



## Master's thesis

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# Estimation of milk yield losses associated with *Mycoplasma bovis* outbreaks in Danish dairy herds



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## Preface

This thesis was developed in the autumn 2014 to reach the Master degree in Veterinary Medicine and was developed at the Section of Animal Welfare and Disease Control in the Department of Large Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen.

The study was part of a larger research project on *Mycoplasma bovis* in Denmark made possible by economical and technical support by the Knowledge Centre of Agriculture, Cattle, Aarhus, Denmark (now SEGES) and the Department of Large Animal Sciences, University of Copenhagen. A big thank you to the entire group for great advice and feedback at our meetings.

The thesis was written as a manuscript for an article, with an extended introduction, and was based on a questionnaire survey conducted in collaboration with two other master students.

The thesis is primarily directed at veterinarians, veterinary students and others with interest in dairy cattle and *Mycoplasma bovis*.

I would like to thank my advisor Liza Rosenbaum Nielsen for great advice, support, inspiration and continuous encouragement during this thesis.

Another unreserved thank to my co-advisor Matt Denwood for help and guidance throughout the statistical process. Thanks to Kaspar Krogh for help developing the questionnaire, for great advice and feedback.

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Thanks to my family and friends and finally yet importantly, the greatest thanks to Jonas Nielsen for love, economic support and patience through my entire study at the University of Copenhagen.

## Abstract

*Mycoplasma bovis* is associated with a variety of diseases in cattle, among others mastitis, pneumonia, arthritis and otitis media. Mastitis is a major problem in dairy herds leading to milk losses and increasing somatic cell counts thereby affecting the farmers' economy. The attention on *Mycoplasma bovis* has increased in Denmark and the objective of this study was to examine if there was a drop in milk yield in herds who experienced an outbreak with *Mycoplasma bovis*.

In total 120 herds, which experienced an outbreak with *Mycoplasma bovis*, and 152 reference herds that did not experience an outbreak, but still had laboratory test results indicative of *Mycoplasma bovis* having been present in the herd during the study period from start 2010 to mid-2014, were included in the study. Expected milk yield was predicted for all available test dates, based on all available milk recordings from the herds up to a year prior to the estimated outbreak date. Milk deviation between predicted and measured milk yield on test dates was used as the outcome variable. A defined 3-month outbreak period for each herd was compared with a similar period the year before and a year after the outbreak using two linear mixed models with random effects of herd, animal and lactation, one model for outbreak herds and one model for reference herds. The model included other predictors that might affect the milk deviations, such as parity, season and year.

A relative small, but significant average milk loss of 62 g ECM/cow per day in the 3-month outbreak period was estimated for the 120 outbreak herds. In comparison, the reference herds were estimated to yield 68 g ECM/cow per day more than predicted in the 3-month period. Hence, the milk yield losses associated with *Mycoplasma bovis* outbreaks are limited, and most likely mainly associated with clinically ill cows and consequential culling of cows during the outbreak. However, it would require additional analyses to evaluate the effect of *Mycoplasma bovis*-associated illness and culling on the total milk production of dairy herds experiencing outbreaks of this infection.

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# Introduction

*Mycoplasma bovis* (*M. bovis*) was first isolated in USA in 1961 but have then spread to European countries over the last 40 years (Nicholas and Ayling, 2003). *M. bovis* was isolated in Denmark in 1981 (Kusiluka et al., 2000), and around 2011 attention on *M. bovis* increased which led to more research on the subject. In 2013 the apparent prevalence in Denmark was 1.7% based on PCR testing with a cut-off of Ct <40. Based on antibody detection using ELISA testing, the prevalence the same year was 7.1% at the animal-level recommended cut-off of ODC% >37 indicative of a positive test-result when testing on bulk tank milk from all Danish dairy herds (Nielsen et al., 2015). No recommendation for cut-off values exist for testing on bulk tank milk and it is currently being investigated.

*M. bovis* belongs to the bacterial class *Mollicutes*, and is characterized by the lack of a cell wall and its small size (Nicholas and Ayling, 2003). The lack of a cell wall means that antibiotics targeting the cell wall has no effect, thus there is a natural resistance towards  $\beta$ -lactams such as penicillin and cephalosporin. *Mycoplasmas* do not synthesize folic acid and are therefore resistant to sulphonamides (Maunsell et al., 2011). The natural resistance towards these antibiotics makes it difficult to treat diseases caused by *M. bovis*, and farmers often discover *M. bovis* in the herds because of unresponsiveness to antibiotic treatment. Clinical and pathological signs of *M. bovis* are not very characteristic and therefore laboratory diagnosis is necessary for detection (Nicholas and Ayling, 2003).

*M. bovis* colonizes the mucosal surfaces where it can persist without causing clinical disease leading to asymptomatic carriers who can shed the bacteria intermittent (Maunsell et al., 2011). The upper respiratory tract and the mammary glands are the most important sites of persistence and shedding of *M. bovis*. Transmission from an infected cow to an uninfected cow is most often by udder-to-udder, via milking machines and milkers' hands but also transmission of respiratory secretions via aerosols and nose-to-nose-contact are routes of transmission (Gonzalez and Wilson, 2003; Maunsell et al., 2011).

*M. bovis* can cause a variety of diseases, including mastitis, pneumonia, arthritis and otitis media. Pneumonia, arthritis and otitis media are the predominant *M. bovis* related diseases in calves and cows are mostly seen with mastitis and arthritis (Pfutzner and Sachse, 1996; Maunsell et al., 2011).

Mastitis caused by *M. bovis* is usually characterised by the glands being swollen and hard but rarely sore with abnormal udder secretions. Often more than one gland are involved with *M. bovis* mastitis and there will be a drop in milk yield in clinically affected cows (Biddle et al., 2003; AlAbdullah and Fadl, 2006; Maunsell et al., 2011).

Mastitis is one of the major problems on dairy farms because it is associated with milk loss that directly affects the farmers' economy (Hertl et al. 2014, Rajala-Schultz et al. 1998). In a study conducted in New York and Pennsylvania, Wilson et al. (1997) estimated the prevalence of mastitis by collecting milk samples from 108,312 cows from January 1991 to June 1995. The milk samples were cultured and it was found that the prevalence of mastitis was nearly 50% amongst all cows, indicating the importance of mastitis. There is no agreement on which pathogens are to be considered as the major pathogens of mastitis. In the study of Wilson et al. (1997) *Mycoplasma* was, together with *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus* spp., considered as the major pathogens. *Mycoplasma* was considered because it lead to large milk losses and large economic losses.

In an outbreak of *M. bovis* with clinical mastitis in a North Italian herd, the clinically diseased cows had a large drop in milk production, the milk quality was reduced as most of the affected cows had a somatic cell count (SCC) above 1 million cells/ml (Radaelli et al. (2011). Haas et al. (2002) found that clinical mastitis had a large effect on SCC. They investigated the effect of clinical mastitis of different pathogens on SCC and found that clinical mastitis led to a SCC above 1.5 million cells/ml during the acute phase for all pathogens. After the clinical phase, the SCC stayed at a higher level than before the case of clinical mastitis ranging from 51.000 to 460.000 cells/ml according to pathogen. Wilson et al. (1997) observed that cows with *M. bovis* mastitis had a linear score SCC at 5.7 corresponding to 650,000 cells/ml in the month when mastitis was detected.

In addition to the milk loss during clinical mastitis with *M. bovis*, the SCC is also important for Danish farmers, as the milk prices in Denmark are differentiated based on milk quality, measured by SCC and other parameters. This means that the farmer will receive a lower price, the higher the SCC in bulk tank milk. Furthermore, Hertl et al. (2014) found that if a cow has had a clinical case of mastitis she was in greater risk of having a subsequent case of clinical mastitis.

To my knowledge, nobody has investigated the milk losses at herd-level in herds with an outbreak of *M. bovis*. The purpose of this thesis was therefore to examine if there is a drop in milk yield in farms with an *M. bovis* outbreak and if possible to quantify the losses. In this current study I was unable to receive information about *M. bovis* on cow level, so I chose to examine whether there is a reduction in milk yield in the Danish dairy herds that have had an outbreak with *M. bovis*. I used the information on milk production before and after the outbreak in the herd as a reference.

## Materials and Methods

### Data collection

In 2011, 2012 and 2013, all Danish dairy farms were surveyed for *M. bovis* by PCR (PCR-Patho-proof, Thermo Fischer Scientific Oy, Finland) once a year on bulk tank milk. In 2013 and 2014, farms were tested four times on bulk tank milk by ELISA (*Mycoplasma bovis* ELISA KIT, Bio-X Diagnostics, Site du Complexe des postes 49, rue J. Wauters, 5580 Jemelle, Belgium). The Knowledge Centre for Agriculture, Cattle, Aarhus, Denmark (from 1<sup>st</sup> of January 2015 named SEGES) monitored the herds and provided a list of farms with positive test results on either PCR or ELISA according to the criteria below.

In August 2014, a questionnaire was developed. A small pilot study was conducted in which some farmers and fellow students were interviewed, to check if the questions were understandable and easy to answer. The questionnaire was adjusted and then set up for online entry of answers in 'Survey-Exact' by AgroTech A/S, Skejby. The inclusion criteria for receiving a questionnaire were: 1) if the farms had reported an outbreak to SEGES; 2) if they have participated in earlier projects regarding *M. bovis* and were test positive; 3) if they had a positive *M. bovis* sample on bulk tank milk in the above mentioned surveillance or 4) if they had a test positive bulk tank milk sample prior to entering a cattle fair in 2014. An ELISA result was considered positive when the corrected optical density percentage (ODC%) compared with a negative control test was  $\geq 37$ , which is the cut-off recommended by the test-kit producer for use at animal level. This cut-off was used because no recommendations exist for bulk tank milk testing. Many herds had an ELISA results  $\geq 37$  so because of limited time available for the project and ongoing analyses indicating that a higher cut-off value would improve specificity of the ELISA used on bulk tank milk samples (Nielsen et al., 2015), the



inclusion criteria were set to be  $\geq 55$  ODC%, and this reduced the number of farms to interview. The cut-off value for PCR testing was set to  $Ct \leq 39$ .

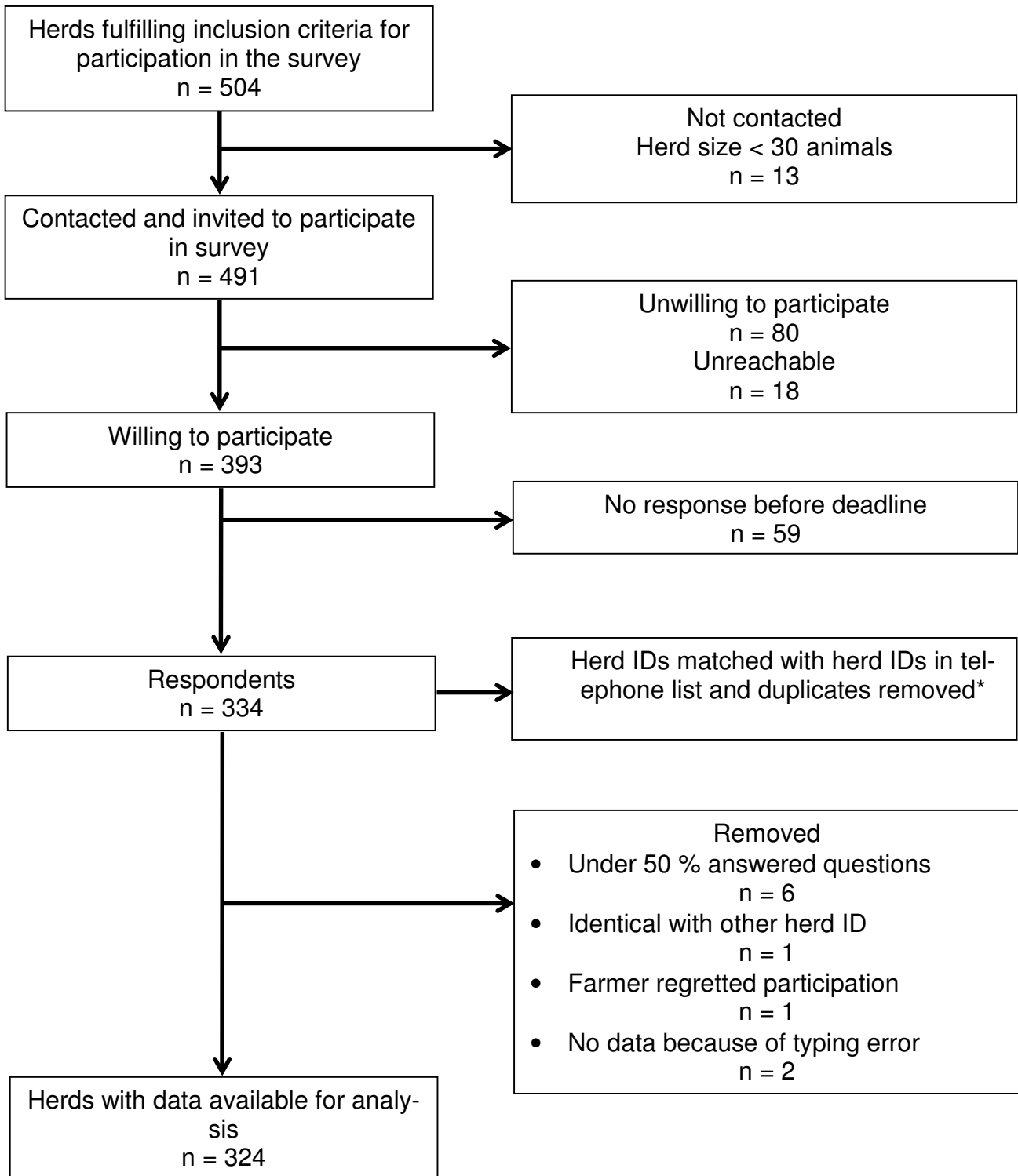
In total 504 herds fulfilled the inclusion criteria. In the pilot study, it was experienced that very small herds had difficulty answering the questions, because no general management procedures were followed and therefore 13 herds were excluded, because they had fewer than 30 animals.

In September-October 2014, 491 farmers were called by one of two interviewers and asked to participate either by phone interview in which the interviewer entered the answers in the online system, or by receiving a link to the online questionnaire by email to fill in the answers him/herself. All farmers that did not respond within a week or did not have an email address, were called again and either got another week to respond or the caller asked the questions and filled in the questionnaire. The questionnaire round ended in the beginning of October and additional data on the participating farms were retrieved from the Danish Cattle Database (DCD).

Of the 491 farmers contacted, 80 farmers were unwilling to participate and we were unable to reach 18 farmers either because lack of contact information or they did not answer the phone. In addition, 59 farmers did not answer the questionnaire before the last day of the questionnaire round. That gave us 334 respondents, but from these it turned out that some farmers had answered the questionnaire more than once and we had received 354 responses. The 20 duplicate responses were compared and the least complete entry was removed. From the 334 respondents, six farmers answered less than 50% of the questionnaire and their answers were therefore excluded. Two responses with different herd ID turned out to be from the same herd and one response was excluded. One farmer retracted his data entry and his response was excluded, and because of typing errors in the herd number, no information was available on two herds and they were excluded. We therefore ended up with 324 herds that were useful for analysis. See flow diagram in Figure 1.

## **Selection of Herds**

In the questionnaire, the farmers were asked if they had had an outbreak with *M. bovis*, and there were four possible answers: 1) No, I have not had sick animals with clinical signs of *Mycoplasma bovis*; 2) No, I have not had an outbreak, but I have had sick animals, which could have been affected by *Mycoplasma bovis*; 3) Yes, I have had an outbreak (sudden or marked increase in



**Figure 1:** Flow diagram of selection of herds from the 504 herds fulfilling the inclusion criteria.  
 \* Herds with more than one herd ID, where matched with the herd ID we had received. When multiple responses, the duplicates were compared, and the least complete entry was removed.

disease occurrence), which could be *Mycoplasma bovis*; 4) Yes, I have had a few sick animals with confirmed infection of *Mycoplasma bovis*. If they answered 3 or 4, they were included in this study as outbreak herds. If they have answered 1 or 2, they were included as reference herds.

In total, 123 farmers had experienced an outbreak with *Mycoplasma bovis* and 201 were considered reference herds. Three of the outbreak herds were excluded because they did not participate in the milk yield recording scheme and therefore no recordings were available for the analysis. One farmer had two farms where the milk recordings were registered under another herd ID than the one we were using, so these two were merged.

Of the 201 reference herds, 36 herds had fewer than 100 cows and one herd had more than 1000 cows. These were excluded to make the reference herds as similar to the outbreak herds as possible. Out of the 164 herds, 12 herds did not participate in the milk yield recording scheme and these were excluded, ending up with 152 reference herds for the analysis.

## **Construction of Variables**

Data on herd information, routinely collected milk yield recordings, treatments of cows and calving information from 2002 to October 2014 was derived from DCD. Information on the outbreaks came from the answers in the questionnaire.

### **Outbreak Information**

*Outbreak start date:* The farmer reported his/her best estimate of the outbreak start date for the outbreak herds. A 'pseudo outbreak start date' was given for the reference herds for comparative purposes. The reference herds with a Ct-value <37 on PCR or ODC%  $\geq$ 55 on ELISA, were given an 'pseudo outbreak start date' on the day of the first positive test-result. Reference herds with a Ct-value 37-39 were given a random 'pseudo outbreak start date' between the first and last outbreak start date in this study with the rand-function in Microsoft Excel (2013). This choice was made because there were indications that small doses of contamination of the bulk tank milk samples with DNA-material from other farms was possible at the laboratory, meaning that these values above the animal-level recommended cut-off could be false-positive PCR-reactions.

*Outbreak end date:* The farmer reported date or the date of the interview if still ongoing outbreak for the outbreak herds. No 'pseudo outbreak end date' was given for the reference herds.

*Outbreak duration:* Calculated for the outbreak herds, as days from outbreak start date to outbreak end date or the date of the interview if still ongoing outbreak.

## **Herd Information**

*Herd:* Herds have a unique herd ID, but were assigned a number between 1 and 272 to keep farm identity anonymous.

*Herd type:* Classified as a conventional or organic herd in the quarter of the year before the outbreak start date.

*Herd size:* The average number of cows registered as present in the herd in the quarter of the year before the outbreak start date in the DCD.

*Herd breed:* Breed was based on the percentage of fat in the milk delivered to the dairy in the quarter of year before the outbreak start date. The percentage of fat in milk differs as smaller breeds such as Jersey, have a higher percentage of fat in the milk. The percentage of fat in the milk for all herds was plotted (not shown). No herds had a percentage of fat between 4.7% and 5%, and the cut-off was set at 5%. Herds delivering milk to the dairy with a fat percentage equal to or above 5% were classified as small breed, and herds with a fat percentage less than 5% were classified as a large breed herd.

*Herds using grassing:* Herds were coded 1 if the cows were reported to have access to pasture and coded 0 if the cows did not have access to grass.

*Salmonella Dublin status:* In Denmark, there is a surveillance program for *Salmonella* Dublin (*S. Dublin*). Three times a year a sample from bulk tank milk is collected from all dairy herds and analysed for presence of antibodies using ELISA. Based on these samples the herds were categorised into two groups. Code 0: Most likely free from *S. Dublin* because of low levels of antibodies in bulk tank milk. Code 1: Signs of infection with *S. Dublin* in the antibody measurements.

*Streptococcus agalactiae status:* Every year milk samples are collected to monitor the prevalence of *S. agalactiae*. If herds were *S. agalactiae* PCR-positive in the outbreak period they were coded 1 and if not they were coded 0.

*Delivered milk:* The amount of milk delivered to the dairy in the quarter of the year with the outbreak start date. Calculated as delivered milk in kg divided by herd size i.e. the average number of cows in the quarter of year.

## Animal Information

*Animal ID:* All live born cattle are ear tagged at birth and registered in DCD.

*Parity:* The cows were divided into three categories based on calving number. First parity (1), second parity (2) and third and higher parity (3+).

*Parity ID:* Based on *Animal ID* and *Parity*.

*Y index:* The Y index is a breeding value for yield that describes the genetic potential for milk, protein and fat production, and it is based on milk records.

*Treatments:* By legislation, the farmers in Denmark have to register when a cow is treated with medicine and only farmers with a mandatory herd health contract can initiate treatments on individual cows and have to register the treatments in DCD. The type of disease treated, was divided into six categories: 1) Mastitis; 2) Calving problem; 3) Digestive or metabolic disease; 4) Hoof or limb lesion; 5) Reproduction disease and 6) Dry-off treatment (Appendix, diseases in Danish). If a cow was treated for any of the first five categories, this was coded 1 for the relevant category for all milk recordings in the lactation, were the treatment occurred regardless of number and timing of treatments in relation to the milk recordings. A cow was also coded 1 in the category of calving problems if she had a 'difficult calving' registration. If the cow was treated at dry-off, she was coded 1 for all milk recordings in the next lactation, since this would be where the effect would be. If the cow had no treatments she was coded 0.

## Milk Yield Information

Of the milk-producing herds in Denmark, 90% participate in a voluntary milk recording scheme where information on individual cow milk yields is recorded routinely six or eleven times a year (RYK, 2015). If a herd has eleven test dates then one milk sample for every milking cow is collected and if the herd only has six test dates then two milk samples from each cow are collected at each test date. The amount of milk is measured and a milk sample is sent to Eurofins Steins Laboratory (currently Vejen, before that Holstebro) where the percentage of fat, percentage of protein and somatic cell count (SCC) is measured. From these data, the Energy Corrected Milk (ECM) was calculated as suggested by Sjaunja et al. (1991):

$$ECM(kg) = Milk(kg) * \frac{0.383 * Fat\% + 0.242 * Protein\% + 0.7832}{3.14}$$

*LogSCC:* SCC was log transformed to make the data more normally distributed.

## Periods

*Outbreak period:* The outbreak period was defined as a 3-month period from the outbreak start date regardless of the reported outbreak duration. This was done to have a similar period for all herds.

*Reference periods:* The reference period consists of two periods; a 3-month period starting one year before and a similar period starting one year after the outbreak start date.

For reference herds, these ‘pseudo-outbreak’ and ‘pseudo-reference’ periods were selected in the same way in relation to the selected outbreak start date.

## Data Analysis

All the analyses were performed in Rstudio 3.1.1 (R Core Team, 2014). Descriptive analyses on herd information and continuous milk information were made on all 120 outbreak herds and 152 reference herds separately. For parity and treatments, the odds ratios and p-values were calculated, and for the continuous variables, the quartiles were calculated.

Milk delivered to the dairy was plotted against time to see if this changed over time. It was plotted with the mean and 95% confidence interval for each quarter of the year from 2009 to 2014 for both outbreak and reference herds (i.e. not only showing the outbreak and/or reference periods).

Matt Denwood developed a multivariable mixed model, used to predict the expected ECM on test dates in the relevant periods based on previous data for all cows in the herds. This was done to make it easier to compare the effect of the outbreaks on milk yield across cows and herds. The model fits an individual curve for each cow based on the ‘Ali-B model’ (Quinn, et al., 2005). In the prediction model, cows that would be dead by the time of the outbreak start date were excluded and cows with milk yields at zero or above 75 kg, or fat percent above 10 % or protein percent above 5 % were excluded to avoid predicting the milk yield of cows based on unlikely and potentially erroneous recordings. Cows with less than 4 days in milk were excluded, because these are not supposed to be in the milk yield recording scheme, because of naturally high SCC.

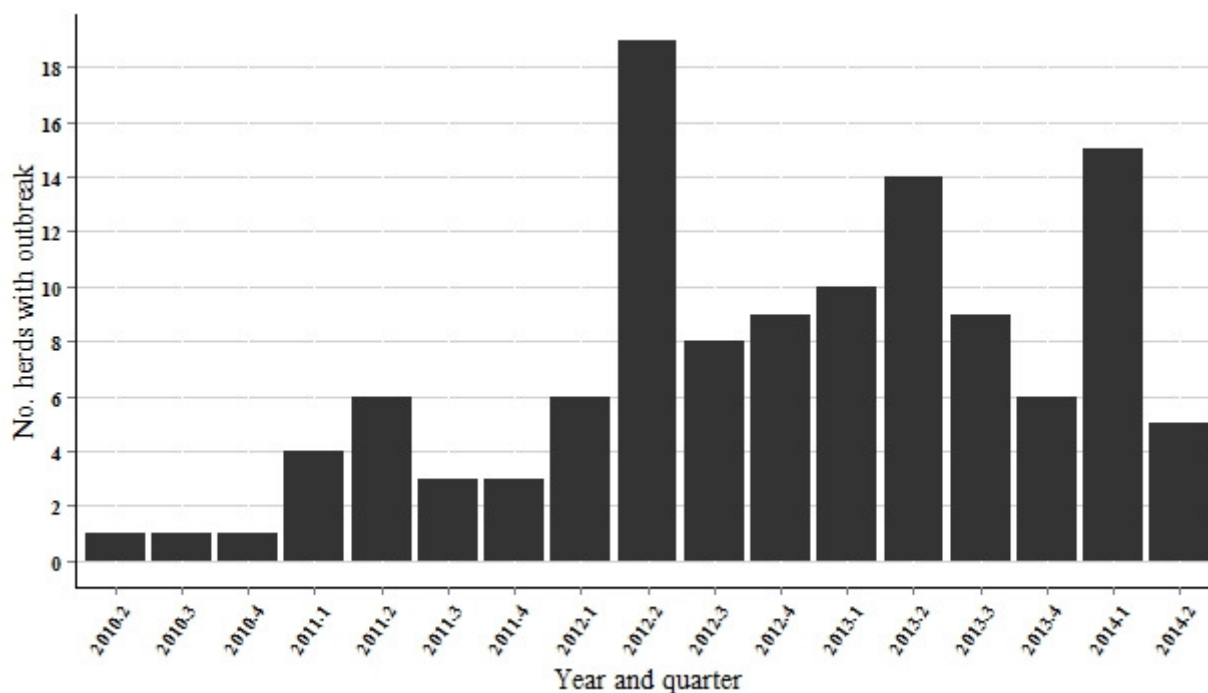
Data until one year before the outbreak start date was used in the ECM prediction model. This cut-off was set to avoid using data in the ECM prediction model that we would use later in the modelling of deviations from the expected milk yield. The best fitting model (assessed using AIC) was:

$$\begin{aligned} \text{PredictedECM} = & \beta + \beta_1\gamma^2 + \beta_2\omega + \beta_3\omega^2 + \beta_4\text{DOB} + \beta_5\text{Yindex} \\ & + \beta_6\text{Breed} + \beta_7\text{Parity} + \beta_8 \sin \theta + \beta_9 \cos \theta + \varepsilon \end{aligned}$$

Where  $\gamma = \frac{\text{DIM}}{305}$ ,  $\omega = \ln\left(\frac{305}{\text{DIM}}\right)$  and  $\theta = 2 * \pi * \frac{\text{DOY}}{366}$ . *DIM* is days in milk, *DOY* is the day of year of the milk recordings and *DOB* is date of birth.

After running the ECM prediction model on the data in the relevant periods, the measured ECM deviation from the predicted ECM on the milk recording day ('milk deviation') was calculated. Then a linear mixed model with parity id, animal id and herd id as random effects was used to investigate whether the milk deviation was different during the *M. bovis* outbreak period than during the reference periods, while adjusting for potential confounders. A stepwise forward selection was used to test the fixed effects of the predictors including all possible two-way interactions. The fixed effects were included in the final model at a 0.1% significance level. All effects were evaluated for both statistical significance and biological relevance. The biological effects of the interactions were evaluated by inspection of graphical displays of the predicted outcome vs. multiple variables. If the biological effects was negligible, the interaction was removed from the model, even if significant. The removed non-significant predictors were reintroduced to the final model to check for confounding. Confounding was considered relevant if a reintroduced variable changed the parameter estimates of any of the fixed effect by more than 20%. The residuals of the final model were using Pearson Correlation.

## Results



**Figure 2:** Timewise distribution of the 120 outbreaks, who experienced an outbreak with *M. bovis* according to the farmer reported outbreak start date, grouped by quarter of year.

The timewise distribution of the 120 outbreaks according to the farmer reported outbreak start dates are illustrated in Figure 2. The 272 herds included in the study contributed with 287,923 milk yield recordings from 44,194 cows in the outbreak herds and 43,478 cows in the reference herds in the outbreak and reference periods. The outbreak herds were on average larger than the reference herds with more cows per herd (Table 1) and more milk recordings per cow, respectively a mean of 3.6 and 3.



**Table 2:** Descriptive statistics on herd-level data for 120 outbreak herds who experienced an outbreak with *Mycoplasma bovis* and 152 reference herds with no reported outbreak. Outbreak data only on outbreak herds. *Streptococcus agalactiae* status not available for reference herds.

	<i>Outbreak herds</i>		<i>Reference herds</i>	
	<b>n</b>	<b>%</b>	<b>n</b>	<b>%</b>
<b>Herd type</b>				
Conventional	106	88.3	135	88.8
Organic	14	11.7	17	11.2
<b>Herd breed</b>				
Large breed	109	90.8	126	82.9
Small breed	11	9.2	26	17.1
<b>Grassing</b>				
Yes	33	27.7	50	32.9
No	86	73.3	102	67.1
<b><i>Salmonella</i> Dublin</b>				
Level 1	102	85	127	83.6
Level 2	18	15	25	16.4
<b><i>Streptococcus agalactiae</i></b>				
PCR-negative	105	87.5		
PCR-positive	15	12.5		
<b>Outbreak type</b>				
Outbreak	76	63.3		
Few sick animals	44	36.7		
<b>Outbreak status</b>				
Outbreak not ended	5	4.2		
Still few sick animal	56	46.7		
Outbreak ended	59	49.2		

**Table 1:** Descriptive statistics on continuous herd-level data for 120 outbreak herds who experienced an outbreak with *Mycoplasma bovis* and 152 reference herds with no reported outbreak.

	<b>Quartiles</b>					
	<b>Min</b>	<b>25%</b>	<b>Median</b>	<b>Mean</b>	<b>75%</b>	<b>Max</b>
<b>Herd size</b>						
<i>Outbreak herds</i>	78.0	172.8	228.5	257.8	310.8	681.0
<i>Reference herds</i>	100.5	142.9	187.1	216.9	250.2	618.8
<b>Milk delivered to dairy (kg/cow/year-quarter)</b>						
<i>Outbreak herds</i>	1418	2059	2305	2266	2503	3030
<i>Reference herds</i>	502	1935	2235	2187	2475	3815
<b>Y index</b>						
<i>Outbreak herds</i>	57	94	100	100	106	134
<i>Reference herds</i>	51	95	101	100	106	134
<b>Outbreak duration (days)</b>						
<i>Outbreak herds</i>	10.0	69.5.0	122.0	191.8	212.5	1096.0

The descriptive statistics on herds are given in Table 2 and indicates that the outbreak and reference herds had the same distribution of conventional and organic herds, and almost the same distribution of herds using grassing and *Salmonella* Dublin status. There were though more small breed herds in the reference group than in the outbreak group. In Table 1, the descriptive statistics are given for the continuous variables on herd level. It shows that the outbreak herds generally delivered more milk per cow to the dairy than the reference herds. This is also shown on Figure 3, which also shows that both outbreak and reference herds delivered more milk per cow to the dairy per quarter over the years, and especially in 2013 and 2014 there was a large increase.

**Table 3:** Descriptive statistics on average Energy Corrected Milk (ECM), milk deviation and log to Somatic Cell Count (logSCC) registered on cow-level for 120 outbreak herds who experienced an outbreak with *Mycoplasma bovis* and 152 reference herds with no reported outbreak.

	Quartiles							
	Min	5%	25%	Median	Mean	75%	95%	Max
<b>ECM (kg/day)</b>								
<i>Outbreak herds</i>								
Outbreak period	0.1	18.1	25.4	30.2	30.7	35.8	44.8	80.6
Reference period a year before	0.5	18.8	25.6	30.0	30.5	35.1	43.9	83.3
Reference period a year after	0.8	18.6	25.8	30.8	31.3	36.3	45.7	78.5
<i>Reference herds</i>								
'Outbreak period' *	1.7	17.9	25.0	29.9	30.3	35.2	44.3	78.3
'Reference period a year before'	0.5	18.0	24.7	29.4	29.9	34.6	43.3	76.3
'Reference period a year after'	0.9	18.3	25.3	30.1	30.6	35.5	44.5	80.2
<b>Milk Deviation</b>								
<i>Outbreak herds</i>								
Outbreak period	-39.5	-10.6	-3.3	0.5	0.3	4.2	10.0	44.2
Reference period a year before	-37.0	-9.2	-2.9	0.3	0.0	3.2	8.1	48.5
Reference period a year after	-36.7	-9.2	-2.4	1.4	1.3	5.1	11.3	36.7
<i>Reference herds</i>								
'Outbreak period'	-40.1	-9.8	-2.7	1.0	0.8	4.6	10.2	42.5
'Reference period a year before'	-34.9	-9.8	-3.2	0.2	0.0	3.5	9.0	42.6
'Reference period a year after'	-34.3	-9.1	-2.1	1.6	1.4	5.2	11.2	39.8
<b>logSCC</b>								
<i>Outbreak herds</i>								
Outbreak period	0.0	2.8	3.6	4.4	4.6	5.4	7.1	9.2
Reference period a year before	0.0	2.8	3.6	4.3	4.5	5.2	6.8	9.2
Reference period a year after	0.0	2.6	3.5	4.3	4.5	5.4	7.0	9.2
<i>Reference herds</i>								
'Outbreak period'	0.0	2.7	3.6	4.4	4.6	5.4	7.1	9.2
'Reference period a year before'	0.7	2.8	3.6	4.4	4.6	5.4	7.0	9.2
'Reference period a year after'	0.0	2.7	3.5	4.3	4.5	5.3	6.9	9.2

\* The outbreak and the reference periods for the reference herds were selected as 'pseudo-outbreak' and 'pseudo-reference' periods for comparative purposes.

The descriptive statistics on animal-level is given in Table 3 and Table 4. Cows in the outbreak herds had a higher average ECM/day than cows in the reference herds, but cows in the reference herds had a higher positive milk deviation than cows in the outbreak herds. Milk deviation is plotted against ECM in Figure 4 for outbreak and reference herds respectively.

Odds ratio is shown for parity and treatments for both outbreak and reference herds (Table 4).

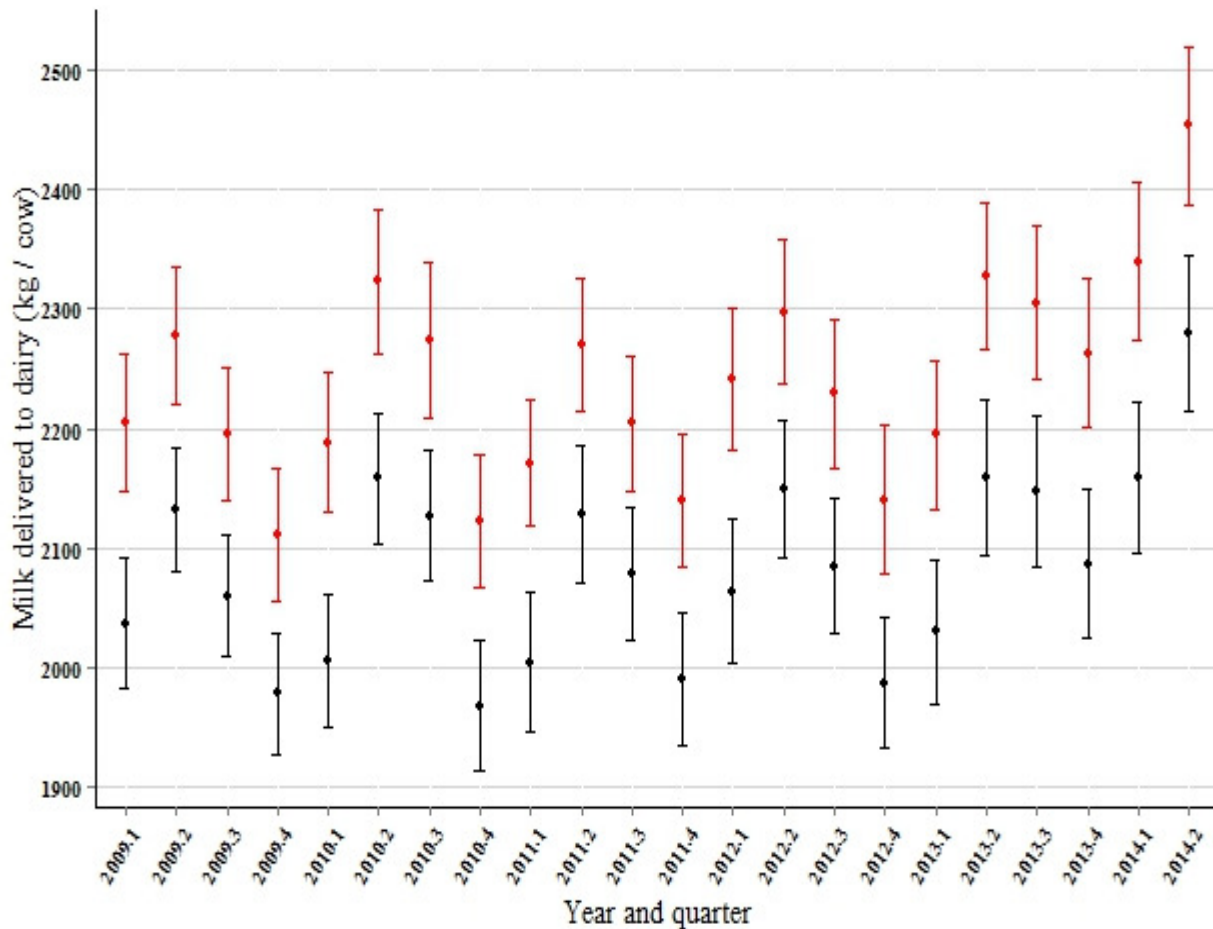
**Table 4:** Descriptive statistics and univariable analyses on parity and treatments registered on cow-level for 120 outbreak herds who experienced an outbreak with *Mycoplasma bovis* and 152 reference herds with no reported outbreak.

	<i>Outbreak herds</i>						<i>Reference herds</i>					
	Outbreak period		Reference period		OR	P-value	'Outbreak period' *		'Reference period' *		OR	P-value
	n	%	n	%			n	%	n	%		
<b>Parity</b>												
1	23259	0.38	39366	0.41			24165	0.38	24137	0.39		
2	18009	0.29	27501	0.28	1.10	<0.001	18244	0.28	17726	0.28	1.04	0.012
3+	20586	0.33	29906	0.31	1.17	<0.001	21689	0.34	20431	0.33	1.07	<0.001
<b>Reproduction</b>												
0	57411	0.93	90409	0.93			60221	0.94	59080	0.95		
1	4443	0.07	6364	0.07	1.10	<0.001	3877	0.06	3214	0.05	1.18	<0.001
<b>Hoof</b>												
0	36335	0.59	58787	0.61			43423	0.68	42067	0.68		
1	25519	0.41	37986	0.39	1.09	<0.001	20675	0.32	20227	0.32	1.01	0.418
<b>Mastitis</b>												
0	39739	0.64	62008	0.64			45397	0.71	44862	0.72		
1	22115	0.36	34765	0.36	0.99	0.493	18701	0.29	17432	0.28	1.06	<0.001
<b>Calving problem</b>												
0	55325	0.89	86896	0.9			57962	0.9	56642	0.91		
1	6529	0.11	9877	0.1	1.04	0.027	6136	0.1	5652	0.09	1.06	0.002
<b>Digestion</b>												
0	57749	0.93	90813	0.94			61034	0.95	59530	0.96		
1	4105	0.07	5960	0.06	1.08	<0.001	3064	0.05	2764	0.04	1.08	0.004
<b>Dry-off</b>												
0	48691	0.79	78795	0.81			52288	0.82	50977	0.82		
1	13163	0.21	17978	0.19	1.19	<0.001	11810	0.18	11317	0.18	1.02	0.239

\* The outbreak and the reference periods for the reference herds were selected as 'pseudo-outbreak' and 'pseudo-reference' periods for comparative purposes.

Results from the final model for the outbreak and the reference herds are given in Table 5. Of the herd level and outbreak predictors shown in Table 1 and Table 2, only milk per cow delivered to the dairy was significant and stayed in the model. *Streptococcus agalactiae*-status was non-significant for outbreak herds, and it was not available on reference herds.

After introducing ECM to the model as a predictor of milk deviation, all the treatment predictors were non-significant and were removed along with logSCC as it graphically showed correlation with ECM. Interactions were statistically significant but were removed as they graphically showed no biological important effect (not shown).



**Figure 3:** Development of amount of milk delivered to dairy per cow for each quarter of year between 2009 and 2014. The red bars illustrate the 120 herds, who experienced an outbreak with *M. bovis*, and the black bars illustrate the 152 herds without an outbreak. All year-quarters from all herds are included, not only year-quarters in the outbreak or reference periods.

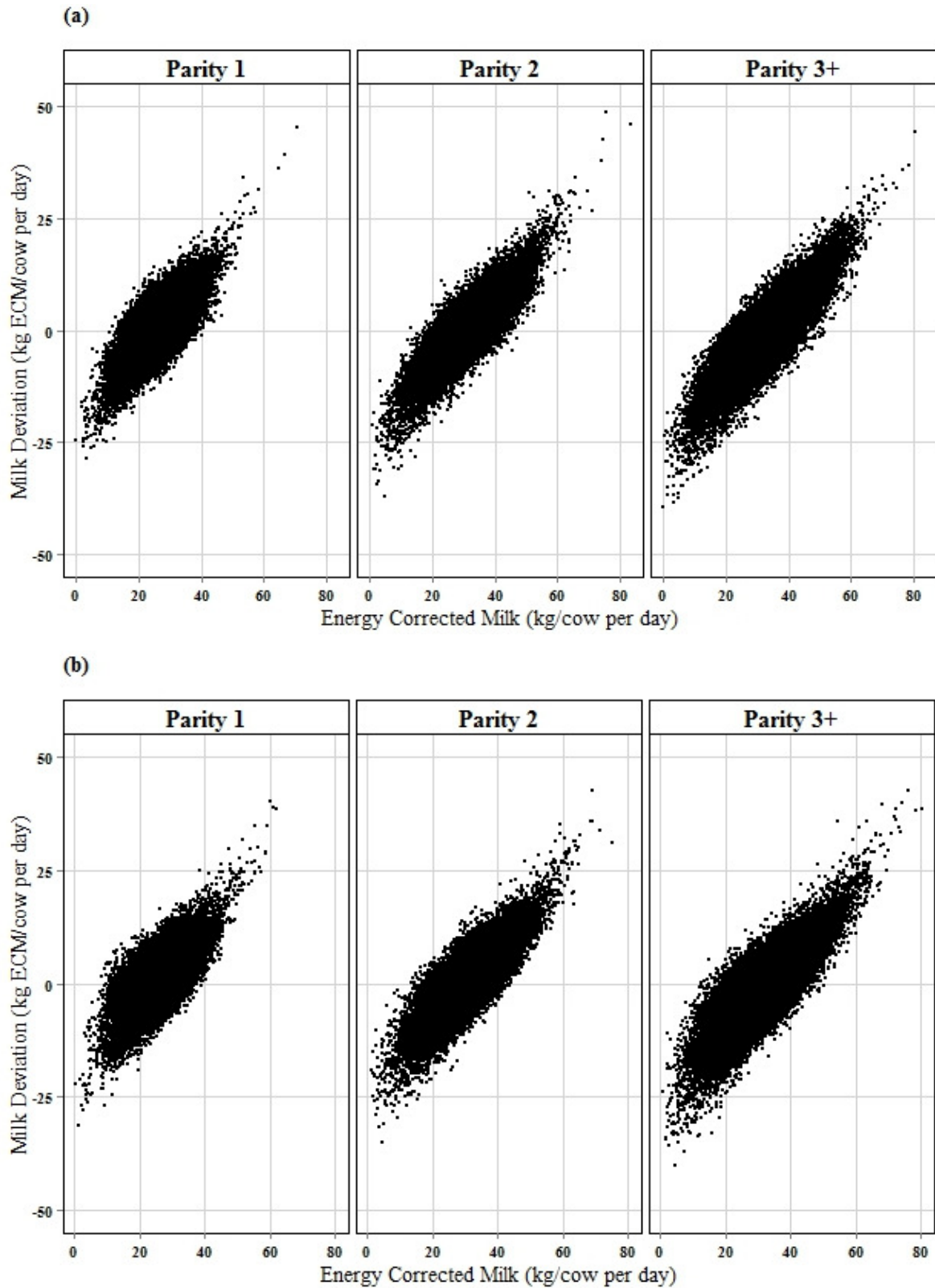
None of the removed non-significant predictors was confounders of the fixed effects in the model. The residuals of the model showed no signs of bias or heteroscedasticity (not shown).

The mean daily milk loss in the 3-month outbreak period in outbreak herds was significant, but small at 62 g of ECM/cow per day (95% CI: 39 to 86 g). The mean daily milk yield for a cow in an outbreak herd was 30.7 kg ECM, so a loss of 62 g ECM would be 0.2% of the mean daily milk yield. For comparison cows in the reference herds produced 68 g ECM/com per day (95% CI: 40 to 97 g) more in the outbreak period than during the reference periods. The effect of year on milk deviation was larger in the reference herds than in the outbreak herds.

All 120 outbreak herds were modelled in the final model. The model was also used on a reduced dataset containing only 79 outbreak herds. This was done to see if the model estimates changed after removing herds with outbreak duration extending in to the reference period the year after, and removing herds with an outbreak in 2014 lacking a reference period a year after. The model estimates did not change to any noteworthy extent, and all outbreak herds were therefore included in the final model.

**Table 5:** Linear mixed model for random and fixed effects on milk deviation for the 120 outbreak herds, who experienced an outbreak with *M. bovis*, and the 152 reference herds with no reported outbreak. Milk deviation is based on predicted Energy Corrected Milk (ECM) on each milk recording days for each cow.

	Outbreak herds		Reference herds			
	Variance	SD	Variance	SD		
<i>Random effects</i>						
Lactation within cow	1.379	1.174	1.906	1.380		
Cow	6.200	2.490	3.730	1.931		
Herd	2.281	1.510	3.104	1.762		
Residual	2.144	1.464	3.577	1.891		
	Estimate	SE	P-value	Estimate	SE	P-value
<i>Fixed effects</i>						
Intercept	-13.810	1.276	< 0.001	-13.240	1.135	< 0.001
Period						
Reference	0			0		
Outbreak	-0.062	0.012	< 0.001	0.068	0.015	< 0.001
Parity						
1	0			0		
2	-4.426	0.019	< 0.001	-4.309	0.025	< 0.001
3+	-6.600	0.025	< 0.001	-6.728	0.028	< 0.001
Season						
Spring	0			0		
Summer	0.389	0.018	< 0.001	0.566	0.020	< 0.001
Fall	0.667	0.023	< 0.001	1.061	0.022	< 0.001
Winter	-0.083	0.018	< 0.001	0.490	0.023	< 0.001
Year						
2009	0			0		
2010	0.859	0.085	< 0.001	0.716	0.821	0.383
2011	1.106	0.088	< 0.001	0.938	0.823	0.255
2012	1.406	0.091	< 0.001	1.344	0.823	0.103
2013	1.934	0.092	< 0.001	2.300	0.823	0.005
2014	2.541	0.094	< 0.001	3.300	0.823	< 0.001
Milk						
ECM (kg)	0.829	0.001	< 0.001	0.763	0.001	< 0.001
Delivered to dairy (per 100 kg)	-0.430	0.042	< 0.001	-0.385	0.035	< 0.001



**Figure 4:** Milk deviation against Energy Corrected Milk grouped by parity for every cow in the 120 outbreak herds (a), who experienced an outbreak with *M. bovis* and the 152 reference herds (b) with no reported outbreak.

## Discussion

This study investigated the milk yield losses associated with *M. bovis* outbreak in 120 Danish dairy herds. A daily milk yield loss of 62 g ECM/cow was found in a defined 3-month outbreak period in the outbreak herds. In a similar period, the reference herds produced 68 g ECM/cow per day more than in the reference period indicating that there is a small milk yield loss associated with the *M. bovis* outbreaks.

Both the outbreak and the reference herds delivered increasing amounts of milk per cow per year-quarter to the dairy over the years and especially in 2013 and 2014 the cows seemed to produce more milk.

The cows in the outbreak herds produced more milk than the cows in the reference herds on average, but the cows in the reference herds had on average a higher positive milk deviation than the cows in the outbreak herds. This indicates that they were able to produce more than their potential or more than expected whereas this was not the case in herds undergoing an outbreak.

### Milk deviation

No other studies on *M. bovis* have investigated milk yield in a way that is comparable to this study. Nielsen et al. (2012) though found that herds with signs of infection with *Salmonella* Dublin had a mean daily milk loss of 3 kg ECM/cow per test day for parity 3 and older cows 7 to 15 months after estimated herd infection. A similar study was performed on cows with positive ELISA tests for paratuberculosis demonstrating a decrease in test day milk yield up to 4 kg ECM compared to cows with repeated negative test results for paratuberculosis (Nielsen et al., 2009). Compared with the studies of Nielsen et al. (2009) and Nielsen et al. (2012) a decrease in milk yield of 62 g ECM/cow per test day during the outbreak period, appear negligible. The difference may be explained by the spread mechanism and pathogenesis of the diseases. *Salmonella* Dublin and paratuberculosis can affect the cow physiologically before or without any clinical signs become apparent, whereas *M. bovis* may not do this to the same extent.

Because the study relied on herd selection based on *M. bovis* test results on bulk tank milk samples, the within-herd prevalences were unknown, and it is therefore not possible to know which cows in the outbreak herds had clinical signs or were subclinical infected. Usually all lactating cows except

dry cows in a herd are in the milk recording scheme, but some sick cows could be missing if they are not milked in the milking parlour, leading to an underestimation of the milk losses. The fact that the average decrease in milk yield was small during the outbreaks could also be a result of high culling rates of the cows with *M. bovis* mastitis. In Denmark in 2014, 22.7% of culled cows were due to low milk production (Raundal and Nielsen, 2014) suggesting that Danish farmers are very focused on milk production. In one study, cows inoculated with *M. bovis* developed mastitis and started to decline in milk production just 5 days after inoculation, and milk production stopped 25 days after inoculation (Bennett and Jasper, 1980). Uhaa et al. (1990) found that cows with a positive antibody response for *M. bovis* on average produced 10% less than cows with a negative antibody response, but it was not known whether some of these cows had been clinically ill. However, no data on isolation or culling and the *M. bovis* status of individual cows were available in the present study, but it could explain, why the estimated difference in milk deviation is very small. This is consistent with the study of Thomas et al. (1982), who found no significant difference in milk yield between herds with an outbreak of *Mycoplasma* and control herds, but they found a 5% higher culling rate in herds with outbreak. Brown et al. (1990) likewise found a marked decrease in milk production of clinically affected cows leading to culling, but they found no significant difference in milk yield between the remaining non-culled *Mycoplasma*-test positive cows, tested by bacterial culture, and uninfected cows.

The fact that the cows in the reference herds had a higher positive milk deviation compared to the cows in the outbreak herds, although they on average produced less milk, could indicate that farmers from reference herds are better at exploiting the cows' potential. As seen in Figure 3, the overall potential for the cows in the reference herds was lower, which might be an intentional strategy to prevent disease in the cows and reduce costs in the production. The Y index was similar for the outbreak and the reference herds, ruling out breeding as the cause of this difference. Management including feeding strategies have a huge impact on milk yield and it is most likely the explanation for the difference.

Both the outbreak and the reference herds had a negative effect on milk deviation by parity, indicating that it is more difficult to get the older cows to produce according to their potential.

Bennedsgaard et al. (2003) showed a greater reduction in milk yield in older cows with mastitis, and it is likely that older cows have higher yield potential, and thereby are more affected by diseases



like mastitis. There is also the possibility that the milk prediction model did not predict the milk yield for older cows as well as for first parity cows.

As all cows except dry cows are part of the milk recording scheme, the very low minimum values for ECM most likely represented sick cows, whose milk production ceased or the milk production was recorded incorrectly, because the cow was not milked in the milking parlour. This would also explain the very low minimum values for milk deviation as these sick cows would be expected to produce more. However, the fact that ECM was included in the model as a predictor took that into account, as a cow with very low ECM would in many cases be a sick cow or a cow being dried off. This is also the most likely reason why the treatment variables could be removed from the model.

The maximum values of milk deviation could represent high-yielding cows with a good persistency of milk production throughout the lactation, which the ECM prediction model was unable to predict, thus getting a very high positive milk deviation.

### **Milk per cow delivered to dairy**

The increase in milk yield per cow found during the years has also been observed by the organisation performing the milk recording scheme (RYK). They reported that there has been an average increase of 525 kg milk/cow per year in the control year 2013/14 (RYK, 2015). RYK said it was the largest increase ever in one year.

The increase could be caused by the fact that in April 2015 the quota for the milk production will cease, and the farmers are therefore pushing the cows so they will produce more milk when the quota ends. Additionally, it could be speculated that this strategy to produce more milk per cow might be part of the reason for the increasing number of *M. bovis* outbreaks in Denmark since 2012 (Figure 1), as suggested by Figure 3, which shows a marked difference in milk delivered to the dairy per cow between outbreak and reference herds. Further investigation on this hypothesis is warranted.

Another reason for the increase in milk yield during the years could be the extra focus on health and biosecurity over the last couple of years, meaning that the farmers have been culling more unhealthy cows, and thus eliminated the low-producing cows. Because of the desire to get higher producing and healthier cows, a breeding progress might have occurred, which also could have affected

the increase in milk yield. Finally, feeding strategies and other optimisation procedures may have been used to gain more milk per cow.

Milk delivered to the dairy per cow was calculated as milk delivered in a quarter divided by the average number of cows recorded as present in the herd during that quarter. It did not adjust for dry or sick cows not producing milk to the tank, which leads to an underestimation of the true value.

Hence, I had to assume that herds had approximately the same proportion of dry cows and sick cows per year-quarter over time, herd size and type of herds.

### **Herd classification**

Both the outbreak and the reference herds were included in the study, based on positive results for *M. bovis* on either PCR or ELISA. This means the reference herds cannot be considered free of *M. bovis*, but the farmers reported that they had not experienced any clinical signs and therefore did not suspect to have *M. bovis* in their herd. Comparing the two groups then tells the difference in milk yield between herds experiencing an outbreak when exposed to *M. bovis*, and those who did not. The latter is not to be confused with a *M. bovis*-free herd. The reference herds then could have had infected cows that were just unnoticed or undiagnosed. Hence, misclassification of herds is a potential source of bias in this study. The reference herds were used because their data was already available, but if I had more time, it would probably be useful to use herds that were test negative for *M. bovis* for comparison.

### **Outbreak date and duration**

The farmers set the outbreak start date during the survey, which means the start date relied in the farmers' memory, which potentially led to recollection bias. The farmers may have experienced an outbreak differently according to the morbidity and mortality in their respective herd. The farmers who experienced the outbreak as a dramatic increase in morbidity, may remember the details about the outbreak more clearly and may be more confident of the outbreak start date. A small reliability evaluation was done by comparing the answers in this study to another ongoing study, where 11 farmers from this study were asked similar questions concerning the outbreak start date. This showed that 6 out of 11 farmers reported different start dates differing by months. If a study similar

to this was to be conducted, it would probably be better to look at the positive samples to check if the farmer reported date makes sense, or to use the positive samples to set the outbreak start date making it more objective and non-dependent on the farmer. This would require frequent repeated sampling of a large proportion of herds at risk of experiencing an outbreak, which in practice may be difficult and expensive to arrange.

The outbreak period was defined as a 3-month period regardless of the outbreak duration reported by the farmer. The true outbreak durations varied from 10 to 1096 days, but a 3-month period was used, as it was easier to compare with a similar period a year before and a year after. Of the 120 outbreak herds, 51 herds either have had an outbreak in 2014 or had an outbreak that lasted more than a year. If the outbreak was in 2014, there was no available data for a reference period in the year after. If the outbreak duration exceeded a year, the reference period the year after, would contain misleading data. I therefore applied the model only to the 79 herds, to investigate if it changed the estimates. The estimates was not changed which shows good robustness of the model, and all the 120 outbreak herds was used in the final model.

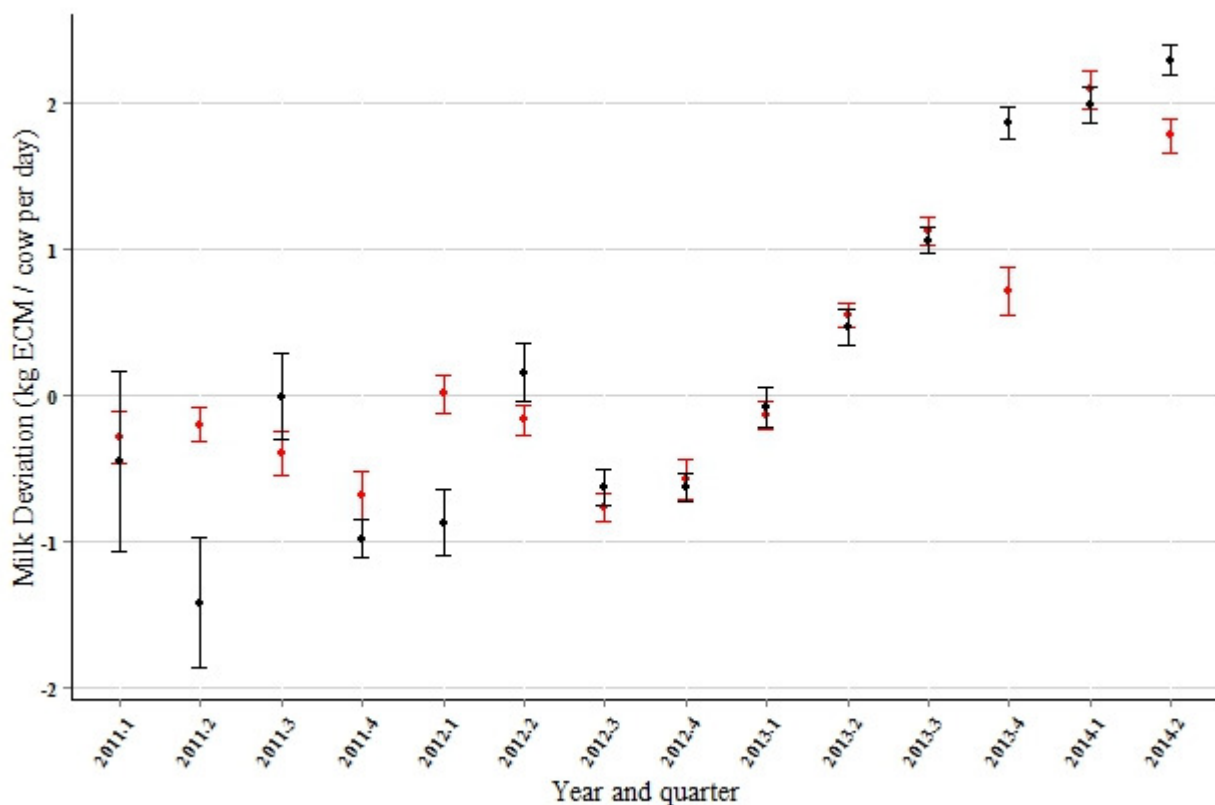
## **Evaluation of the model**

Estimating an effect of a disease on milk yield is difficult, as there are many factors affecting milk yield, thus milk recordings from different cows cannot be directly compared. Parity, breed and DIM are some of the factors strongly influencing milk yield, and the ECM prediction model was therefore made to get a comparable outcome. The ECM prediction model was based on a large amount of data over a period of 12 years, where the increasing milk yield tendency over time was taken into account, which makes it reliable. The 'Ali-B' model used in the ECM prediction model is one of many models that can be used to predict a lactation curve per cow based on few test dates per cow. The 'Ali-B' model was evaluated amongst others by Quinn et al. (2005) who found the 'Ali-B' model, the most consistent at predicting the individual daily milk yield.

Rajala-Schultz et al. (1999) compared the yields of cows with mastitis, with yields of healthy cows and found that on average the cows developing mastitis was higher yielding. They showed that mastitis significantly affected the milk yield but they found no significant difference between cows with mastitis and healthy cows, as mastitis simply lowered the milk yield to the same level as the healthy

cows. This indicates, that comparing with healthy cows might not be the best way to estimate the losses and that the losses might be underestimated. Therefore, I compared the herds to themselves in a reference period and thereby ruling out herd differences such as the overall milk production level.

The ECM prediction model adjusted for the effect of year, but plotting the milk deviation according to time showed a large increase in 2013 and 2014 (Figure 6), indicating that the model underestimated the effect of year. In the beginning of the study, I tried to compare the milk yield during an outbreak with at similar period a year before. As the ECM prediction model underestimated the effect of year, I change the model to include a reference period the year after the outbreak start date to try to adjust for the increase in milk yield.



**Figure 5:** Development of milk deviation per cow grouped by quarter of year between 2011 and 2014. The red bars illustrate the cows in the 120 herds, who experienced an outbreak and the black bars illustrate the cows in the 152 herds with no reported outbreak. All milk recordings from all cows are included, not only milk recordings in the outbreak or reference periods

## **Strength and weaknesses**

The strengths of the study is the large amount of data available over a long period of time, and that the herds were not selected based on herd type, breed or size. Of the outbreak herds, 9.2% had small breeds and in Denmark in 2013/14, 10% of the dairy herds were Jersey breed. This means the herds in the study resemble the Danish dairy herds with regard to breed distributions. The average herd size in the same years was 166 cows per herd. This is a lot smaller than the study herds, which is expected as reference herds with a herd size below 100 cows were excluded to make them more similar to outbreak herds. However, the herd size did not affect milk deviation when included in the model.

The study did not account for different management procedures and management is very important for milk yield, as especially feeding affects the milk yield a lot. I tried to adjust for unexplained between-herd variance by using herds as a random effect. I also adjusted for other diseases by using all available treatment records for the cows in the study, to rule out effects of other diseases. There is a risk of imperfect treatment registration though, and in addition, there is a large variation in treatment strategies amongst herds. Some farmers treat all cows with the slightest change in milk quality or SCC, while others wait to see if the infection clears itself.

## **Overall perspectives**

The characteristics of the herds in the study are a lot similar to other Danish dairy herds, which makes it well representing for the Danish dairy cattle population. It could also apply to other countries with similar intensive dairy production systems.

## **Conclusion**

A significant milk loss of 62 g ECM/cow per day, in a 3-month period following an outbreak with *M. bovis*, was estimated in the study regarding 120 Danish dairy herds experiencing an outbreak with *M. bovis* between 2010 and 2014.

A general trend was found in both the outbreak and the reference herds. They all delivered more milk per cow to the dairy between 2012 and 2014, indicating the cows are generally producing more milk than earlier years.

## References

- AlAbdullah, H.A. & Fadl, E.A. (2006): Mycoplasma mastitis in dairy cattle herds in Saudi Arabia. *Veterinary Record*. Vol. 159:3, pp. 88-89. 18 ref.
- Benedsgaard, T.W., Enevoldsen, C., Thamsborg, S.M. & Vaarst, M. (2003): Effect of mastitis treatment and somatic cell counts on milk yield in Danish organic dairy cows. *Journal of Dairy Science*. Vol. 86:10, pp. 3174-3183.
- Bennett, R.H. & Jasper, D.E. (1980): Bovine mycoplasmal mastitis from intramammary inoculations of small numbers of *Mycoplasma bovis*: local and systemic antibody response. *American Journal of Veterinary Research*. Vol. 41:6, pp. 889-892. 23 ref.
- Biddle, M.K., Fox, L.K. & Hancock, D.D. (2003): Patterns of mycoplasma shedding in the milk of dairy cows with intramammary mycoplasma infection. *Journal of the American Veterinary Medical Association*. Vol. 223:8, pp. 1163-1166. 20 ref.
- Brown, M.B., Shearer, J.K. & Elvinger, F. (1990): Mycoplasmal mastitis in a dairy herd. *Journal of the American Veterinary Medical Association*. Vol. 196:7, pp. 1097-1101. 16 ref.
- Gonzalez, R.N. & Wilson, D.J. (2003): Mycoplasmal mastitis in dairy herds. (Mastitis). *Veterinary Clinics of North America, Food Animal Practice*. Vol. 19:1, pp. 199-221. 109 ref.
- Haas, Y.d., Barkema, H.W. & Veerkamp, R.F. (2002): The effect of pathogen-specific clinical mastitis on the lactation curve for somatic cell count. *Journal of Dairy Science*. Vol. 85:5, pp. 1314-1323.
- Hertl, J.A., Schukken, Y.H., Welcome, F.L., Tauer, L.W. & Groehn, Y.T. (2014): Pathogen-specific effects on milk yield in repeated clinical mastitis episodes in Holstein dairy cows. *Journal of Dairy Science*. Vol. 97:3, pp. 1465-1480.
- Kusiluka, L.J.M., Ojeniyi, B. & Friis, N.F. (2000): Increasing prevalence of *Mycoplasma bovis* in Danish cattle. *Acta Veterinaria Scandinavica*. Vol. 41:2, pp. 139-146. 30 ref.
- Maunsell, F.P., Woolums, A.R., Francoz, D., Rosenbusch, R.F., Step, D.L., Wilson, D.J., *et al* (2011): *Mycoplasma bovis* infections in cattle. *Journal of Veterinary Internal Medicine*. Vol. 25:4, pp. 772-783. 89 ref.
- Microsoft Corporation (2013): Microsoft® Excel® 2013 (15.0.4675.1001) MSO (15.0.4675.1002) 32-bit. Microsoft® Office Proofing Tools .
- Nicholas, R.A.J. & Ayling, R.D. (2003): *Mycoplasma bovis*: disease, diagnosis, and control. *Research in Veterinary Science*. Vol. 74:2, pp. 105-112. many ref.
- Nielsen, P.K., Petersen, M.B., Nielsen, L.R., Halasa, T. & Toft, N. (2015): Latent class analysis of bulk tank milk PCR and ELISA testing for herd level diagnosis of *Mycoplasma bovis*.

- Nielsen, S.S., Krogh, M.A. & Enevoldsen, C. (2009): Time to the occurrence of a decline in milk production in cows with various paratuberculosis antibody profiles. *Journal of Dairy Science*. Vol. 92:1, pp. 149-155.
- Nielsen, T.D., Green, L.E., Kudahl, A.B., Ostergaard, S. & Nielsen, L.R. (2012): Evaluation of milk yield losses associated with Salmonella antibodies in bulk tank milk in bovine dairy herds. *Journal of Dairy Science*. Vol. 95:9, pp. 4873-4885.
- Pfutzner, H. & Sachse, K. (1996): Mycoplasma bovis as an agent of mastitis, pneumonia, arthritis and genital disorders in cattle. *Revue Scientifique Et Technique - Office International Des Epizooties*. Vol. 15:4, pp. 1477-1494. 64 ref.
- Quinn, N., Killen, L. & Buckley, F. (2005): Empirical algebraic modelling of lactation curves using Irish data. *Irish Journal of Agricultural and Food Research*. Vol. 44:1, pp. 1-13. 43 ref.
- R Core Team (2014): R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Radaelli, E., Castiglioni, V., Losa, M., Benedetti, V., Piccinini, R., Nicholas, R.A.J., *et al* (2011): Outbreak of bovine clinical mastitis caused by Mycoplasma bovis in a North Italian herd. *Research in Veterinary Science*. Vol. 91:2, pp. 251-253. 12 ref.
- RajalaSchultz, P.J., Grohn, Y.T., McCulloch, C.E. & Guard, C.L. (1999): Effects of clinical mastitis on milk yield in dairy cows. *Journal of Dairy Science*. Vol. 82:6, pp. 1213-1220. 21 ref.
- Raundal, P. & Nielsen, J. (2014): *Afgangsårsager for døde og for tidligt slagtede køer, 20-12-2014, SEGES P/S*, [cited 06-01-2015]. Available at the Internet. <<https://www.landbrug-sinfo.dk/Kvaeg/Tal-om-kvaeg/Sider/Afgaarsag201411.aspx>>.
- RYK (2015): *RYK - Ydelseskontrol*, SEGES P/S, [cited 06-01-2015]. Available at the Internet. <<https://www.landbrugsinfo.dk/kvaeg/ryk/sider/startside.aspx>>.
- Sjaunja, L.O., Baevre, L., Junkkarinen, L., Pedersen, J. & Setala, J. (1991): A Nordic proposal for an energy corrected milk (ECM) formula. *Performance Recording of Animals: State of the Art*. Vol. 1991, pp. 156-157, 192. 1 ref.
- Thomas, C.B., Jasper, D.E. & Willeberg, P. (1982): Clinical bovine mycoplasmal mastitis. An epidemiologic study of factors associated with problem herds. *Acta Veterinaria Scandinavica*. Vol. 23:1, pp. 53-64. 9 ref.
- Uhaa, I.J., Riemann, H.P., Thurmond, M.C. & Franti, C.E. (1990): A cross-sectional study of blue-tongue virus and Mycoplasma bovis infections in dairy cattle. I. The association between a positive antibody response and production efficiency. *Veterinary Research Communications*. Vol. 14:6, pp. 461-470. 15 ref.
- Wilson, D.J., Gonzalez, R.N. & Das, H.H. (1997): Bovine mastitis pathogens in New York and Pennsylvania: prevalence and effects on somatic cell count and milk production. *Journal of Dairy Science*. Vol. 80:10, pp. 2592-2598. 27 ref.

# Appendix

<b>ID</b>	<b>Code</b>	<b>Disease text</b>	<b>Category</b>
120001	1	Brunstmangel	Not relevant
120002	2	Børbetændelse	Calving problem
120003	3	Cyster	Reproduction disease
120004	4	Efterbyrd	Calving problem
120005	5	Forundersøgelse	Not relevant
120006	6	Blødning	Not relevant
120007	7	Brunst	Not relevant
120008	8	Brunstinduktion	Not relevant
120009	9	Reprolidelse, andet	Reproduction disease
120011	11	Yverbetændelse	Mastitis
120012	12	Yverbetændelse, goldperioden	Mastitis
120013	13	Goldningsbehandling	Dry-off treatment
120014	14	Yverbetændelse efter læsion	Mastitis
120015	15	Yverbetændelse, akut	Mastitis
120016	16	Pattetråd	Mastitis
120017	17	Patteopstikning	Mastitis
120018	18	Patteamputation	Mastitis
120019	19	Yverlidelse, andet	Mastitis
120020	20	Løbeudvidelse	Digestion or metabolic disease
120021	21	Ketose	Digestion or metabolic disease
120022	22	Kælvningsfeber	Calving problem
120023	23	Løbedrejning	Digestion or metabolic disease
120024	24	Fordøjelsesforstyrrelse	Digestion or metabolic disease
120025	25	Fremmedlegeme	Digestion or metabolic disease
120026	26	Sur vom (vomacidose)	Digestion or metabolic disease
120027	27	Løbekatarrh/forgiftning	Digestion or metabolic disease
120028	28	Tarmbetændelse	Digestion or metabolic disease
120029	29	Ford./stofskiftelidelse, andet	Digestion or metabolic disease
120030	30	Græsforgiftning	Digestion or metabolic disease
120031	31	Trykning	Hoof or limb lesion
120032	32	Klovbrandbyld	Hoof or limb lesion
120033	33	Sålesår	Hoof or limb lesion
120034	34	Balleforrådnelse	Hoof or limb lesion
120035	35	Hudbetændelse	Hoof or limb lesion
120036	36	Såleblødning	Hoof or limb lesion
120037	37	Tyk has	Hoof or limb lesion
120038	38	Ledbetændelse	Hoof or limb lesion
120039	39	Lemmelidelse, andet	Hoof or limb lesion



<b>ID</b>	<b>Code</b>	<b>Disease text</b>	<b>Category</b>
120040	40	Halelæsion	Not relevant
120041	41	Lungebetændelse	Not relevant
120042	42	Infektion	Not relevant
120044	44	Tånekrose	Hoof or limb lesion
120045	45	Magnet ilagt	Not relevant
120046	46	Børstave ilagt	Calving problem
120047	47	Abort	Reproduction disease
120048	48	Nydannelse	Hoof or limb lesion
120049	49	Lidelse andet	Not relevant
120051	51	Diarre	Digestion or metabolic disease
120052	52	Coccidiose	Digestion or metabolic disease
120053	53	Navlebetændelse	Not relevant
120054	54	Leverikte	Digestion or metabolic disease
120055	55	Lungeorm	Not relevant
120056	56	Løbetarmorm	Digestion or metabolic disease
120057	57	Ormebehandling	Not relevant
120058	58	Skab	Not relevant
120059	59	Kalve under 6 mdr. andet	Not relevant
120065	65	Cyster, hormonbeh.	Not relevant
120066	66	Skedebetændelse	Reproduction disease
120067	67	Fødselsinduktion	Not relevant
120068	68	Inaktive æggestokke	Not relevant
120069	69	Forbrunst	Not relevant
120070	70	Efterbrunst	Not relevant
120072	72	Fluemastitis	Mastitis
120079	79	Kvie over 6 mdr. andet	Not relevant
120080	80	Klovbeskæring	Not relevant
120081	81	Klovbeskæring ++	Not relevant
120082	82	Klovbeskæring +	Not relevant
120083	83	Klovbeskæring -	Not relevant
120084	84	Klovbeskæring --	Not relevant
120085	85	God drikkelyst	Not relevant
120086	86	Godt middel drikkelyst	Not relevant
120087	87	Knap middel drikkelyst	Not relevant
120088	88	Manglende drikkelyst	Not relevant
120089	89	Tyr over 6 mdr. andet	Not relevant
120090	90	Børkrængning	Reproduction disease
120091	91	Børslyngning	Reproduction disease
120092	92	Kejsersnit	Calving problem
120093	93	Pattehudsbetændelse	Mastitis
120094	94	Yverbetændelse, brandig	Mastitis
120095	95	Yverbetændelse, subklinisk	Mastitis

<b>ID</b>	<b>Code</b>	<b>Disease text</b>	<b>Category</b>
120096	96	Løbedrejning, højresidig	Digestion or metabolic disease
120097	97	Løbedrejning, venstresidig	Digestion or metabolic disease
120098	98	Trommesyge	Digestion or metabolic disease
120099	99	Projekt	Not relevant
120110	110	Drægtig	Not relevant
120112	112	Fødselshjælp	Calving problem
120113	113	Ikke drægtig	Not relevant
120120	120	Endetarmskrængning	Not relevant
120121	121	Drægtighedssyge	Reproduction disease
120122	122	Manglende opblokning	Calving problem
120123	123	Kobberforgiftning	Not relevant
120125	125	Kobbermangel	Not relevant
120127	127	Ondartet klovsyge	Hoof or limb lesion
120128	128	Bændelorm	Digestion or metabolic disease
120129	129	Fluelarver (maddiker)	Not relevant
120130	130	Mundskurv (ORF)	Not relevant
120131	131	Øjenbetændelse	Not relevant
120132	132	Øjenlåg indadvendt	Not relevant
120133	133	Mellemørebetændelse	Not relevant
120135	135	Asymmetrisk klov	Hoof or limb lesion
120136	136	Proptrækkerklov	Hoof or limb lesion
120137	137	Overgroet klov	Hoof or limb lesion
120138	138	Sakseklov	Hoof or limb lesion
120139	139	Dobbeltsål	Hoof or limb lesion
120140	140	Klovbeskæring ++	Not relevant
120141	141	Klovbeskæring +	Not relevant
120142	142	Klovbeskæring -	Not relevant
120143	143	Klovbeskæring --	Not relevant
120144	144	Hul væg, løs hvid linje	Hoof or limb lesion
120145	145	Hul væg, byld i hvid linje	Hoof or limb lesion
120146	146	Balleforrådnelse, svær	Hoof or limb lesion
120147	147	Klovspalte, betændelse	Hoof or limb lesion
120148	148	Klovspalte, nydan, side	Hoof or limb lesion
120149	149	Klovspalte, nydan, midt	Hoof or limb lesion
120150	150	Såleknusning, let	Hoof or limb lesion
120151	151	Såleknusning, forbind	Hoof or limb lesion
120152	152	Såleknusning, dobb.sål	Hoof or limb lesion
120153	153	Laminitis, rød sål	Hoof or limb lesion
120154	154	Laminitis, R/H linie	Hoof or limb lesion
120155	155	Laminitis, Br/H linie	Hoof or limb lesion
120156	156	Digital dermatitis	Hoof or limb lesion
120157	157	Digital vorte	Hoof or limb lesion

<b>ID</b>	<b>Code</b>	<b>Disease text</b>	<b>Category</b>
120158	158	Snabelklov	Hoof or limb lesion
120159	159	Halthedsscore	Not relevant
120160	160	Kastration/studning	Not relevant
120161	161	Urinvejsinfektion	Not relevant
120162	162	Nyrebækkenbetændelse	Not relevant
120163	163	Blærebetændelse	Not relevant
120164	164	Løbesår/mavesår	Digestion or metabolic disease
120165	165	Fedtlever	Digestion or metabolic disease
120166	166	Cryptosporidiose	Digestion or metabolic disease
120167	167	Aktinomykose	Not relevant
120168	168	Vinterostertagiose	Not relevant
120169	169	Salmonella (dublin)	Not relevant
120170	170	Paratuberkulose	Not relevant
120171	171	Vomforrådnelse (vomalkalose)	Digestion or metabolic disease
120172	172	Tarmslyng	Digestion or metabolic disease
120173	173	Blindtarmsdilatation	Digestion or metabolic disease
120174	174	Cerebrokortikal nekrose	Not relevant
120175	175	Bughindebetændelse, peritonitis	Digestion or metabolic disease
120176	176	Kalvedifteritis	Not relevant
120177	177	Aflivning, kreatur	Not relevant
120178	178	Ringorm	Not relevant
120179	179	Yverbetændelse med lammelse	Mastitis
120180	180	Operation, anvendt medicin	Not relevant
120181	181	Operation, efterbehandling	Not relevant
120182	182	Dødsattest	Not relevant
120183	183	Mæidiprøver	Not relevant
120184	184	Selen og vitaminbehandling	Digestion or metabolic disease
120185	185	vitamin- og jernbehandling	Digestion or metabolic disease
120186	186	Tyrering isat	Not relevant
120187	187	Væskemangel/væsketerapi	Not relevant
120188	188	Mælkeprøve udtaget	Not relevant
120189	189	BVD-prøve udtaget	Not relevant
120190	190	Blodprøve udtaget	Not relevant
120199	199	Manglende mælkenedlægning	Reproduction disease
120200	200		Not relevant
120201	201	Hæmolyserende streptokokker, gr. A. streptokokker	Mastitis
120202	202	Streptokokagalactia - gr. B. streptokokker	Mastitis
120203	203	Coliforme stave	Mastitis
120204	204	Streptokokdysgalactia	Mastitis
120205	205	E-coli	Mastitis
120206	206	Gær	Mastitis

<b>ID</b>	<b>Code</b>	<b>Disease text</b>	<b>Category</b>
120207	207	Hæmolyserende streptokokker	Mastitis
120208	208	Listeria monocytogenes	Mastitis
120209	209	CNS	Mastitis
120210	210	Koagulasenegative stafylokokker, pen.	Mastitis
120211	211	Lactococcus spp	Mastitis
120212	212	Enterococcus spp	Mastitis
120213	213	Arcanobacterium pyogenes	Mastitis
120214	214	Corynebacterium bovis	Mastitis
120215	215	Staph. Aureus	Mastitis
120216	216	S. aureus, pen. res.	Mastitis
120217	217	Strept. Uberis	Mastitis
120218	218	Str. uberis, mucoide variant	Mastitis
120219	219	Mælkeprøve, anden bakterie	Mastitis
120220	220	Ingen vækst	Not relevant
120221	221	Klebsiella	Mastitis
120222	222	Micrococcus spp	Mastitis
120223	223	Bacillus spp	Mastitis
120224	224	Proteus	Mastitis
120225	225	Mycoplasma bovis	Mastitis
120250	250	Vaccine 1	Not relevant
120251	251	Vaccine 2	Not relevant
120260	260	Sera	Not relevant
120261	261	Råmælksmåling	Not relevant
120262	262	Overførsel af råmælksantistoffer	Not relevant
120263	263	Tyrekvie	Not relevant
120270	270	Optrapning/steaming up	Not relevant
120271	271	Abortinduktion	Not relevant
120272	272	Atypisk mælkefeber	Not relevant
120273	273	Brud på knogle	Not relevant
120274	274	Sterilisation	Not relevant
120275	275	Lus	Not relevant
120280	280	Fluekontrol	Not relevant
120300	300	Rådgivningsbesøg	Not relevant
120310	310	Afhorning af kalve	Not relevant
120320	320	Intern pattelukning	Not relevant
120330	330	Bluetongue, vaccineret mod	Not relevant
120340	340	Mastitis Staf/E.coli, vaccination	Not relevant
120801	801	IBR negativ blodprøve	Not relevant
120802	802	Leukose negativ blodprøve	Not relevant
120803	803	Brucellose negativ blodprøve	Not relevant
120804	804	Tb prøve negativ hudtest	Not relevant
120805	805	BVD virus negativ blodprøve	Not relevant

<b>ID</b>	<b>Code</b>	<b>Disease text</b>	<b>Category</b>
120806	806	BVD antistov negativ blodprøve	Not relevant
120807	807	BVD antistof positiv blodprøve	Not relevant
120809	809	Camfylobacter negativ forhudsskylleprøve	Not relevant
120810	810	Camfylobacter negativ skedeskylleprøve	Not relevant
120811	811	Para Tb negativ blodprøve	Not relevant
120812	812	Leptospirose negativ blodprøve	Not relevant
120813	813	Leptospirose - Interrogans, castellonis, pyrogenes, tarassovi og wolffi	Not relevant
120814	814	Ringvac.	Not relevant
120815	815	BVD virus neg. sædstrå	Not relevant
120816	816	IBR negativ sædstrå	Not relevant
120817	817	Para Tb negativ sædstrå	Not relevant
120819	819	Para Tb positiv blodprøve	Not relevant
120820	820	Chlamydia negativ blodprøve	Not relevant
120821	821	IBR,Leukose, Brucellose, BVD virus og antistof: Neg.blodprøve. Tb: neg.hudtest	Not relevant
120822	822	IBR,Brucellose,BVD Virus og antistof:Neg.blodprøve. Camfylobacter og Tricomonas:neg.forhudsskyllepr.	Not relevant
120823	823	Camfylobacter neg skedeskylleprøve og tricomonas negativ skedeskylleprøve	Not relevant
120824	824	Tricomonas negativ skedeskylleprøve	Not relevant
120825	825	Rotavac, ringvac. og tb. neg. hudtest	Not relevant
120831	831	Q fever- negativ blodprøve	Not relevant
120832	832	Brucella agg + CF, negativ blodprøve	Not relevant
120833	833	Leptospirose- hardjo, hebdomis, grippo og ictero	Not relevant
120834	834	Leptospirose- can, grippo, hardjo, ictero og Pomona	Not relevant
120835	835	Bluetongue antistof negativ	Not relevant
120836	836	Bluetonguevirusnegativ	Not relevant
120840	840	Schmallenberg ELISA negativ	Not relevant
120841	841	Schmallenberg SN negativ	Not relevant
120842	842	Schmallenberg PCR blod negativ	Not relevant
120843	843	Schmallenberg PCR sæd negativ	Not relevant
120844	844	Schmallenberg positiv	Not relevant
120850	850	Serum prøve udtaget	Not relevant
120856	856	Prøve udtaget BVD antistof negativ blodprøve	Not relevant
120861	861	Prøve udtaget Para Tb negativ blodprøve	Not relevant
120872	872	Prøve udtaget IBR,Brucellose,BVD Virus, antist.:Neg.blodpr. Camfylob. og Tricom.:neg.forh.skyllepr.	Not relevant

<b>ID</b>	<b>Code</b>	<b>Disease text</b>	<b>Category</b>
120873	873	Prøve udtaget Camfylobacter neg skede- skylleprøve og tricomonas negativ skede- skylleprøve	Not relevant
125831	881	Prøve udtaget Q fever- negativ blodprøve	Not relevant
120883	883	Prøve udtaget Leptospirose- hardjo, hebdo- mis, grippo og ictero	Not relevant
120884	884	Prøve udtaget Leptospirose- can, grippo, hardjo, ictero og Pomona	Not relevant
120885	885	Prøve udtaget Bluetongue antistof negativ	Not relevant
120890	890	Rotavec-Corona Vac.	Not relevant
120891	891	Bovipast RSP, Intervet	Not relevant