Shaka and Nansen, 1979). Nielsen *et al.* (1973) found the same pattern in an experiment with tracer calves, with most liver flukes isolated from the animals getting infected in the period July until October. In May and June, the metacercaries will be a result of winter infection in the snail, in July and August the metacercaries will be a mix of winter and summer infection of the snail, whereas summer infection in the snail will result in metacercaries on pasture in September and October (Shaka and Nansen, 1979). As mentioned previously, the life cycle of the fluke, and with that the infection pressure, is influenced

by ambient temperature and humidity resulting in variation in infection pressure from one year to another dependent on rainfall and temperature (Nielsen *et al.*, 1973; Henriksen and Pilegaard-Andersen, 1979; Shaka and Nansen, 1979).

Due to the pre-patent period of the liver fluke, the period where the highest prevalence of positive faecal samples, indicating the highest prevalence of adult liver flukes in the cattle, will be December to March. This will usually be the period of the year with the highest number of liver fluke recordings at slaughter and the period with the highest amount of positive faecal samples (Henriksen and Pilegaard-Andersen, 1979).

Investigations of liver fluke infested livers based on abattoir recordings in Danish cattle in 1969-1972 found a mean prevalence of 16.5% (15-20%) infested livers from cattle older than two years (Riising *et al.*, 1973). There was a significant variation between geographical regions of the country with the highest prevalence in Jutland. In the following years the problems with liver flukes decreased, most likely due to increased drainage and focus on the subject (Henriksen and Pilegaard-Andersen, 1979). With a reform in the European Union in 1992 recommending an environmentally friendly use of set aside-land, the problems with liver flukes were expected to reappear. An investigation of slaughterhouse recordings found an overall increase in the prevalence of liver flukes from 3.2% to 8.3% in beef cattle and from 0.9% to 2.7% in dairy cattle over the years 2000 to 2003 (Thamborg *et al.*, 2005).

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CHAPTER 2 - Manuscript 1

Association between *Salmonella* Dublin and liver abscesses in the Danish feedlot production

ABSTRACT

The objective of this study was to investigate the association between *Salmonella* Dublin (*S.* Dublin) and liver abscesses in the Danish feedlot production. *Salmonella* test status was recalculated from data from the Danish Surveillance Programme for *S.* Dublin, whereas data on liver abscesses was based on recordings from the meat inspection at slaughter. The study was divided into two parts, one part investigating risk factors for the individual animal having liver abscesses recorded at slaughter and the other investigating risk factors for the herd having a high prevalence (>10%) of liver abscesses recorded at slaughter. In neither of the two studies *Salmonella* test status of the herd was found to be associated with liver abscesses. The prevalence of liver abscesses in bulls in this study was 12.5% whereas the prevalence of liver abscesses in feedlot herds included in the study varied from 0 to 48%. Due to convergence problems, the model on animal level risk factors could not be performed the way we intended. Instead the model was run ten times on randomly selected subsets of the full dataset. There was a large variation in the results of the ten runs which made it difficult to make any final conclusions from that part of the study.
Production type (slaughter calf compared to young bull production) and herd size measured as the number of year-calves under 180 days were found to be associated with a high prevalence (>10%) of liver abscesses in the herd and there was a significant interaction between them. Herds with mainly slaughter calf production were more likely to have a high prevalence of liver abscesses detected at slaughter than herds with young bull production for small herd sizes, whereas for large herd sizes, the young bulls were more likely to have a high prevalence.
In conclusion, an association between *S.* Dublin and liver abscesses was not found in this study neither on individual animal nor herd level. This association has not previously been investigated, but recordings of other diseases at meat inspection such as lung diseases are known risk factors. An investigation of *S.* Dublin measured on animal level as a risk factor for having liver abscesses continue to be of interest as convergence problems in the model used in the present study prevented a conclusive result.

INTRODUCTION

Salmonella Dublin

Salmonella enterica ssp. *enterica* serotype Dublin (*S*. Dublin) is host adapted to cattle (Wray and Davies, 2000). Currently 19.6% of non-milk producing properties are considered not free of *S.* Dublin either due to antibody value, purchase or contact with other herds classified as not free or unknown status (Anonymous, 2007b). Recently, a campaign to eradicate *S.* Dublin in the Danish cattle industry before 2014 was launched.

Growth of *Salmonella* in the gastrointestinal tract is favoured if the pH rises (Mattila *et al.*, 1988). A rise in abomasal pH is seen in young calves fed with milk replacer (Ahmed *et al.*, 2002). The shedding of *Salmonella* in calves entering a new herd was investigated by Wray *et al.* (1987).The first day *Salmonella* was isolated from 0.7% of the calves. Within the first 3 weeks after entry, 51% were shedding *Salmonella* in their faeces. After 4 weeks the infection decreased. Another experiment showed 61% calves shedding the bacteria within the first 7 days after purchase, before the infection declined to a low level during the fourth week (Hinton *et al.*, 1984). Normally, the volatile fatty acid-concentration in the rumen of grown cattle inhibits the growth of *Salmonella* (Chambers and Lysons, 1979). A period of starvation reduces the VFA concentration and growth of *Salmonella* occurs (Mattila *et al.*, 1988). After recovery of clinical illness some calves may become carriers and play a role in spreading of the disease (Gitter *et al.*, 1978). Use of milk replacer and frequent purchase of animals are common in the feedlot industry and these factors are important in relation to salmonella in the feedlot production.

Liver abscesses

Liver abscesses are bacterial infections of the liver (Cullen and MacLachlan, 2001). It is most commonly observed in feedlot cattle where ruminitis, resulting from ruminal acidosis, is the primary site of infection (Nagaraja and Chengappa, 1998). The etiological agent is often *Fusobacterium Necrophorum* (Lechtenberg *et al.*, 1988). Clinical symptoms are rare (Brink *et al.*, 1990).

An aggressive feeding strategy in the feedlot cattle is often used to achieve a high daily weight gain. The calves are fed with large amounts of glucose and starch rich concentrate such as grain, and only

little forage. This feeding strategy predisposes acidosis and is thereby a risk factor for getting liver abscesses. Besides a high daily weight gain, the concentrate diet is favourable due to both the low cost per calorie and the operational efficiency in feedlot mills (Brown *et al.*, 2006).

A study of recordings of liver abscesses at slaughter found the average prevalence of liver abscesses in individual animals to be 11.3% in Denmark in 2002 (Kjeldsen *et al.*, 2002). Large variation between the herds was detected. In the 10% of herds with the lowest prevalence, the prevalence was below 1.6%, whereas among the 10% herds with the highest prevalence, it was above 18.2%.

A study found that slaughter calves which had liver abscesses at slaughter were more likely to have other recordings at meat inspection, for example of lung disease, compared to animals without liver abscesses (Kjeldsen *et al.*, 2002). Also at herd level, an association between a high prevalence of other recordings at meat inspection and a high prevalence of liver abscesses was found.
Other factors have been found to be associated with having liver abscesses at individual as well as herd level. Examples of these are month of slaughter (Kjeldsen *et al.*, 2002), whether the animal had been moved between herds (Kjeldsen *et al.*, 2002), carcass weight (Brink *et al.*, 1990) and breed (Nagaraja *et al.*, 1996; Kjeldsen *et al.*, 2002).

The production system in feedlot herds could result in a high risk of getting infected with *Salmonella*. Liver abscesses are common in the feedlot industry and apparently associated with having other diseases. With the wish to eradicate *S.* Dublin in the Danish cattle industry it is relevant to investigate a potential association with liver abscesses. Knowledge of the association could provide motivation for the farmer to reduce and eradicate salmonella because of a potential simultaneous profit in relation to liver abscesses.

Aim of study

The aim of this study was to investigate a possible association between having liver abscesses and a positive antibody status of *Salmonella* on herd level indicative of active spread of *S.* Dublin in the herd. Through statistical analyses of database recordings, the study investigates the risk of individual animals developing liver abscesses identifiable at slaughter and the possible effect of the *Salmonella* classification status of the herd. A second analysis was made at herd level. The study

investigates, whether or not herd prevalence of liver abscesses is influenced by the *Salmonella* classification status of the herd.

MATERIALS AND METHODS

Data sources

All properties in Denmark are registered with a specific CHR number. The properties with cattle are integrated in the Danish Cattle Database (DCD). This database contains information at individual animal level and herd level of all registered cattle and properties with cattle in Denmark are available. Data for the study was extracted from the DCD as three separate dataset. One contained information about all cattle slaughtered form January 1st to May 10th 2007. Information about breed, date of birth, date of slaughter, source herd, departure herd, abattoir and records of liver abscesses at meat inspection were stated for each individual animal. The second dataset contained information at herd level about calves per year, type of herd and purchased cattle based on data from 2006. The third dataset contained calf mortality rates at herd level. Data about *Salmonella* status of each properties originated from the National Surveillance Programme for *S.* Dublin.

Study animals and study herds

Only bulls were included in the studies. Each animal was defined as being either a slaughter calf or a young bull. Figure 1 illustrates the number of slaughtered bulls distributed by age. This plot was used to define animal product as slaughter calf or young bull. At day 210 there is a steep rise on the curve. This was used to define the minimum age of a slaughter calf. There is a decline in slaughtered bulls at the age of 310 days. This correlates with the economical advantage of slaughtering the bull before it is 310 days old (Anonymous, 2007a). Thus, for this study a maximum age of 310 days was chosen for slaughter calves and a minimum age of a young bull was 311 days. At 730 days there is a small abrupt decline. This was used as the maximum age of a young bull. In summary, a slaughter calf was defined as a bull aged 210-310 days and a young bull was defined as a bull aged 311-730 days at slaughter. In order to be included in the study, the bull had to originate from a departure herd which was defined as a feedlot herd. A feedlot herd was defined as a herd that had delivered more than 20 slaughter calves or young bulls for slaughter from January to May 2007. In model B only animals slaughtered at abattoirs with electronic recordings of finds were included.

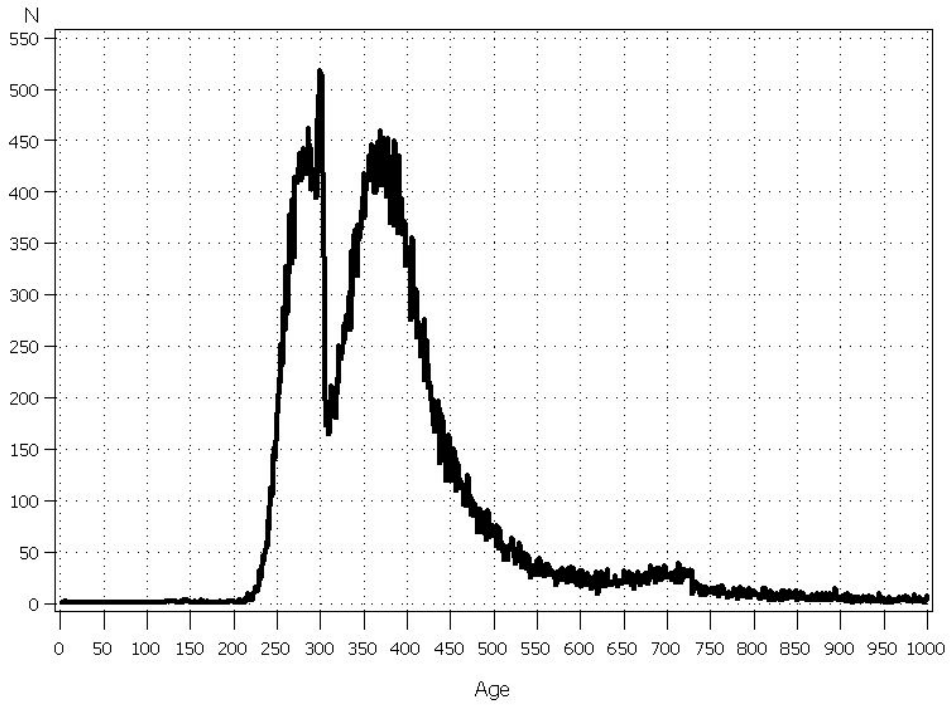

Figure 1. Distribution of number of slaughtered bulls (N) under 1000 days of age from January 1st to May 10th, 2007.

Epidemiological study design

Two models were used in the study of association between *Salmonella* test status and liver abscesses. In model A, we tested risk factors for having liver abscesses at slaughter on single animal level. In model B, we tested risk factors for having a high prevalence of liver abscesses on herd level. The variables in the analyses were defined for a given period from May 2005 to May 2007. Figure 2 illustrates the collection periods of some of the data. A detailed description of each variable included in the analyses is given in the following section.

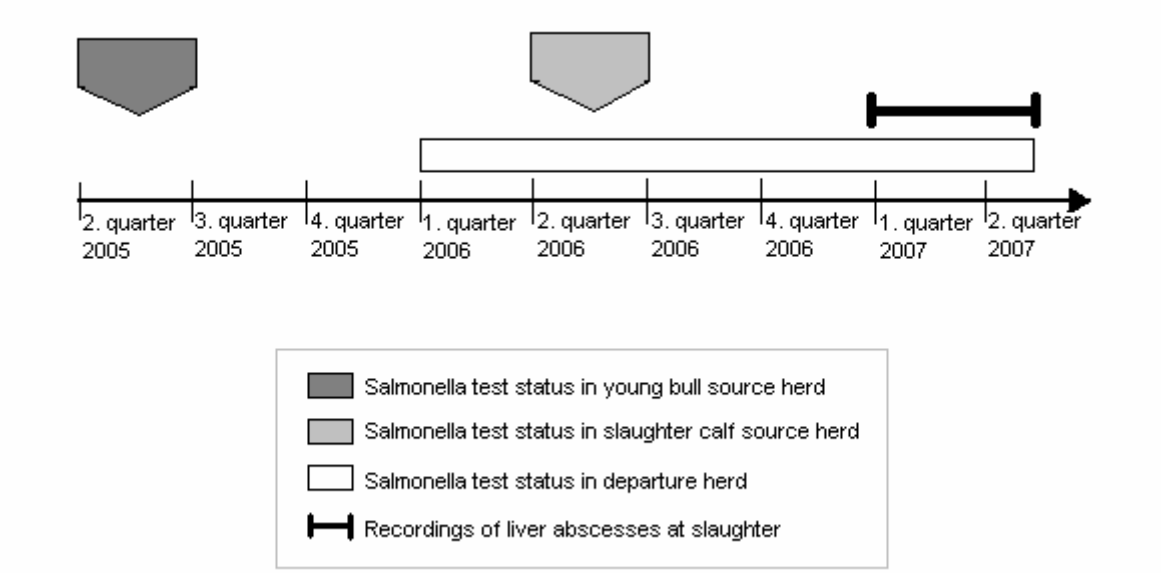

Figure 2. Data collection periods for the variables *Salmonella* test status and liver abscesses. The analyses were based on recordings of liver abscesses in slaughtered bulls. Based on the age of these, the period for calculating *Salmonella* test status in source and departure herd was estimated.

Data editing and descriptive statistics

Model A: Risk factors for liver abscesses at animal level

Data on the following variables were extracted from the slaughter recordings from animals slaughtered in the period January 1st to May 10th 2007:

Recordings of liver abscesses. In Denmark all carcasses and organs are examined by the veterinary meat inspection. All abnormal findings are supposed to be recorded. Liver abscesses are recorded with code 375. In model A, this was the outcome variable of interest and it was coded as a categorical variable (yes/no) indicating if the animal had one or more chronic liver abscesses or no liver abscesses at slaughter.

Age. The age of each animal calculated from date of birth to date of slaughter. This variable was used to define if the animal should be included in the study.

Abattoir. The abattoirs are registered with a specific CHR number. The findings at meat inspection can be recorded using two different methods, electronic via a touch screen and manually via paper notes. Information about method of registration for each abattoir was available from the Danish Veterinary and Food Administration (Anonymous, 2007c).

Breed. Breed was categorized on four levels as Large breed (SDM, RDM and DRH), Jersey, Crossbreeds or Beef cattle.

Period of slaughter. This variable was defined based on date of slaughter. It was divided into four periods. January, February, and March were separate periods with April (entire month) and May (1st to 10th) as a joined fourth period.

Weight. The weight of each animal recorded at slaughter. One animal had an unrealistic value recorded (weight of 9999.9 kg), this value was stated missing.

Specific registration number (CHR) for departure herd. For each single animal included in the analysis, CHR number of departure herd was given and used as a random effect in the model to account for intra-herd correlation. From each CHR number five animals were randomly selected to be included in the analysis.

Data on *Salmonella* status in the source herd and departure herd was extracted from the Danish Surveillance Programme for *S.* Dublin. In the Surveillance Programme the classification of herds are based on ELISA tests of BTM (bulk tank milk) and blood samples. From the ELISA tests an ODC% (optical density calibrated) is calculated, we use the ODC% to recalculate a herd test status:

Salmonella test status in the source herd. Animals defined as slaughter calves were housed in their source herds in 2006, whereas animals defined as young bulls were housed in their source herds in 2005. The *Salmonella* test status was calculated as positive or negative from BTM, because the source herds were usually dairy herds. The herd was test negative if the average ODC% in the last four BTM samples was less than 25 ODC% and the increase in the most recent sample was less than 20 ODC% compared to an average of the previous three samples. If either of the cut-off criteria described above was exceeded, the herd was defined as positive. For the calf source herd, the *Salmonella* test status was calculated from the last four samples of BTM before the second quarter of 2006, whereas *Salmonella* test status for source herd of young bulls was calculated from the last four samples before the second quarter of 2005 (Figure 2).

Salmonella level in the departure herd. Herds were principally meat producing and therefore *Salmonella* test status was calculated from ELISA measurements of blood samples. This variable had 2 levels, high and low. The herds categorized as 'low' where herds with less than 25% positive samples and 'high' if they had more than 25% positive samples. The *Salmonella* test status for the departure herd was calculated on behalf of samples taken during the period January 1st 2006 to May 10th 2007. For feedlot production herds on properties which also had a milk-producing herd on the property, *Salmonella* test status was based on BTM results and no status based on blood samples.

These herds would have missing values for *Salmonella* test status in the departure herd in the analysis.

Data on calf mortality in source and departure herd, respectively, originated from calculations on data from the Danish Cattle Database. The data containing calf mortality percent was calculated for a year from May 1st to April 30th 2007. Calculations were performed by Jørgen Nielsen, The Danish Dairy Board.

Source herd mortality. The source herd calf mortality percent was calculated as the mortality among animals from 0-14 days of age. The variable was stated as missing in one herd because of an unrealistically high mortality percent (mortality percent of 100).

Departure herd mortality. The departure herd calf mortality percent was calculated as the mortality among animals from 0-180 days of age. The variable was stated as missing in one herd because of an unrealistically high mortality percent (mortality percent of 100).

Herd size. As an estimate for departure herd size we used 'year calves less than 180 days' calculated on data from 2006. This resulted in a scale that enabled comparison of herds.

Model B: Risk factors of getting a high prevalence of liver abscesses on herd level

Data on the following variables were based on the dataset with recordings at slaughter of animals slaughtered in the period January 1st to May 10th 2007.

Herd prevalence of liver abscesses. The prevalence of liver abscesses in each herd was calculated as number of animals with liver abscesses at slaughter out of the total number of slaughtered animals. Prevalence was categorized in two groups with appearance ≤10% liver abscesses defined as low and appearance >10% liver abscesses as high.

Production. A herd was defined as a slaughter calf production if more than 50% of the bulls slaughtered were between 210 and 310 days of age, and as a young bull production if more than 50% of the bulls slaughtered were between 311 and 730 days of age.

Salmonella level in the departure herd. Herds were principally meat producing and therefore *Salmonella* test status was calculated from ELISA measurements of blood samples. This variable had four levels, unknown, negative, low and high. 'Unknown' if less than four samples existed, 'negative' if the herd had 0 positive samples, 'low' if the herd had from 0 to 25%, and 'high' was if the herd had more than 25% positive samples. The *Salmonella* test status was calculated on behalf

of samples taken in the period of January 1st 2006 to May 10th 2007. For feedlot production herds on properties which also had a milk-producing herd *Salmonella* test status was based on BTM results and not on blood samples. For these herds, the analysis would be missing values for *Salmonella* test status in the departure herd.

Calf mortality in departure herd. Data on calf mortality in departure herd originated from calculation on data from the DCD. The data containing calf mortality percent was calculated for a year from May 1st to April 30th 2007. Calculations were performed by Jørgen Nielsen, The Danish Dairy Board. The departure herd calf mortality percent was calculated as the mortality among animals from 0-180 days of age. The variable was stated as missing in two herds because of an unrealistically high mortality percent (mortality percent of 100).

Entrance of animals. This variable was based on whether the herd had purchased any bull calves aged 0-180 days in 2006. Some dairy herds raise their own bull calves for meat production. They represent the herds with no purchase.

Herd size. As an estimate for departure herd size we used 'year calves less than 180 days' calculated on data from 2006. This resulted in a scale that enabled comparison of herds. Data originated from the DCD.

Statistical method of analysis

A logistic analysis with departure herd as random effect was used to identify risk factors for liver abscesses at animal level. The GLIMMIX procedure in SAS® v. 9.0 was used. The variable *Salmonella* test status in departure herd is at herd level and therefore stated as a random effect as well. The model could not be tested with all animals included due to convergence problems. This is believed to be caused by a large variation in the dataset. Instead, the analysis was run 10 times with five animals randomly selected from each herd.

A logistic analysis was used to identify risk factors for a high within-herd prevalence of liver abscesses and the herd level set of data was used. Herd prevalence of liver abscesses (≤10%, >10%) was the outcome. The GENMOD procedure in SAS® v. 9.0 was used.

The dispersion parameter in goodness of fit for each model was used as model control. This parameter should be close to 1 (Ersbøll *et al.*, 2004).

In both logistic analyses all variables were included in the initial model. Stepwise backward elimination was used to remove non-significant variables. Changes in estimates were checked to look for confounding between variables and, if any was found, they were tested in the model by replacing the analogous. Interaction between the variables was checked in the final model. The significance level criteria for staying in the model were $P < 0.05$. The distribution was binominal with a Logit as the specified Link function for both analyses.

RESULTS

Model A: Risk factors of getting liver abscesses as a single animal

Descriptive statistics

The prevalence of liver abscesses among the bulls in this study is 12.5%.

In table 1 the qualitative categorical variables and their levels are listed. The total number of animals is shown on each level, as well as the percentage and the total number of animals with liver abscesses. The highest variation in animals with liver abscesses is seen between the levels of breed and recording system at abattoir.

Variable and level	N	Animals with liver abscesses (n)	Animals with liver abscesses (%)
Salmonella test status in departure herd			
High	8.864	1.309	14.8
Low	39.038	4.757	12.2
Salmonella test status in source herd			
High	10.983	1.424	13.0
Low	38.334	4.884	12.7
Breed			
Beef	915	50	5.5
Crossbreed	5.512	539	9.8
Jersey	804	31	3.9
Large breed	44.442	5.839	13.1
Period of slaughter			
January	13.092	1.464	11.2
February	11.017	1.330	12.1
March	14.717	1.952	13.3
April & May	12.866	1.714	13.3
Recording system at abattoir			
Electronic	45.160	5.950	13.2
Manuel	6532	510	7.8

Table 1. Descriptive analyses of qualitative categorical variables in model A

In table 2 quantitative variables in the model are listed. They are described by their minimum and maximum value, median, mean and standard deviation for all animals, for animals with and without liver abscesses respectively. Animals with liver abscesses generally came from herds that were 15% larger than the herds where animals without liver abscesses came from. Also noteworthy is weight and age.

Variable	Min. value	Max. value	Median	Mean	Standard deviation
Herd size					
All herds	0	836.9	119.3	185.4	179.2
Animals without liver abscesses	0	836.9	113.7	182.0	179.1
Animals with liver abscesses	0	836.9	149.0	209.5	178.4
Calf mortality pct. in departure herd					
All herds	0	56.2	4.6	7.0	8.5
Animals without liver abscesses	0	56.2	4.6	7.1	8.5
Animals with liver abscesses	0	56.2	4.5	6.8	8.4
Calf mortality pct. in source herd					
All herds	0	50.0	3.1	3.8	3.5
Animals without liver abscesses	0	50.0	3.1	3.8	3.5
Animals with liver abscesses	0	38,1	3.1	3.7	3.3
Weight					
All herds	79	514	209.0	220.3	35.0
Animals without liver abscesses	79	514	210.0	221.3	35.4
Animals with liver abscesses	122	495	201.0	213.4	31.3
Age					
All herds	211	730	333.0	341.9	72.7
Animals without liver abscesses	211	730	335.0	343.6	73.7
Animals with liver abscesses	214	702	310.0	329.8	63.5

Table 2. Descriptive analyses of quantitative variables in model A

Analytic statistics

The results of model A is an overview of ten test runs with only 5 animals per herd. The number of animals delivered for slaughter from each herd varies from 20 to 644.

The parameter estimates and significance levels of risk factors are described in Table 3. The *P*-value for *Salmonella* test status in the departure herd ranges form 0.1083 to 0.9234 and the estimate ranges between negative and positive. The *P*-value for herd size is between 0.0005 and 0.2393; seven out of ten are significant. The estimates are all positive. The *P*-value for weight is between 0.0004 and 0.1874; eight out of ten are significant. The estimates are all negative. Abattoir has a *P*-value ranging from 0.0236 to 0.9139; only one out of ten is significant. The estimates are all positive, except when the *P*-value is highest where the estimate changes to negative. The *P*-value for breed is between 0.0009 and 0.4873; four out of ten are significant. The estimates vary but for breeds, Jersey always has the lowest estimate.

Test no.	Intercept	*Salmonella*[a]		Herd size		Weight		Abattoir[b]		Breed				
	β	β	*P*-value	β	*P*-value	β	*P*-value	β	*P*-value	β_{Beef}	β_{Cross}	β_{Jersey}	β_{Large}	*P*-value
1	-1.41	-0.02	0.9234	0.18	0.0005	-0.50	0.0078	0.35	0.0562	0.03	-0.01	-0.83	0	0.4873
2	-1.52	0.03	0.8556	0.16	0.0057	-0.44	0.0291	0.23	0.2254	-0.68	-0.21	-0.70	0	0.2448
3	-1.31	0.02	0.9224	0.14	0.0153	-0.49	0.0141	0.22	0.2405	-0.79	-0.10	-2.34	0	0.0582
4	-1.51	0.14	0.4270	0.10	0.0659	-0.31	0.1038	0.07	0.6970	-0.54	-0.41	-1.02	0	0.0482
5	-1.26	0.03	0.8757	0.12	0.0299	-0.54	0.0066	0.32	0.0805	0.11	-0.29	-2.35	0	0.0561
6	-1.31	-0.11	0.5454	0.12	0. 0362	-0.45	0.0195	0.32	0.0772	-0.48	-0.48	-1.18	0	0.0116
7	-0.66	0.05	0.7758	0.07	0.2393	-0.74	0.0004	0.16	0.3999	-0.31	-0.64	-1.43	0	0.0118
8	-1.24	0.27	0.1083	0.12	0.0215	-0.59	0.0022	0.42	0.0236	-0.15	-0.23	-1.14	0	0.1799
9	-1.75	-0.22	0.2554	0.13	0.0293	-0.25	0.1874	0.31	0.0956	-1.17	-0.56	-2.44	0	0.0009
10	-1.01	0.02	0.9013	0.10	0.0873	-0.50	0.0105	-0.02	0.9139	-0.16	-0.18	-2.43	0	0.0838

Table 3. The parameter estimates and significance levels of *Salmonella* test status in departure herd, herd size, weight, abattoir and breed in model A.

a: *Salmonella* test status in departure herd

b: Recording system at the abattoir

β: Estimate

Model B: Risk factors of getting a high prevalence of liver abscesses on herd level

Descriptive statistics

The average prevalence of liver abscesses at herd level is 12.4% in the study herds. The prevalence ranges from 0 to 48%.

In table 4 the qualitative categorical variables and their levels are listed. The total number of herds is shown for each variable level and percentage of herds with a prevalence of liver abscesses greater than 10. There seems to be a substantial difference between the two production types, slaughter calf and young bull, of having liver abscesses.

Variable and level	N	Herds with >10% liver abscesses (n)	Herds with >10% liver abscesses (%)
Salmonella test status in departure herd			
High	73	51	69.9
Low	107	66	61.7
Negative	200	107	53.5
Unknown	48	23	47.9
Entrance of bull animals in 2006			
No	56	25	44.6
Yes	443	253	57.1
Production type			
Slaughter calves	185	137	74.1
Young bulls	324	141	44.9

Table 4. Descriptive analyses of qualitative categorical variables in model B

In table 5 quantitative variables in the model are listed. They are described by their minimum and maximum value, median, mean and standard deviation for all herds, for herds with ≤10% liver abscesses and >10% liver abscesses respectively. The difference in mean of herds with more than 10% liver abscesses and herds with less than 10% liver abscesses is 40%.

Variable	Min. value	Max. value	Median	Mean	Standard deviation
Herd size					
All herds	0.3	836.9	64.8	104.9	112.6
Herds with ≤10% liver abscesses	0.9	836.9	48.2	76.8	93.1
Herds with >10% liver abscesses	0.3	700.7	82.0	127.2	121.6
Herd calf mortality					
All herds	0.0	53.1	4.5	7.2	10.0
Herds with ≤10% liver abscesses	0.0	53.1	4.7	6.7	7.9
Herds with >10% liver abscesses	0.0	52.9	4.5	7.0	8.3

Table 5. Descriptive analyses of quantitative variables in model B

Analytic statistics

The parameter estimates and significance levels of risk factors for herd prevalence of liver abscesses are shown in Table 6. There was a significant interaction between production type and herd size ($P = 0.0194$). Figure 3 illustrates the association between herd size and herd prevalence of liver abscesses for the two production types. The odds of having more than 10% liver abscesses in the herd were higher for herds producing slaughter calves compared to young bull production if the herd size was under 255, whereas the odds were lower if the herd size was larger than 255 (calculations not shown).

Variable and level	Estimate	SE	*P*-value#
Intercept	-0.6959	0.2031	
Production			< 0.0001
Slaughter calf	1.5329	0.3361	
Young bull	0	-	
Herd size (per 100 heads)	0.81	0.28	0.0010
Herd size*Production type			0.0194
Slaughter calf	-0.0060	0.0031	
Young bull	0	-	

Table 6. Risk factors associated with herd liver abscess prevalence. #P-value estimated by the score statistics for type 3 contrasts in the generalized estimating equation analysis

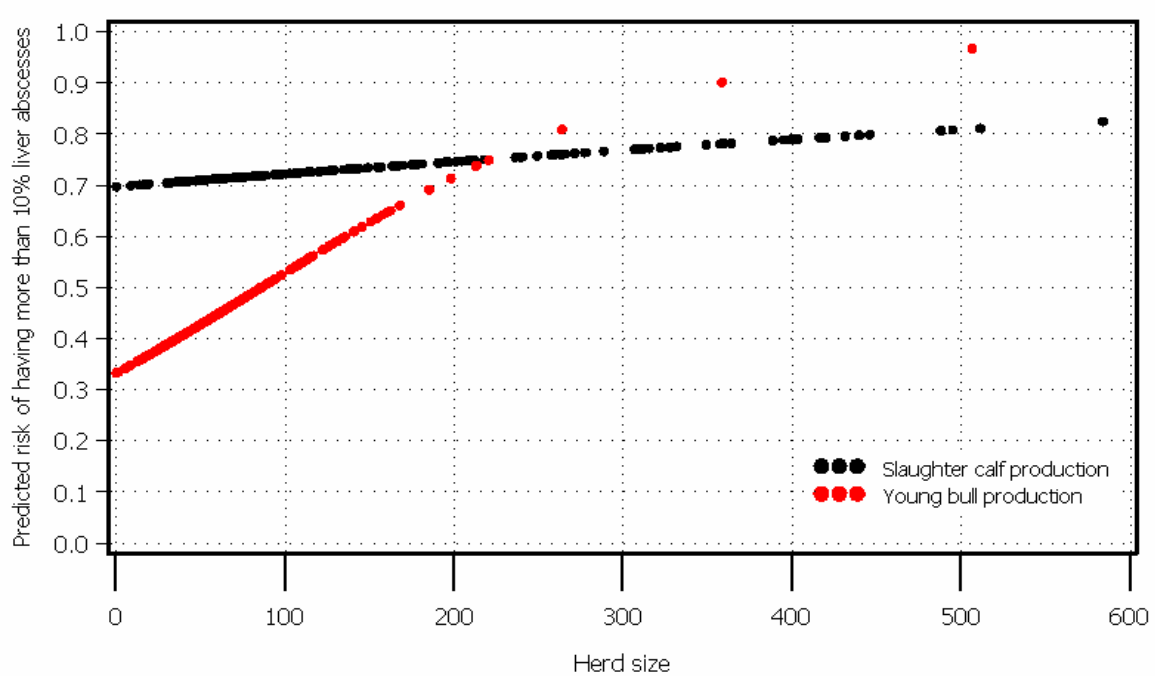

Figure 3. Graphical illustration of the relation between the risk of having liver abscesses and herd size for slaughter calf production and young bull production respectively. Herd size is estimated by year-calves under 180 days of age.

DISCUSSION

Herd classification and variables

Data source

There are no recordings of the validity of the veterinary meat inspection in Denmark. Visits at four of the abattoirs with an electronic registration system and interviews with the specially trained technicians and the veterinarians working at these abattoirs were meant to provide a basic understanding of the data about liver abscesses. As a routine, the liver is examined at the veterinary meat inspection. An electronic touch screen makes the recordings convenient and the recordings go directly to the Danish Cattle Database (DCD). Based on our observations, we estimate the recordings on liver abscesses at the abattoirs with electronic registration systems to be reliable.

Type in errors can always occur in databases. The information in the DCD is reported from farmers, veterinarians, abattoirs etc. The risk of type in errors increases because of the large amount of people who are in touch with this database. The datasets we use in these analyses are based on a large number of individual animals and herds, reducing the likelihood of errors affecting the result.

Study herds and animals

Despite the substantial registration system in Denmark where all herds are registered with a CHR number, feedlot herds are not registered as a specific production type. The definitions of feedlot herd we have made in this analysis are very simple. Number of slaughtered bulls of a defined age determined if the herd was included in the analysis. We assumed that herds which had delivered more than 20 bulls aged 210 to 730 days for slaughter in the study period were feedlot herds. These could be other production types without intensive feeding strategies.

There was a large variation in the number of animals each herd had delivered for slaughter in the study period. Therefore we set a criterion of a minimum of twenty slaughtered bulls per herd for the animal or herd to be included in the studies. This was done in order to get a more accurate herd prevalence of liver abscesses. More than two animals should have recordings of liver abscesses from a herd with only twenty animals slaughtered for the herd to be categorized with a high prevalence of liver abscesses. Despite this criterion the prevalence of liver abscesses might not be the true prevalence for the herd, because animals slaughtered in the period might not represent the herd.

Study design and variables

In the study of risk factors for liver abscesses on single animal level the variables of *Salmonella* test status and calf mortality percentage in the source herd were included. These variables were calculated for defined limited periods. The age of slaughter calves and young bulls varied and therefore the single animal might not have been in the source herd in the specific period when the variable value was calculated. This may mean that the animal was not influenced by the used value. This is especially a problem for animals defined as young bulls because of a variation in age from 311 to 730 days whereas the age variation among slaughter calves is only 210 to 310 days. For *Salmonella* test status in the source herd, one period was used for animals defined as slaughter calves whereas another period was used for animals defined as young bulls. However, very few herds change test status, so it is unlikely that the source herd had a different status than the one used in the study when the animal was in the herd. As to calf mortality percent, only one value was calculated and used for both slaughter calves and young bulls. We do not expect a big variation in the calf mortality percent because it was calculated for a whole year (May 2006 to May 2007).

A study by (Kjeldsen *et al.*, 2002) found a seasonal variation in the prevalence of liver abscesses recorded at slaughter with the highest prevalence in autumn. In our study we only used recordings from January to May. It is possible that there is a seasonal variation which we could not include in our study. It is also possible that herds which had a low prevalence of liver abscesses in our study would have had a high prevalence if recordings from a different season were used.

In the study we defined study herds as either slaughter calf production or young bull production. Most herds delivered only slaughter calves or young bulls, but some delivered both products for slaughter. Herds with mixed production were defined as slaughter calf production if more than 50% of the animals delivered for slaughter were slaughter calves. Likewise, herds were defined as young bull production if more than 50% of the animals delivered for slaughter were young bulls. Most of the herds with mixed production delivered more than 50% slaughter calves. This is not surprising because of the economical advantage gained when calves slaughtered obtain a special status as Danish veal (Anonymous, 2007a).

Model results

Model A

In this analysis, we wanted to test risk factors of the individual animal having liver abscesses. There was a cluster effect of herd. Three other variables in the analysis were on herd level and should be included in the random statement as well, but only *Salmonella* test status in the departure herd was included along with departure herd. There was a large variation in number of slaughtered animals per herd as well as a large variation between the distributions of animals on levels of some variables. The analysis was performed with five randomly selected animals from each herd.

The number of animals delivered for slaughter per herd varied from 20 animals to 644. Random selection of five animals from each herd was not optimal because that sample size was not big enough to be representative for the herd. Also, herds which had delivered many animals for slaughter in the study period were not represented with enough animals in the analysis. This could be a problem when we consider that herd size was a significant risk factor of having a high prevalence of liver abscesses in the herd in model B. Instead of five animals from each herd, a

percentage of animals from each should have been randomly selected. Had we used this method, it is likely that we would have experienced convergence problems.

By running the analysis ten times in a row with different random selection of five animals each time, we found a big variation in the *P*-values and estimates for the variables included in the model. A variable could be found significant in one round and insignificant in the next. There was no pattern in the relation between *P*-values for the variables. Five variables were included in the model to illustrate the variation in model results. These were salmonella in departure herd, herd size, weight, type of recording system on abattoir and breed. As a conclusion, a sample of five animals from each herd does not represent the true distribution of animals on each variable level.

Salmonella test status in departure herd was not significant in any of the ten test runs of the analysis. Therefore *Salmonella* is not considered to be associated with having liver abscesses. It is possible that applying the *Salmonella* test status calculated on herd level to the individual animal is the reason why we did not find an association. We consider it most likely that no association occurs because the pathogenesis of these two infections differs widely.

Herd size was significant in seven out of ten test runs and we consider it likely that it is a significant risk factor for the single animal having liver abscesses. This correlates with the results of model B, where the variable herd size was significant.

There was a strong indication of weight being significant because eight out of ten *P*-values were under 0.05. The estimates were all negative indicting that the likelihood of having liver abscesses was higher as the weight decreased. It is a difficult variable to draw conclusions from, because the association could be vice versa. Brink *et al.* (1990) did not find associations between live weight and liver abscesses, but found associations between hot carcass weight and liver abscesses. They stated that the hot carcass weight was reduced because the animal utilized energy to produce tissue which was condemned at slaughter. Based on this study, he concluded that animals with severe liver abscesses had reduced weight gain estimated from hot carcass weight and feed efficiency.

From the descriptive statistics, we expected the recording system at the abattoir to be significant, but it was only significant in one out of ten test runs. The reasons for this non-significant result

could be multiple. Individual variation in electronic and manual recording systems, respectively, might affect the result because of the limited number of animals. Only 14.5% of the animals were slaughtered at abattoirs with manual recording systems. The number of animals slaughtered on a manual system may not be enough to show a difference or it is possible that the non-significant result might be true. In the investigation of liver abscesses in Danish slaughter calves by (Kjeldsen *et al.*, 2002), they did not find the possibility of having liver abscesses to be affected by abattoir.

Four out of ten *P*-values of breed showed significance and two others were very close to the significance level of 0.05. In most incidences the estimates demonstrated a higher prevalence of liver abscesses for large breeds compared to other breeds. The biggest difference was seen between Jersey and large breed. Holsteins compared to beef cattle are found to have a higher incidence of liver abscesses (Nagaraja *et al.*, 1996). Holsteins have a higher level of digestive disturbances (Vogel and Parrott, 1994) and they, too, have a higher feed intake than beef breeds on the same starting weight (Hicks *et al.*, 1990). Because liver abscesses are highly related to ruminal acidosis, the digestive disturbances might be an explanatory reason why Holsteins have a higher prevalence. Differences in breed were also found by (Kjeldsen *et al.*, 2002), but there were only few observations in Jersey and beef compared to large breed. One more Jersey or beef cattle with liver abscesses would change the prevalence considerably.

The result of our statistical analysis was that risk factors for getting liver abscesses as a single animal could not be tested in this way. We still find it interesting to investigate *Salmonella* as a risk factor for getting liver abscesses. It would be interesting to use *Salmonella* results for the individual animal instead of using herd *Salmonella* status to test *Salmonella* as a risk factor. This would overcome some of the model problems with too many variables being on herd level.

It would also be interesting to test other risk factors for getting liver abscesses. It might be possible to select animals from a limited number of herds with fairly similar herd size. Thereby we would expect that the random effect of departure herd could be included in the model, because all herds would contribute with fairly similar numbers of animals in the analysis.

Model B

Risk factors for having a high prevalence of liver abscesses in the herd was analysed in model B. Herd *Salmonella* test status was found not to be associated with prevalence of liver abscesses in the herd. Odds are that no association exists, because the two infections are very different in cause and are affected by different management factors.

An interaction between type of production and herd size was shown ($P = 0.0194$). This affected the results in a way that a production of slaughter calves in a small herd were more likely to have a high prevalence of liver abscesses, whereas a young bull production in a large herd were more likely to have a high prevalence of liver abscesses.

The production type was found to be associated with prevalence of liver abscesses ($P < 0.0001$). We believe that the variable production type contains the management with the intensive feeding strategy. It is well known from literature that intensive feeding containing large amounts of grain increases the prevalence of liver abscesses (Nagaraja *et al.*, 1996). Because liver abscesses are eventually resorbed, we question whether young bulls have fewer liver abscesses than calves or if it is because liver abscesses in young bulls are resorbed and therefore not seen at meat inspection. In a survey of experimentally induced liver abscesses scar formation of abscesses was seen between 45 and 180 days (Jensen *et al.*, 1954).

In our study we found herd size to be significant ($P = 0.001$). Larger herds were more likely to have a higher prevalence of liver abscesses. We have not found previous literature demonstrating this significant difference in herd size. Kjeldsen *et al.* (2002) found a higher prevalence of liver abscesses in individual animals compared to average herd prevalence. They presumed that difference was seen because of more incidences of liver abscesses in large herds.

CONCLUSION

In conclusion, an association between *S.* Dublin and liver abscesses was not found in this study. An investigation of *S.* Dublin measured on animal level as a risk factor for having liver abscesses continues to be of interest as the large variation of the results in the model used in the present study

prevented a conclusive result. On herd level, *Salmonella* test status was not found to be associated with having more than 10% liver abscesses. Type of production and size of the herd was found to be associated with the risk of having a high prevalence (> 10%) of liver abscesses in the herd.

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CHAPTER 3 - Manuscript 2

Association between *Fasciola hepatica* and *Salmonella* Dublin in Danish dairy herds

ABSTRACT

The main objective of this study was to investigate the association between infection with *Fasciola hepatica* (liver fluke) and *Salmonella* Dublin (*S.* Dublin) in Danish dairy cattle herds. *Salmonella* test status was recalculated from data from the Danish Surveillance Programme for *S.* Dublin whereas liver fluke status for each herd was based on recordings from the meat inspection at slaughter.

In one model, herds with more than 2% liver flukes recorded at slaughter from January to May 2007 were more likely to be *Salmonella* test positive in the first quarter of 2007 than herds with 0-2% liver flukes (OR=2.1; 95% CI: 1.6-2.9, $P < 0.0001$). Geographical region in Denmark was included to control for regional cluster effects of *Salmonella* prevalence based on knowledge from previous work. Due to convergence problems interactions with region could not be tested. Large herds had a higher risk of being *Salmonella* test positive (OR = 1.5 per 100 heads; 95%CI: 1.2-1.8, $P < 0.0001$). In two alternative models, risk factors of changing *Salmonella* test status from negative to positive (indicating infection) and from positive to negative (indicating recovery) were also investigated. Again, geographical region of the herd was included to control for regional cluster effects and was a significant risk factor of changing *Salmonella* test status from negative to positive. Also, herd size was associated with changing *Salmonella* test status from negative to positive (OR = 1.7 per 100 heads; 95%CI: 1.2-2.2, $P = 0.0031$). Odds were 1.7 (95%CI: 1-2.9) times higher for changing from *Salmonella* test positive to negative among herds with 0-2% liver flukes compared to herds with more than 2% liver flukes ($P < 0.04$). This association has not been investigated before in other studies. It was concluded that this result could explain why association between liver flukes and *S.* Dublin exists, because herds with liver flukes were less likely to recover from *S.* Dublin.

Organic status and purchase of animals was found not to be associated with neither *Salmonella* test status nor a change in test status in this study.

INTRODUCTION

Salmonella enterica subspecies *enterica* Dublin (*S.* Dublin) is a host-adapted salmonella serotype in cattle (Radostits *et al.*, 2000). It is a serious food born infection (Helms *et al.*, 2003) as well as an important infection in cattle. Currently, about 16% of Danish dairy cattle herds are infected with *S.* Dublin (Nielsen, 2007). The Danish cattle industry has decided that *S.* Dublin should be eradicated in Danish cattle before 2014. The eradication programme is based on the National Surveillance programme of *S.* Dublin which was introduced in 2002. The basis of the programme is breaking the transmission routes both between and within herds, primarily by optimizing hygiene and management.

Infection with *Fasciola hepatica* (*F. hepatica*; the large liver fluke) is a parasite with a worldwide distribution. In cattle it is most often asymptomatic and sub-clinical (Torgerson and Claxton, 2007). An investigation of abattoir recordings in Denmark found an overall increase in the prevalence of liver flukes from 0.9% to 2.7% in dairy cattle over the years 2000 to 2003 (Thamsborg *et al.*, 2005).

Several studies have investigated the association between infection with liver flukes and infection with *S.* Dublin. An epidemiological study in 1973 found a decreased incidence of *S.* Dublin and *F. hepatica* occurring in parallel (Dijkstra, 1973). This was interpreted as a sign that the two infections could be related. On single animal level (Aitken *et al.*, 1978b) as well as on herd level (Richardson and Watson, 1971; Vaessen *et al.*, 1998), the two infections have been found to be associated in experimental and observational studies respectively. On the contrary, Taylor and Kilpatrick (1975) concluded that the association between *S.* Dublin and liver flukes was the result of the two agents being influenced by similar climatic and management conditions.

Only limited investigations have been made about the association between the two infections in Denmark. A recent observational study in Danish dairy herds on single animal level found no association between liver flukes and *S.* Dublin (Karlsen, 2007), however there was very little active *S.* Dublin in the herds included in that study. No studies have examined the herd level associations between these two infections in Denmark, and no studies have looked at the differences in probability of infection and recovery of *S.* Dublin in fluke infested and fluke free herds on a large scale neither inside nor outside of Denmark.

With the suspected increase of liver flukes in Danish cattle these years and the desire to eradicate *S.* Dublin in the Danish cattle industry, it is relevant to revisit previous and test new hypotheses concerning the association between *F. hepatica* infection and *S.* Dublin. A better knowledge of the association between the two would be useful in terms of optimizing the eradication programme for *S.* Dublin, and lead to a better understanding of the underlying pathogenesis.

Aim of study

The aim of the present study is to investigate if there is an association between infection with *F. hepatica* and *S.* Dublin on herd level in Danish dairy cattle herds. The aim is analysed using three different perspectives, in relation to herd liver fluke prevalence:

A. The overall association between *Salmonella* test status and liver fluke status at herd level
B. The probability of changing to a test positive *Salmonella* status (indicative of becoming infected) at herd level
C. The probability of changing to a test negative *Salmonella* status (indicative of recovery from infection) at herd level

MATERIALS AND METHODS

Data sources

In Denmark, all data on cattle are joined in the Danish Cattle Database (DCD). Each property with cattle has a specific number (CHR-number) and all live born cattle are tagged with an earmark giving the animal a unique identification number. Recordings about birth, movements, production, clinical treatments, recordings at slaughter etc. about each individual animal are recorded in the CHR throughout the entire course of the animal's life. Based on these recordings, herd level data such as herd size, location and movement of cattle are calculated for each property. In Denmark, all carcasses and organs are checked by veterinarians or specially trained technicians. All abnormal findings are supposed to be recorded at the slaughter line and transferred to the DCD.

Data defining the characteristics of the herds (herd size, purchase, and type of production) were taken from year 2006, whereas abattoir recordings were from January 1st to May 10th 2007. Geographical information on all farms was obtained from the Map and Land Register Authority in Denmark. Records of organic herds were obtained from The Danish Plant Directorate per January

1st 2007. Information about herd *Salmonella* test status originated from the National Surveillance Programme for *S.* Dublin which is an integrated part of the DCD.

Study herds

To be included in the study, herds had to meet the following criteria: Firstly, the herd had to be defined as a dairy herd. Information about milk production was collected from the DCD, and a herd having weekly recordings of somatic cell counts from bulk tank milk (BTM) was defined as a dairy herd. Herds which had ceased milk production within the past six months as well as herds currently delivering milk were included. Secondly, a minimum of ten slaughtered female cattle at abattoirs with electronic recordings of findings in the study period (January 1st until May 10th 2007) were necessary for the herds to be included in the study. Thirdly, to avoid a single recording of liver flukes at the abattoir in defining a herd as fluke infested, only herds with agreement concerning liver fluke status in 2006 and 2007 were included in the study.

Epidemiological study design

Three models were set up in the study. In model A, we tested risk factors of having *Salmonella* test positive herd status in the first quarter of 2007 (January to March). In model B, we tested risk factors of changing *Salmonella* test status from negative to positive (indicates infection) from one quarter of the year to the next quarter. In model C, we tested risk factors of changing *Salmonella* test status from positive to negative (indicates recovery) from one quarter to the next.

The design in model A is illustrated in Figure 1. Liver fluke records at six abattoirs in the study period (January to May 2007) were used to define a liver fluke status for each herd. Other risk factors were defined based on the herd data from 2006.

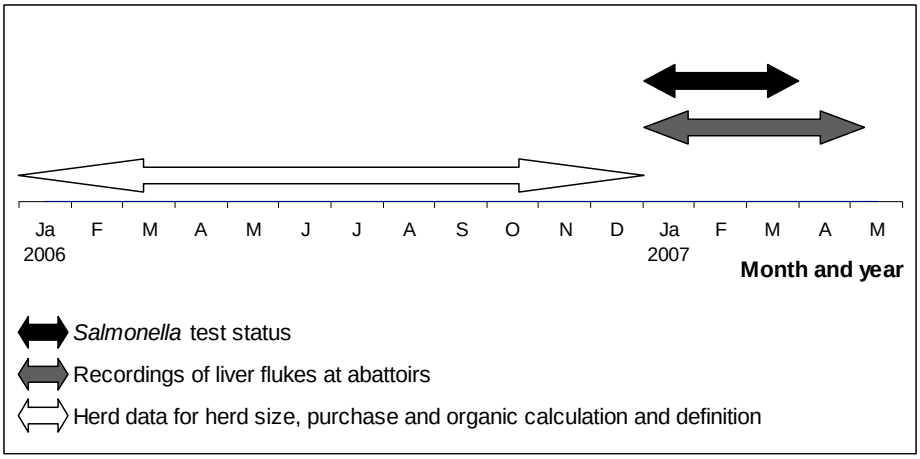

Figure 1. Data collection periods for model A

Figure 2 illustrates the study design for model B and C which were similar in data collection. Variables were tested as risk factors of changing *Salmonella* test status, negative to positive or positive to negative respectively, from one year-quarter to the next. Liver fluke records in the abattoir study period (2007) were taken as an expression of liver fluke infection occurring in the herd in July to October 2006. This is the period of the year with the highest infection pressure on pasture. Other risk factors were defined based on the herd data from 2006.

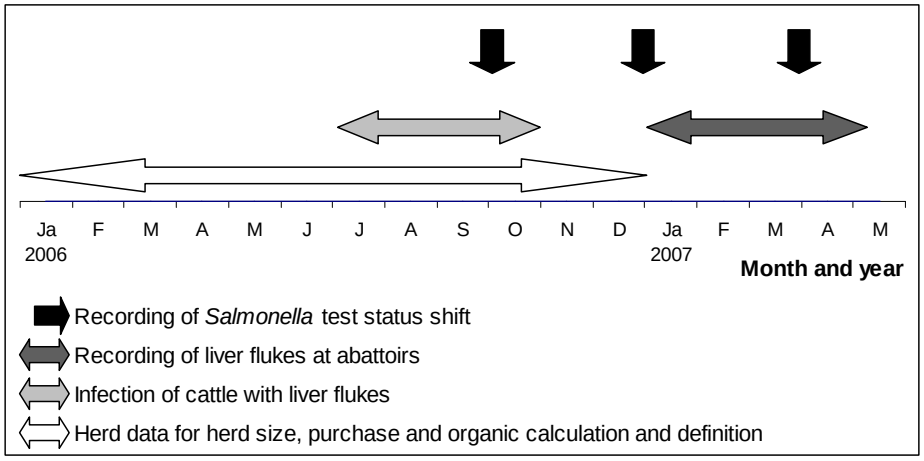

Figure 2. Data collection periods for model B and model C

Data editing and descriptive statistics

Salmonella status. Data from the National Surveillance Programme database was used to recalculate *Salmonella* herd test results for the dairy herds included in the study. The programme is based on ELISA ODC% (calibrated optical density) in BTM samples. Warnick *et al.* (2006) evaluated the test programme validity. For this study, a *Salmonella* test status for each herd was calculated for each quarter of the year in the study period. The status was defined as *Salmonella* test negative or *Salmonella* test positive for each year-quarter. The herd was test negative if the average ODC% in the last four BTM samples was less than 25 ODC% and the increase in the most recent sample was less than 20 ODC% compared to an average of the previous three samples. If either of the cut-off criteria described above was exceeded the herd was defined as positive. Four quarters of a full year were included in the study: Third (July to September) and fourth (October to November) quarter of 2006 and first (January to March) and second (April to June) quarter of 2007.

Liver fluke status. Individual animal numbers, departure herd identification number, abattoir number and records of liver fluke for all animals slaughtered in the period January 1st to May 10th 2007 were extracted from the DCD. The liver fluke herd status was calculated as the number of female cattle with liver fluke records from the herd out of the total number of slaughtered female cattle from the herd. The status was categorised as neglect able (0-2%) or positive (more than 2%).

Geographical region. Herds were classified into the following seven regions: Islands, East Jutland, South Jutland, West Jutland, North Jutland South, North Jutland North and North Western Jutland. Division of the country into the seven regions originated from the Danish Cattle Federation. Because of few positive herds in some of the regions Islands and East Jutland as well as North Jutland North and North Western Jutland, these were joined as two regions giving a total of five regions in two of the models (B and C).

Herd size. The sum of "year-heifer-calves under 180 days of age", "year-heifers above 180 days of age" and "year-cows" in each herd in 2006 was used as an estimate of herd size. All female animals were included in the calculation of herd size, because only female animals are included in the analysis. Due to the way year-animals are calculated, a herd can have a herd size of less than ten though all herds in the study had more than ten animals slaughtered in the study period.

Purchase of female animals. Purchase of animals was included as a dichotomous variable defined as the entrance of female cattle or not into the herd in 2006 (open or closed herd).

Organic. Information about herds with organic status was obtained from The Danish Plant Directorate. The status is determined by manual comparison of lists from the Danish Milk Board containing names and addresses of persons in their systems and authorisation numbers of properties registered as organic producers in the Danish Plant Directorate.

Statistical Method of Analysis

Three separate multivariable logistic analysis were used to analyse risk factors associated with *Salmonella* test status in first year-quarter of 2007 (Model A) or a change in test status between two year-quarters from test negative to test positive (Model B) and from test positive to test negative (Model C), respectively.

The dispersion parameter in goodness of fit of each model was used as model control. This parameter should preferably be close to 1 (Ersbøll *et al.*, 2004).

Initially, all risk factors were included in the model. Non-significant risk factors were excluded one at a time using stepwise backwards elimination. A significance level criterion for variables to remain in the final model was $P < 0.05$. Significant risk factors were tested for interaction mutually and with all other risk factors. Due to insufficient number of herds on some variable levels, some interactions with the following variables could not be performed: Fluke status, region, organic status and purchase. Odds ratio with 95% confidence interval were calculated for significant variables and associations were illustrated graphically.

The statistical computer programme SAS® version 9.0 was used for data editing and statistical analysis. The GENMOD procedure was used for all three logistic analysis models. The distribution was binominal with a specified Logit link function.

RESULTS

Model A: Salmonella test status first quarter of 2007

Descriptive statistics

In Table 1 qualitative categorical variables in model A are listed. The total number of animals is shown on each level as well as the number and percentage of herds with *Salmonella* test positive status. In table 2, descriptive statistics of the continuous variable herd size is given.

Variable and level	N	*Salmonella* test positive herds (n)	*Salmonella* test positive herds (%)
Fluke-herd-status			
0-2 %	1364	225	16.5
> 2%	378	110	29.1
Region			
East J	218	17	7.8
Islands	131	3	2.3
NW Jutland	279	42	15.05
North J-N	148	17	11.49
North J-S	212	84	39.62
South J	431	115	26.68
West J	323	57	17.65
Purchase			
No	927	170	18.34
Yes	815	165	20.25
Organic			
No	1528	279	18.26
Yes	214	56	26.17

Table 1. Descriptive statistics of qualitative categorical variables in model A (Risk factors for *Salmonella* test status in the first quarter of 2007)

Variable	Min. value	Max. value	Mean	Median	Standard deviation
Herd size					
All herds	6.7	1478.8	131.2	121.8	77.2
Salmonella negative herds	6.7	767.0	124.8	119.7	60.1
Salmonella positive herds	7.4	1478.8	157.8	135.0	122.3

Table 2. Descriptive statistics of the continuous variable in model A (Risk factors for *Salmonella* test status in the first quarter of 2007)

Analytical statistics

The parameter estimates and significance levels of risk factors for herd *Salmonella* test status in the first quarter of 2007 are shown in Table 3. The odds were 2.1 (95% CI: 1.6-2.9) times greater for being *Salmonella* test positive among herds with more than 2% liver flukes compared to herds with 0-2% liver flukes ($P < 0.0001$). There was also a significant association between herd size and *Salmonella* test status. Per increase in herd size of 100, the odds of being *Salmonella* test positive were 1.5 (95% CI: 1.2-1.8). Figure 3 illustrates the association between risk of being *Salmonella* test positive and herd size for the two levels of liver fluke infection. The geographical distribution

of test negative and test positive herds in first quarter of 2007 are illustrated in Figure 4. There was a significant difference in the likelihood of being *Salmonella* test positive depending on which region the herd belonged to, with herds in region North Jutland South being most likely and herds in region Islands being least likely to be *Salmonella* test positive. Figure 5 illustrates the association between risk of being *Salmonella* test positive and herd size for each region. Interaction between fluke status and herd size, region and herd size, region and purchase, herd size and organic and herd size and purchase was non-significant. Other interactions could not be tested in the model. Figure 6 illustrates the geographical distribution of herds with 0-2% liver flukes and herds with more than 2% liver flukes. Visual comparison of figure 4 and figure 6 indicate that there is no clear consistence between regions having high prevalence of *Salmonella* test positive herds also having a high prevalence of liver fluke infected herds.

Variable and level	Estimate	SE	*P*-value*	Odds ratio	95 % confidence interval for odds ratio
Intercept	- 3.59	0.62			
Liver fluke			<0.0001		
2 % <	0.76	0.15		2.1	1.6 to 2.9
0-2 %	0	-		1	-
Region			<0.0001		
North Jutland (S)	3.05	0.60		21.1	6.5 to 69.1
South Jutland	2.62	0.60		13.8	4.3 to 44.2
West Jutland	2.10	0.60		8.2	2.5 to 26.7
North West Jutland	1.83	0.61		6.2	1.9 to 20.7
North Jutland (N)	1.20	0.65		3.3	0.9 to 11.9
East Jutland	1.20	0.64		3.3	1.0 to 11.6
Islands	0	-		1	-
Herd size (per 100 heads)	0.42	0.09	<0.0001	1.5	1.2 to 1.8

Table 3. Risk factors associated with *Salmonella* test result in the first quarter of 2007 in the Danish Surveillance Programme for *S.* Dublin. *P-value estimated by the score statistics for type 3 contrasts in the generalized estimating equation analysis

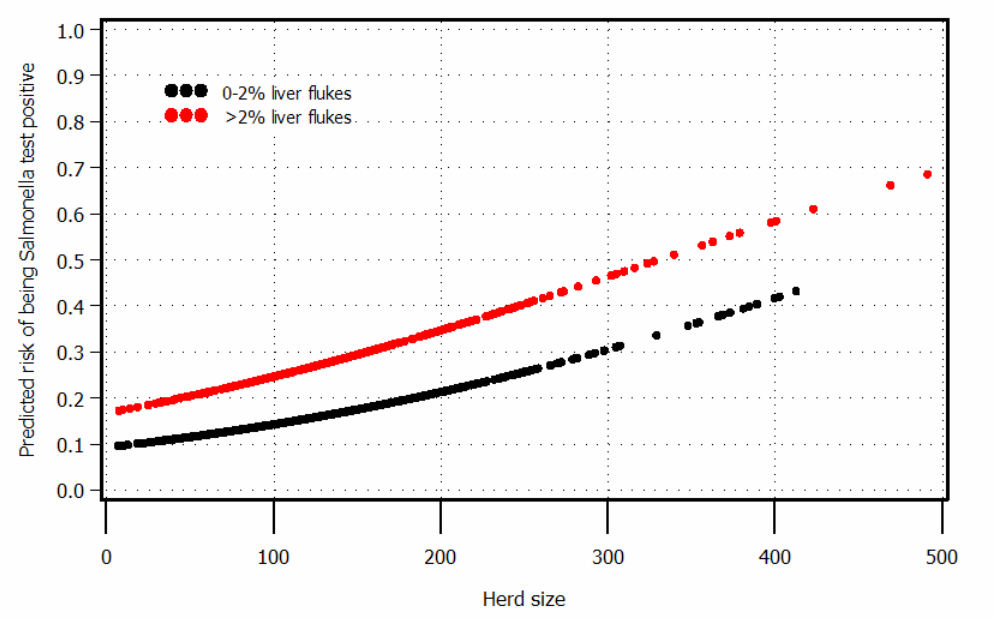

Figure 3. The relation between risk of being *Salmonella* test positive in the first quarter of 2007, herd size (measured as female-year-animals) and herd liver fluke prevalence.

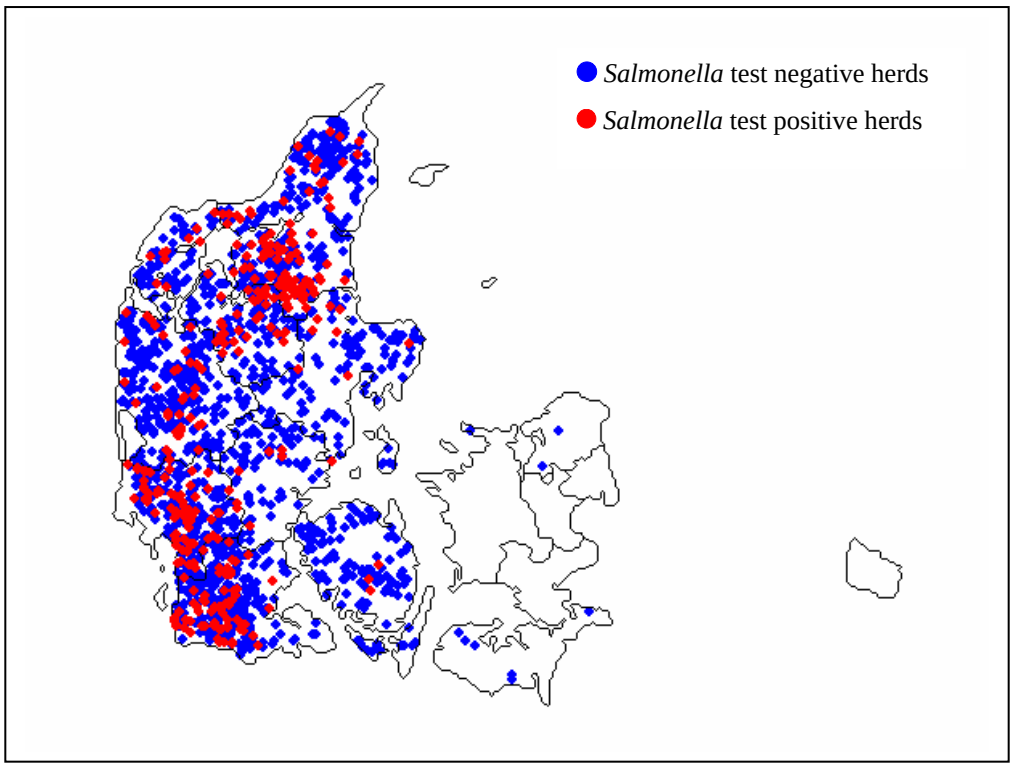

Figure 4. Geographical distribution of herds in model A by *Salmonella* test status

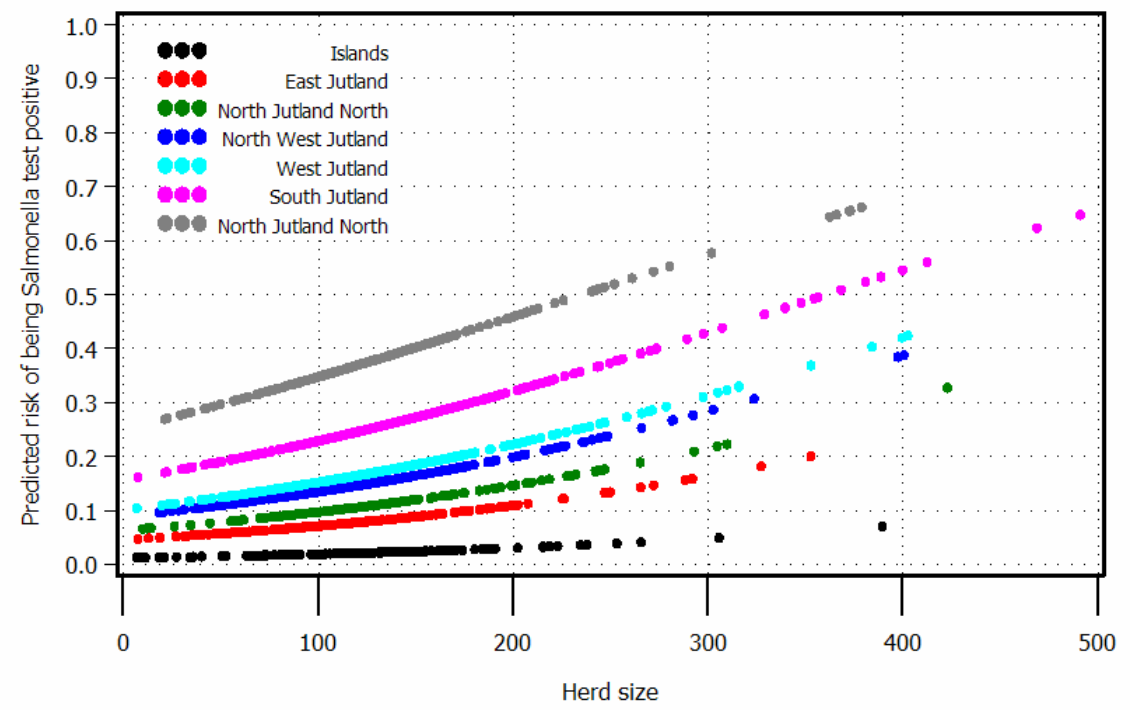

Figure 5. The relation between risk of being *Salmonella* test positive and herd size for herds in each of the seven geographical regions

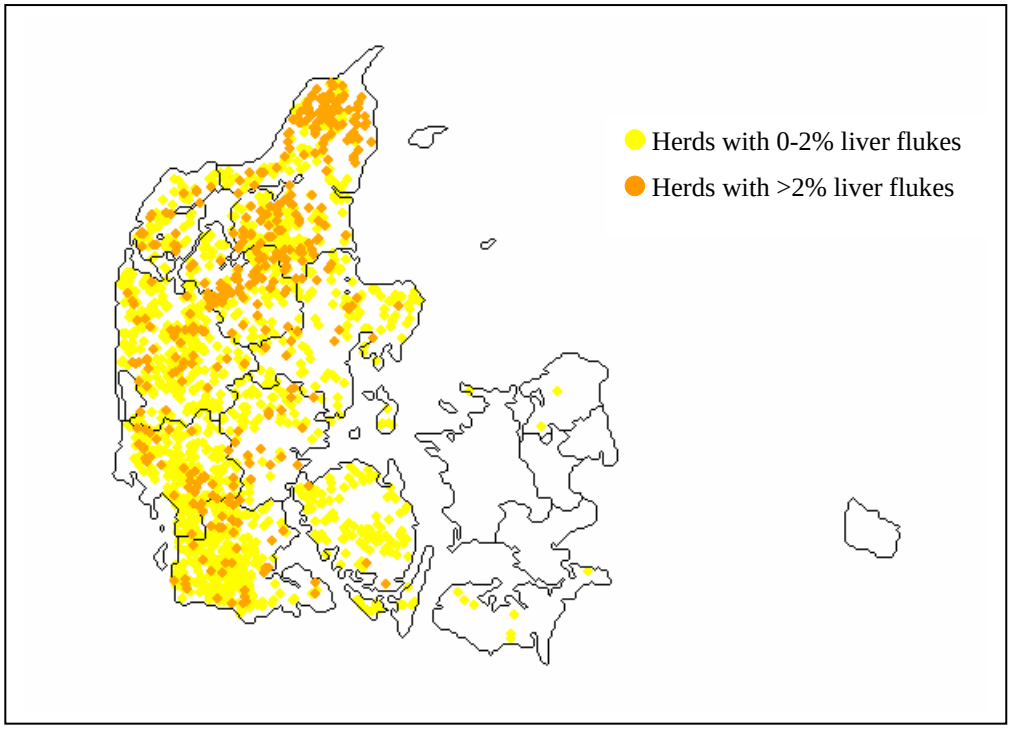

Figure 6. Geographical distribution of herds in the study by liver fluke status

Model B: Change from negative to positive herd Salmonella test status, indicating new infection

Descriptive statistics

In Table 4 qualitative categorical variables in model B are listed. The total number of animals is shown on each level as well as the number and percentage of herds changing from *Salmonella* test negative to positive from one year-quarter to the next. In table 5 descriptive statistics of the continuous variable herd size is given.

Variable and level	N	Herds neg to pos n	Herds neg to pos %
Fluke-herd-status			
0-2 %	3358	46	1.37
2% <	784	16	2.04
Region			
East J-Islands	971	5	0.51
NW Jutland-North J-N	1085	13	1.20
North J-S	364	10	2.75
South J	929	18	1.94
West J	793	16	2.02
Purchase			
No	2223	26	1.17
Yes	1919	36	1.88
Organic			
No	3679	55	1.49
Yes	463	7	1.51

Table 4. Descriptive statistics of qualitative categorical variables of model B (Previously *Salmonella* test negative herds)

Variable	Min. value	Max. value	Mean	Median	Standard deviation
Herd size					
All herds	6.7	767.0	125.3	120.0	58.4
Salmonella test negative herds	6.7	767.0	124.9	119.8	57.7
Salmonella test negative to positive herds	18.3	566.9	152.2	135.4	91.6

Table 5. Descriptive statistics of the continuous variable herd size in model B (Previously *Salmonella* test negative herds)

Analytical statistics

The parameter estimates and significance levels of risk factors for herds changing *Salmonella* test status from negative to positive between two quarters of a year are shown in Table 6. There was a significant difference in the likelihood of changing *Salmonella* test status from negative to positive depending on which region the herd was placed in with herds in North Jutland South being most likely and herds on Islands and in East Jutland being least likely of changing to positive status. The geographical distribution of herds which changed from *Salmonella* test negative to *Salmonella* test positive and herds which remained *Salmonella* test negative between two quarters of a year are illustrated in Figure 7. There was a significant association between herd size and the likelihood of changing *Salmonella* test status from negative to positive between two year quarters. Per increase in herd size of 100 the odds of becoming *Salmonella* positive was 1.7 (95% CI: 1.2-2.2). Figure 8 illustrates the association between risk of changing from *Salmonella* test negative to test positive and herd size for each region graphically. Interaction between herd size and fluke status, herd size and region, herd size and organic, herd size and purchase was non-significant. Other interactions could not be tested in the model.

Variable and level	Estimate	SE	*P*-value*	Odds ratio	95 % confidence interval for odds ratio
Intercept	-5.91	0.50			
Region			0.0106		
North Jutland (S)	1.64	0.55		5.2	1.8 to 15.2
West Jutland	1.34	0.52		3.8	1.4 to 10.5
South Jutland	1.24	0.51		3.5	1.3 to 9.4
NW Jutland & N Jutland (N)	0.84	0.53		2.3	0.8 to 6.5
East Jutland & Islands	0	-		1	-
Herd size (per 100 heads)	0.50	0.15	0.0031	1.7	1.2 to 2.2

Table 6. Risk factors associated with a change in classification from negative to positive in the Danish Surveillance Programme for *Salmonella* Dublin. *P-value estimated by the score statistics for type 3 contrasts in the generalized estimating equation analysis

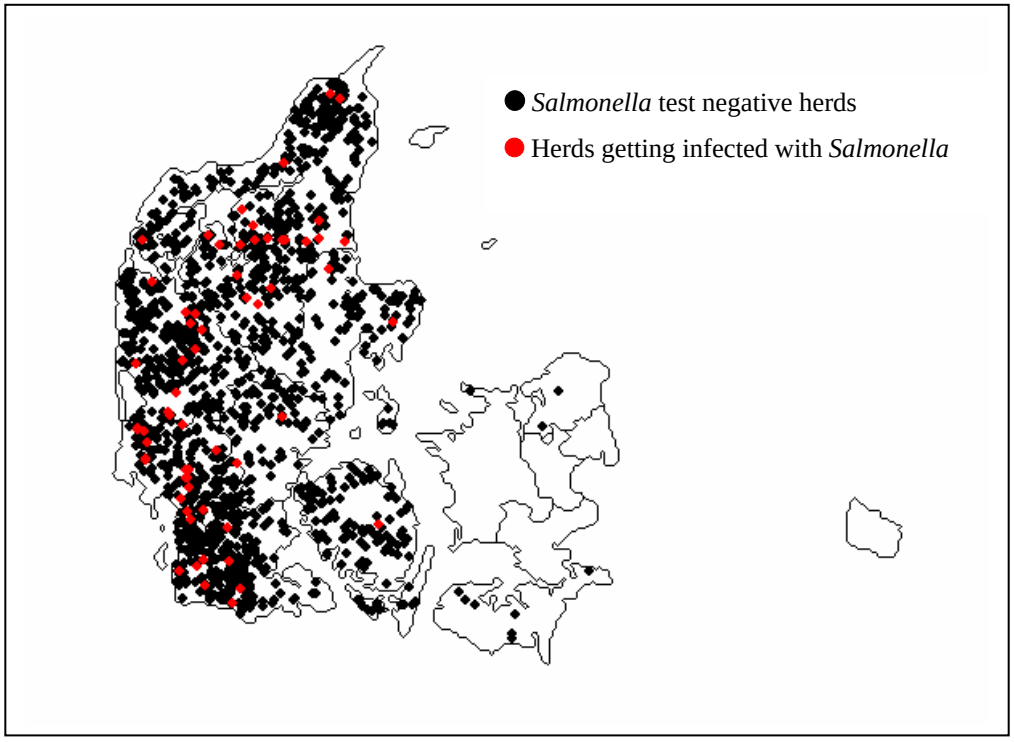

Figure 7. Geographical distribution of consistently *Salmonella* test negative herds and herds getting infected with *Salmonella*

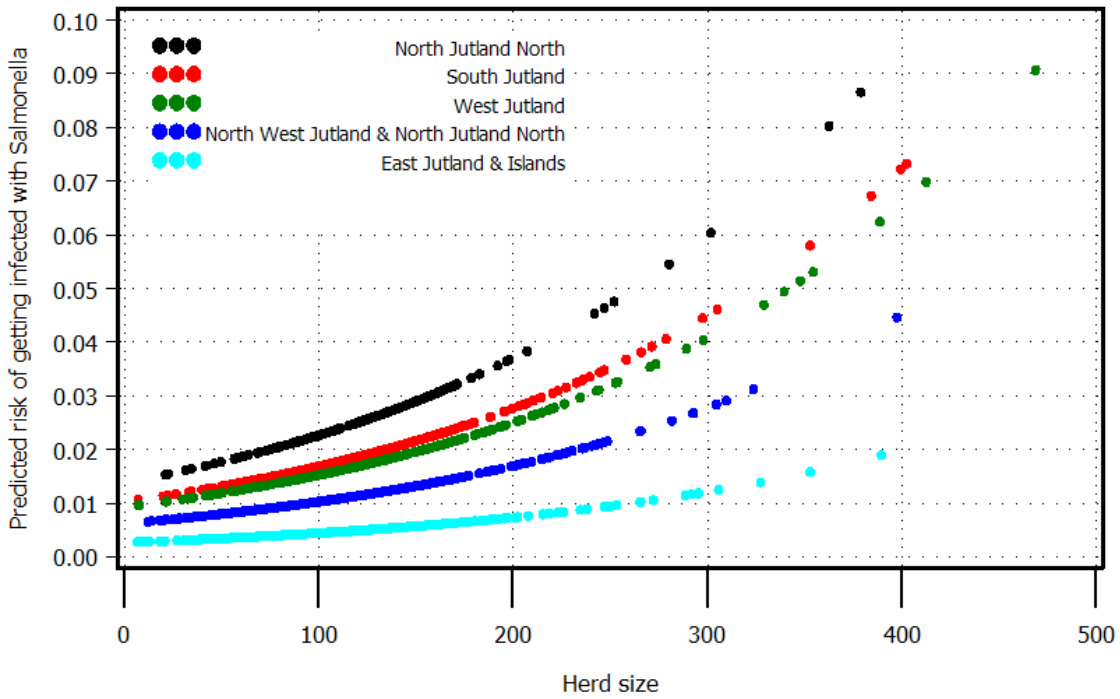

Figure 8. The relation between the likelihood of changing from *Salmonella* test negative to *Salmonella* test positive (predicted risk of getting infected with *Salmonella*) and herd size for herds in each geographical region

Model C: Change from positive to negative herd Salmonella test status, indicating recovery

Descriptive statistics

In Table 7 qualitative categorical variables in model C are listed. The total number of animals is shown on each level as well as the number and percent of herds changing from *Salmonella* test positive to negative from one year-quarter to the next. In table 8 descriptive statistics of the continuous variable herd size is given.

Variable and level	N	Herds pos to neg n	Herds pos to neg %
Fluke-herd-status			
0-2 %	691	64	9.26
> 2%	334	19	5.69
Region			
East J-Islands	59	7	11.86
NW Jutland-North J-N	180	18	10.00
North J-S	262	14	5.34
South J	357	32	8.96
West J	167	12	7.19
Purchase			
No	520	41	7.88
Yes	505	42	8.32
Organic			
No	851	68	7.99
Yes	174	15	8.62

Table 7. Descriptive statistics of qualitative categorical variables of model C (Previously *Salmonella* test positive herds)

	Min. value	Max. value	Mean	Median	Standard deviation
Herd size					
All herds	7.4	1478.8	157.5	134.1	123.6
Salmonella test positive herds	7.4	1478.8	158.6	134.5	124.8
Salmonella test positive to negative	18.3	767.0	145.2	127.0	109.6

Table 8. Descriptive statistics of the continuous variable herd size in model C (Previously *Salmonella* test positive herds)

Analytical statistics

The parameter estimates and significance levels of risk factors for herd *Salmonella* status in the first quarter of 2007 are shown in Table 9. Odds were 1.7 (95%CI: 1-2.9) times higher for changing

from *Salmonella* test positive to negative among herds with 0-2% liver flukes compared to herds with more than 2% liver flukes ($P < 0.04$). Figure 9 illustrates the association between the chance of changing from *Salmonella* test positive status to test negative status and herd size for the two levels of liver fluke infection, though herd size was not a significant variable in the model. The geographical distribution of herds which changed from *Salmonella* test negative to *Salmonella* test positive and herds which remained *Salmonella* test positive between two quarters of a year are illustrated in Figure 10. Interactions could not be tested in the model.

Variable and level	Estimate	SE	*P*-value*	Odds ratio	95 % confidence interval for odds ratio
Intercept	-2.81	0.24			
Liver fluke			0.0431		
0-2 %	0.53	0.27		1.7	1.0 to 2.9
2 % <	0	-		1	-

Table 9. Risk factors associated with a change in classification from positive to negative in the Danish Surveillance Programme for *Salmonella* Dublin. *P-value estimated by the score statistics for type 3 contrasts in the generalized estimating equation analysis

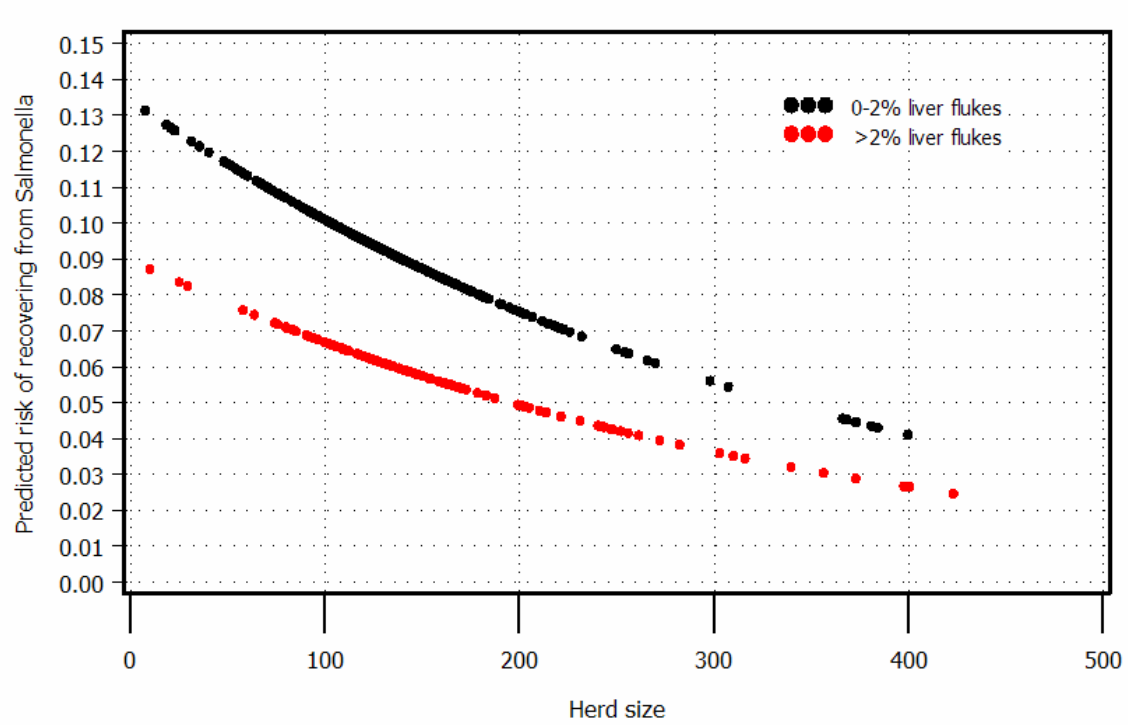

Figure 9. The relation between the likelihood of herds changing from *Salmonella* test positive to *Salmonella* test negative status (predicted risk of recovering from *Salmonella*), herd liver fluke prevalence and herd size**.** Notice that herd size is not significant in the model.

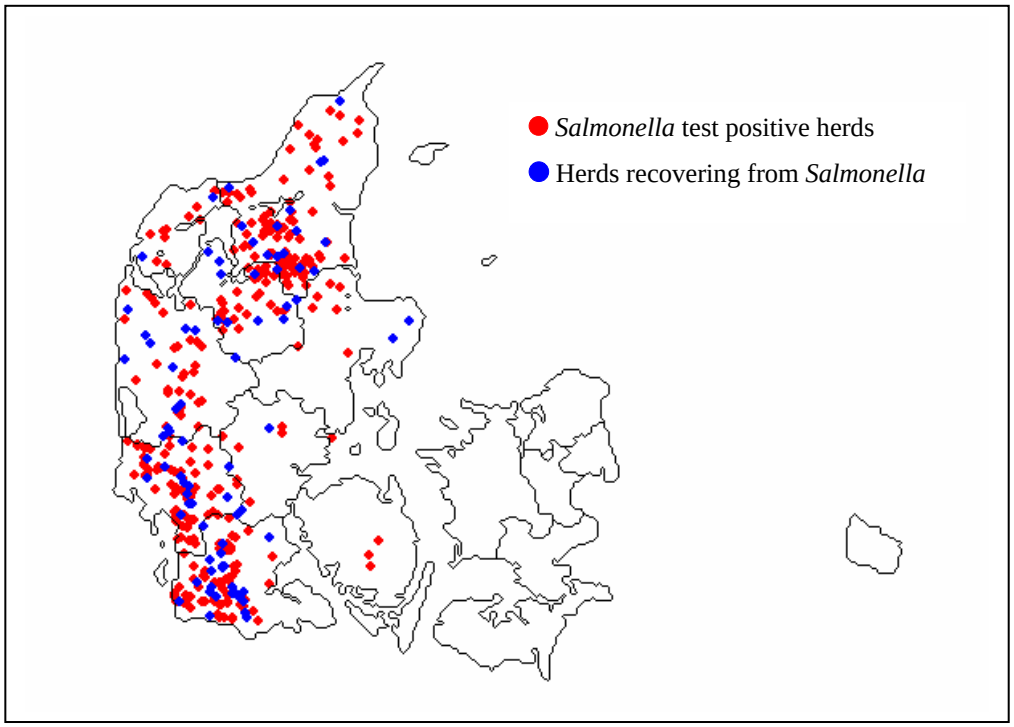

Figure 10. Geographical distribution of consistently *Salmonella* test positive herds and herds recovering from *Salmonella*

DISCUSSION

The particular strength of this study was the large number of herds included and the design being under natural conditions and not in an isolated experimental set up.

Data editing and reduction of data

In this study, we used recordings of liver flukes at slaughter to estimate the herd liver fluke prevalence. An estimation of the sensitivity of meat inspection in Denmark exists neither for liver fluke nor for other recordings. An investigation of the quality of liver fluke recordings in Switzerland found the diagnostic sensitivity of meat inspection to be 63.2% (Rapsch *et al.*, 2006). The sensitivity was estimated by comparing liver fluke recordings at slaughter with results of ELISA tests of the living animals. In the spring of 2007 we visited four cattle abattoirs in Jutland to interview and talk to the veterinarians and technicians in order to get an idea of the sensitivity of the meat control. We concluded that recordings of liver flukes at slaughter were reliable enough to use in this study, because we did not use the herd prevalence of liver flukes as a continuous variable.

Only animals slaughtered at abattoirs with electronic recordings of findings were included in the study. This criterion was settled on because we found a significant difference in the likelihood of the single animal being recorded as having liver flukes when comparing abattoirs with electronic recording system to abattoirs with manual recording system (results not shown). Animals from the six abattoirs in Denmark with electronic recordings of findings were included in the study and it is possible that the threshold for recording liver flukes is not identical at all six abattoirs. The difference in prevalence of liver flukes between the regions could be true, but it is also possible that animals from a particular region are mainly slaughtered at an abattoir with high sensitivity in recordings. This was not included in our study, but an investigation of the variation between the differences in sensitivity of abattoir recordings are of interest.

A criterion of ten slaughtered female animals in the study period was set for herds to be included in the study. This limit was set because we used the prevalence of liver fluke infested animals from the herd to categorise the herd liver fluke prevalence. A herd with only one slaughtered animal showing liver flukes would be defined as having a liver fluke prevalence of 100%. In the study herds were divided into two categories based on liver fluke prevalence, 0-2% (liver fluke negative or neglect able number of animals with liver flukes) or >2% (liver fluke positive). This could result in errors because one or few animals with liver flukes could cause the herd to be classified as liver fluke positive. Another possible error is that this or these animals were purchased into the herd and that they were already infected at this time. Therefore the infection did not originate from the herd which delivered them to slaughter. In this study we assumed that the animals were primarily infected with liver flukes in the autumn 2006 but since liver flukes can persist in cattle up until 26 months (Ross, 1968), they could originate from an earlier infestation. In order to increase the reliability of the herd liver flukes status, only herds with agreement between status of liver flukes in 2006 and 2007 were included in the study.

Study design

In this study we investigated liver flukes as a risk factor for infection with *Salmonella* Dublin. A risk factor should appear before the dependable variable. Cattle are most susceptible to infection with *S.* Dublin as young calves (Wray and Davies, 2000). Infection with liver flukes requires that the animal ingest metacercaries along with grass and vegetation and it is therefore unlikely that they get infected while they are calves. We can not reject that the causality between liver flukes and *S.* Dublin are oppose our assumption.

A problem in the study could be that not all herds are equally likely to change *Salmonella* test status. A test negative herd with a history of *Salmonella* test positive in the most recent years is more likely to be test positive in a later test than a herd which has not been tested positive recently (Wray *et al.*, 1989). Therefore herds are not necessarily randomly selected in this study and therefore do not have similar risk of changing *Salmonella* test status.

Model results

Several studies have investigated the association between *Fasciola hepatica* and *S.* Dublin in cattle on single animal level. Aitken *et al* (1978b) found that the lethal dose of *S.* Dublin was lower in fluke-infected animals compared to fluke-free animals and also that fluke-infected animals stayed infected with *Salmonella* for a longer period of time than fluke-free animals. This last result is interesting in terms of recovering from *Salmonella*, since carrier animals seem to be an important factor in cattle herds that stay persistently infected with *S.* Dublin (Wray and Davies, 2000). A potential problem with the experimental design in their study was the use of intravenous (IV) injection of high doses of *Salmonella* Dublin to infect the experimental animals. Infection through blood is not the natural way of infection for *S.* Dublin. A later experiment with liver fluke infection followed by oral infection with *S.* Dublin did not find an association between the two infections, nor the risk of becoming a persistent faecal shedder of *S.* Dublin (Hall *et al.*, 1981). Therefore, it is questionable if the results from the study with intravenous injection of *S.* Dublin can be extrapolated directly to natural conditions.

Herd liver fluke status was found to be associated with *Salmonella* test status on herd level in our study. Herds with more than 2% liver flukes were more likely to be *Salmonella* test positive than herds with 0-2% liver flukes. This result corresponds with two previous studies of the possible association. Richardson and Watson (1971) found that salmonellosis was more prevalent on fluke-infested farms compared to fluke-free farms. A scarcity in this study was that fluke-status was not measured directly on each farm but on the basis of use of known or suspected fluke-contaminated land and the use of routine treatment of flukes. Another study also found an association between liver flukes and *S.* Dublin (Vaessen *et al.*, 1998) but fluke status was not measured directly in this study either since it was based on interviews. The authors of this study suggested the possibility that farmers from *Salmonella* positive herds were more conscious of liver flukes. A strength of our study compared to the two previously described is that we measured fluke-status directly for each herd.

In our study we found liver flukes not to be a significant risk factor for the herd getting infected with *Salmonella*. A study found that the association between liver flukes and *S.* Dublin in single animals was dependent on the period of time between the two infections (Aitken *et al.*, 1978a) and therefore our result of no association could be caused by our study design. It is likely that liver flukes are not associated with the risk of getting infected with *S.* Dublin. On the contrary, herd liver fluke prevalence was found to be associated with changing *Salmonella* test status from positive to negative, which was interpreted as the herd recovering from *Salmonella*. This association has not previously been detected. Herds with 0-2% liver flukes were more likely to recover from *Salmonella* than herds with more than 2% liver flukes. This result could explain the existence of an association between *S.* Dublin and liver flukes, because herds with liver flukes are more likely to stay infected than fluke-free herds. The answer as to why an association between liver fluke status and the chance of recovering from *S.* Dublin exists could be found in the study performed by Aitken *et al.* (1978b). They found that liver fluke-infected cattle carried *S.* Dublin in the tissues for longer than liver fluke-free cattle. The presence of carrier animals in cattle herds is considered an important factor for *Salmonella* positive herds staying infected (Veling *et al.*, 2000; Wray and Davies, 2000; Nielsen *et al.*, 2004). Therefore the result of the present study could be interesting and relevant in terms of controlling and eradicating *S.* Dublin.

Geographical region in Denmark was included to control for regional cluster effects of *Salmonella* prevalence based on knowledge from previous work (Nielsen *et al.*, 2007). The present study found geographical region of origin to be associated with *Salmonella* test status. This agrees with the fact that not all regions have the same prevalence of test positive herds. Also, region was found to be associated with the likelihood of the herd getting infected with *Salmonella*. The association could be explained by the results published by Wedderkopp *et al.* (2001) who found that the prevalence of positive herds in the region was associated with the likelihood of the herd changing from test negative to test positive. Unlike Nielsen *et al.* (2007) we found region not to be associated with the chance of recovering from *Salmonella*. The regions in their study were not completely similar to our study which could explain why they found an association, whereas we did not.

Unfortunately, interactions between liver fluke prevalence and region could not be tested in our model. Taylor and Kilpatrick (1975) suggested that the association between *S.* Dublin and liver

flukes was caused by the two infections being influenced by similar external factors. It is possible that *S.* Dublin and liver flukes thrive best in a similar environment and are therefore more prevalent in similar areas of the country. A future investigation of interaction between region, liver flukes and *S.* Dublin could be of interest, making it even more optimal if the country was to be divided into even smaller regions than in our study because there is a large variation in the environment within each of the seven regions in this study.

Herd size was found to be associated with being *Salmonella* test positive as well as the risk of the herd getting infected. Large herds were more likely to be and to change to *Salmonella* test positive. It is possible that herd size by itself is not a risk factor but that it is an indirect measure of management such as it was suggested by Nielsen *et al.* (2007), who also found herd size to be associated with the risk of the herd getting infected. Investigating management as a factor in the epidemiology of *S.* Dublin is of great interest.

In our study, purchase of animals was found not to be a risk factor if getting infected with *Salmonella*. A previous study found that closed herds were less likely of introducing *S.* Dublin into the herd (van Schaik *et al.*, 2002), but purchase of animals was not found to be a significant risk factor. Nielsen *et al.* (2007) found purchase of animals from test positive herds to be a risk factor for getting infected with *Salmonella*. One reason why we did not find purchase to be a risk factor for getting infected could be that we only tested purchase of animals in general and not purchase of animals from farms with a particular *Salmonella* test status. Also, our study found neither association between organic status and *Salmonella* test status, nor the risk of getting infected or the chance of recovering from *Salmonella*. This corresponds with a previous study which found no difference in *Salmonella* spp. shedding on conventional compared to organic farms (Fossler *et al.*, 2004), but unlike Nielsen *et al.* (2007) who found organic herds to be less likely to recover from *Salmonella*.

Part of the motivation for performing the present study was the fact that an eradication programme for *Salmonella* in the Danish industry is currently in progress. Therefore it is worth considering how the results of the study could be utilised in this connection. The study found that fluke-free herds were more likely to recover from *Salmonella*. This is interesting in relation to the eradication programme because treatment of liver flukes in liver fluke-infected herds could be used as a tool to

increase the likelihood of the herd recovering from *Salmonella*. Also, the result showing that geographical region of origin is associated with *Salmonella* test status as well as the risk of getting infected is useful in the eradication programme, because it seems very likely that local factors are decisively supportive to the chance of eradicating *Salmonella*.

CONCLUSION

This study found an overall association between *S.* Dublin and the herd prevalence of liver flukes ($P < 0.0001$). Herds with more than 2% liver flukes were more likely to be *Salmonella* test positive than herds with 0-2% liver flukes. Also we found recovering from *Salmonella* to be associated with herd liver fluke prevalence ($P = 0.04$). On the basis of these results we conclude that herds with 0-2% liver flukes are more likely to recover from *Salmonella* compared to herds with more than 2% liver flukes. This gives a possible explanation of the overall association between the two infections since it seems like liver flukes predispose for the herd to remain infected. The study found no association between herd prevalence of *Salmonella* and the risk of getting infected with *Salmonella*.

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CHAPTER 4

Overall Conclusion and Perspectives

OVERALL CONCLUSION

No association between *Salmonella* Dublin (*S.* Dublin) and liver abscesses was found in the investigation of *S.* Dublin and liver abscesses in the Danish feedlot production. The results of the model at animal level were not of a quality to make a definitive conclusion. The conclusion is that risk factors for getting liver abscesses as a single animal could not be tested in this model. In the other model, testing the association at herd level, no association between *Salmonella* test status and the prevalence of liver abscesses was found. Because the two infections are very different in cause and are affected by different management factors we consider it to be most likely that they are not associated. At herd level production type (slaughter calf vs. young bull production), herd size and interaction between these were found to be associated with herd prevalence of liver abscesses.

In the second study we investigated the association between *Fasciola hepatica* (liver flukes) and *S.* Dublin in Danish dairy herds. An overall association between *S.* Dublin and the herd prevalence of liver flukes was found ($P < 0.0001$). Herds with more than 2% liver flukes were more likely to be *Salmonella* test positive than herds with 0-2% liver flukes. This agrees with the results of previous studies of the association on individual as well as herd level. In the second model we found the prevalence of liver flukes not to be associated with the herd getting infected with *S.* Dublin. In the last model we found recovering from *Salmonella* to be associated with herd liver fluke prevalence ($P = 0.04$). We conclude that herds with 0-2% liver flukes are more likely to recover from *Salmonella* compared to herds with more than 2% liver flukes. Also this gives a possible explanation of the overall association between the two infections since it seems like liver flukes predispose for the herd to remain infected. Geographical region in Denmark was included to control for regional cluster effects of *Salmonella* prevalence based on knowledge from previous work. Region was associated with *Salmonella* test status as well as getting infected with *S.* Dublin. Unfortunately interactions with region could not be tested.

We think that the results of the present studies can be used in the process of eradicating *S.* Dublin in the Danish cattle industry. The association between *S.* Dublin and liver flukes, and specially the knowledge of the relation between having liver flukes in the herd and remaining infected with *S.* Dublin, can be utilised to optimise the eradication programme. On the other hand the knowledge that liver abscesses and *S.* Dublin does not seem to be associated means that focus may be put on other factors affecting *S.* Dublin.

PERSPECTIVES

Our studies were based on recordings of liver abscesses and liver flukes at meat inspection. We observed a variation in recordings originating from abattoirs with electronic recording system compared to abattoirs with manual recording of slaughter finds. Recording of finds from meat inspection at abattoirs in Denmark provide an impressive set of data on Danish cattle, which the industry could gain from. These data may often reflect the prevalence of disease in the cattle industry and an effort should be made to utilise these data as good as possible. Currently no official studies of the sensitivity of meat inspection in Denmark exist. In order to optimise the data originating from the meat inspection, it would be of interest to conduct an evaluation of the meat inspection in Denmark, for one thing at the single abattoir for another of the variation between abattoirs. Such an investigation would improve the possibilities of using the recordings and data in future studies and making reliable and certain conclusions. Therefore such an investigation would be of great interest.

Despite the result of no association between *Salmonella* test status and liver abscesses found at herd level we still find it interesting to investigate *Salmonella* as a risk factor for getting liver abscesses. Based on the problems in our model it would be relevant to use *Salmonella* results for the individual animal instead of using herd *Salmonella* status to test *Salmonella* as a risk factor for the individual animal developing liver abscesses.

In this thesis we looked at liver abscesses in relation to *S.* Dublin because of the National Surveillance programme for *S.* Dublin. The results of our statistical analyses showed that herd size and production type (slaughter calf and young bull) were associated with liver abscesses. We assume that herd size and production type, which we found to be associated with liver abscesses at herd level, are an expression of the intensive feeding strategy, which is known to be predisposing for liver abscesses. There was a strong indication of a lower total weight if the animal had liver abscesses. We think that liver abscesses in the feedlot production are a problem for the cattle industry both ethically and economically. Therefore it is recommendable to look into other risk factors for getting liver abscesses and to put focus on the condition.

Data of liver flukes and *Salmonella* test status in our study does not cover a whole year. It is possible that use of data from other years would give different results of the association. Including

data of variations in temperature and rainfall from year to year in a future analysis of the association would be of interest, because the two infections are both influenced by variations in climate. Maybe the association between the two infections is related to their pathogeneses which vary throughout the year. Also, with both liver flukes and *S.* Dublin infections being seasonal, it is possibly that the association demonstrated in this study, can not be found when using data from other seasons. Including season as a variable in the model would be of interest. We think that the association between the two infections should be investigated even further. Collection of data over several years would make it possible to include more variables in the model. Also, a larger set of data would make it possible to investigate interactions between risk factors.