Bio-economic modeling of bovine intramammary infections

Bio-economisch modellering van intramammaire infecties in runderen

(*met een samenvatting in het Nederlands*)

Proefschrift

ter verkrijging van de graad van doctor

aan de Universiteit Utrecht

op gezeg van de rector magnificus,

prof. dr. J. C. Stoof,

ingevolge het besluit van het college voor promoties

in het openbaar te verdedigen

op maandag 22 juni 2009 des ochtends te 10.30 uur

Door

Tariq Halasa

geboren op 15 april 1979

te Karak, Jordan

Promotor:	**Prof. dr. J. A. Stegeman**
Co-promotoren:	**Dr. ir. H. Hogeveen**
	Dr. M. Nielen
	Dr. T. van Werven

Halasa, T. 2009. Bio-economic modeling of bovine intramammary infections.

PhD Thesis, Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands
(with summary in English and Dutch)

ISBN: 978-90-393-5050-8

The printing of this thesis was sponsored by:

- Department of Farm Animal Health, Utrecht University.
- Business Economics Group, Wageningen University.
- Intervet/Schering-Plough Animal Health.
- The Dutch Udder Health Center (UGCN).

U G C N

uiergezondheidscentrum nederland

Contents

Chapter 1

General Introduction

Dairy production is of great economic interest for the EU economy, with a considerable number of people being employed in the dairy industry. In the year 2006 the EU harbored approximately 26 million dairy cows contributing to approximately 25 % of the world exports of milk products (ARD-EC, 2007). Moreover, dairy production is a significant part of human culture and tradition (Lund et al. 2006). Mastitis frequently impairs the welfare of a cow due to pain (Fregonesi et al. 2001). Moreover, mastitis is one of the most important factors negatively affecting the longevity of dairy cows (Seegers et al. 2003). The quality of milk is negatively affected as a result of mastitis, leading to bad milk processing abilities (Hogeveen and Østerås, 2005). Mastitis also has a negative effect on the image of dairy production chain; healthy milk from healthy cows (Hogeveen and Østerås, 2005). Although these factors have an economic impact on dairy production, more losses may occur on the dairy producer level, due to culling of the infected cow, veterinary costs, extra-labor costs, disposed milk, reduced slaughter value, and reduced reproductive performance (Hogeveen and Østerås, 2005). Literature estimates of the cost of mastitis vary largely (Seegers et al. 2003), mainly because mastitis associated-cost factors differed between studies. Clearly, a framework of cost factors of mastitis to be considered for economic calculations does not exist, making it hard to provide a comprehensive review of published studies calculating the cost of mastitis and the benefits of improved management in bovine dairy herds. An agreed upon framework would make it easier to compare different studies, and could help decision making in relation to mastitis.

The high social and economic importance of mastitis has directed a large research effort in the last decades. The complex biological transmission processes underlying mastitis were also studied. There are two main transmission routes of mastitis pathogens, the cow-to-cow transmission, which is frequently referred to as contagious transmission, and the environmental transmission route (Zadoks et al., 2002). In the contagious transmission, a transmission rate parameter is calculated reflecting the potential number of new infections caused by an infectious animal per unit of time (Backer et al., 1989). Studies by Lam et al. (1996) and Zadoks et al., (2001 and 2002) provided estimates of transmission rates of important intramammary infection (**IMI**) causative agents. For the environmental route of infection, the prevalence or the cumulative incidence of infection are usually used to reflect the number of new infections. Existing simulation models do not include transmission of IMI between cows. Moreover, because IMI is caused by several pathogens, co-existence and competition between pathogens should be modeled, as well as the different routes of infection.

Two important states of IMI should also be modeled, clinical and subclinical IMI. Clinical IMI is an obvious cause of economic damage to dairy farms (Seegers et al., 2003) and its negative effects could last to the end of the lactation of a cow (Grohn et al., 2004). On the other hand, subclinical IMI is an obscure cause of loss to dairy farmers (Seegers et al., 2003). It is associated with elevated somatic cell count (**SCC**), where SCC level is often used as indication of the severity of the subclinical case (Seegers et al., 2003). Despite the frequent use of SCC to represent subclinical cases, no consensus has yet been reached to define

subclinical cases based on SCC elevation, which makes it difficult to assess associated losses and costs.

Hence, a model of mastitis that includes the above mentioned IMI attributes may be important to support decision making in relation to IMI control and prevention. A dynamic approach that can consider the complex nature of IMI with the high associated uncertainty should be used (Allore et al. 1999). Discrete-event models may offer the best approach to reflect reality, study the dynamics of living resources and perform economic calculations (Allore et al. 1999; Law, 2007). Moreover, such models make it possible to combine different scientific disciplines (biology, economics, and mathematics) in what would be called bio-economic modeling. The resulting bio-economic model is expected to improve cost assessment of IMI as an important prerequisite to economic assessment of IMI control strategies. In addition, the model may be a useful tool to estimate economic effects of IMI control to support decision making in bovine dairy herds.

The dry period (**DP**) is an important stage during the production cycle of the dairy cow. It was shown that the DP is a high risk period for occurrence of new IMI (Hassan et al., 1999; Bradley and Green, 2004; Green et al, 2005). Several studies reported the relation between dry cow interventions and IMI (Hassan et al., 1999; Green et al, 2005; Green et al., 2007). Few studies reported an economic analysis on interventions to control IMI during the DP (Oliver et al., 2003; Berry et al., 2004; Huijps and Hogeveen, 2007). A variation between the estimated economic outcomes was observed. Moreover, economic impact of some important interventions was not measured. More important, the effect of IMI transmission during lactation was not taken into account in previously published estimates. Thus economic assessment of interventions to control IMI during the DP could be a useful practical application of the proposed bio-economic model and could help farm decisions to achieve beneficial application of DP interventions. To reach this objective, careful assessment of the efficacy of IMI interventions during the DP is necessary. Due to the large number of studies that quantified the preventive and curative efficacy of IMI interventions during the DP, meta-analysis would facilitate comprehensive and precise quantification of the efficacy of IMI interventions during the DP.

The main objective of this thesis was to develop a bio-economic model of IMI caused by *S. aureus*, *S. uberis*, *S. dysgalactiae*, and *E. coli* based on recent knowledge of pathogen-specific transmission dynamics, and to provide a useful application of the model. To reach this objective, several studies were conducted with the following goals:

1- Provide an economic framework of the factors associated with mastitis and mastitis management. Moreover, provide a comprehensive review of the cost and cost-benefits of mastitis management published since 1990.

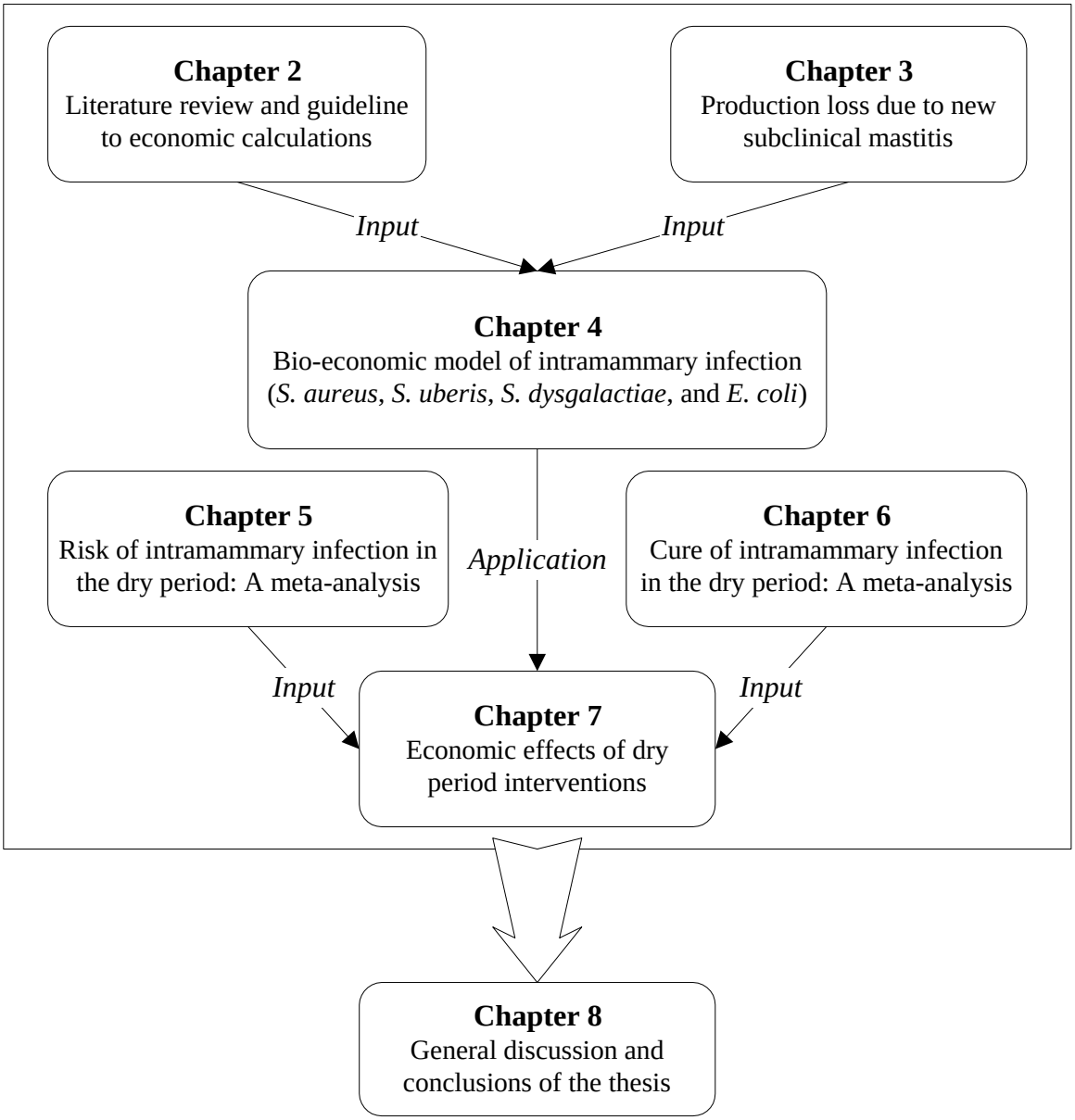
2- Estimate the milk production loss due to new subclinical mastitis in Dutch dairy herds.

3- Develop and describe a bio-economic model of intramammary infections (IMI) that includes the dynamics of IMI between cows to calculate the cost of pathogen-specific IMI in bovine dairy herds.

4- Estimate the protective effect of different applied interventions against new IMI during the DP and early lactation using meta-analysis quantification of existing scientific literature.

5- Estimate the cure rate of IMI during the DP and early lactation after dry cow therapy using meta-analysis quantification of existing scientific literature.

6- Estimate the economic effects of different IMI interventions during the DP.



The framework of this thesis is represented by the above flow chart. The thesis will provide (in chapter 2) a comprehensive review of published literature in relation to the cost of mastitis and the economic effects of mastitis management. Moreover, an explicit discussion of mastitis associated cost factors, that should be considered when attempts are made to conduct economic calculations in relation to mastitis, is presented. Definition of new subclinical mastitis and quantification of milk, fat, and protein losses due to new subclinical mastitis are presented in chapter 3 based on an analysis of a large Dutch data set. Chapter 4 presents the

development of a bio-economic model of IMI to calculate the economic damage due to pathogen-specific IMI in bovine dairy herds. In chapters 5 and 6, meta-analyses on 1) the risk of new IMI during the DP using different interventions, and 2) the cure of existing IMI using dry cow therapy, based on peer-reviewed literature are presented. Chapter 7 provides cost-benefit analyses of IMI interventions during the DP as an application of the bio-economic model. Finally in chapter 8 concerns that have not been discussed throughout the earlier chapters are provided. This chapter is finished with conclusions based on the work presented in this thesis.

References:

Agricultural in the European Union, statistical and economic information. 2008. In, agricultural and rural development, the European Commission. www.ec.europa.eu/agriculture/agrista/2007/table_eng/index.htm.

Allore, H.G., Erb, H.N. 1999. Approaches to modeling intramammary gland infections in cattle. Prev. Vet. Med. 39:279-293.

Backer, N.G., 1989. Analysis of infectious disease data, Chapman and Hall, London, UK.

Berry, E. A., H. Hogeveen, and J. E. Hillerton. 2004. Decision tree analysis to evaluate dry cow strategies under UK conditions. J. Dairy Res. 71:409-418.Broom, D.M. 1991. Animal welfare: Concepts and measurement. J Anim Sci 16:4167-4175.

Bradley, A. J., and M. J. Green. 2004. The importance of the non-lactating period in the epidemiology on intramammary infection and strategies to prevention. Vet. Clin. Food Anim. 20:547–568.

Fregonesi, J.A., Leaver J.D. 2001. Behavior, performance and health indicators of welfare for dairy cows housed in straw yard or cubicle systems. Livest Prod Sci 68:205–216.

Green, M.J., Green, L.E., Bradley, A.J., Burton, P.R., Schukken, Y.H., Medley, G.F. 2005. Prevalence and associations between bacterial isolates from dry mammary glands of dairy cows. Vet Rec. 156:71-77.

Green, M. J., A. J. Bradley, G. F. Medley, and W. J. Browne. 2007. Cow, farm, and management factors during the dry period that determine the rate of clinical mastitis after calving. J. Dairy Sci. 90:3764–3776.

Grohn, Y.T., Wilson, D.J., Gonzalez, R.N., Hertl, J., Schulte, H., Bennett, G., Schukken, Y.H. 2004. Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. J. Dairy Sci. 87:3358–3374.

Hassan, Z., Daniel R.C.W., O'Boyle D., Frost A.J. 1999. Effects of dry cow intramammary therapy on quarter infections in the dry period. Vet. Rec. 635-639.

Hogeveen, H., and Østerås O. 2005. Mastitis management in economic framework. Page 41, in Proc. 4th International Mastitis Seminar, IDF, Mastitis in Dairy Production, Current Knowledge and Future Solutions. Wageningen Academic Publisher, Wageningen, The Netherlands.

Huijps, K., Hogeveen H. 2007. Economic evaluation of dry cow therapy. J. Dairy Sci. 90:1225-1234.

Kossaibati, M.A., Esslemont, R.J. 1997. The costs of production diseases in dairy herds in England. Vet. J. 154:41-51.

Lam, T.J.G.M. 1996. Dynamics of bovine mastitis: A field study in low somatic cell count herds. Ph.D. Dissertation, Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

Law, A.M., 2007. Simulation modeling and analysis, McGraw-Hill international, New York, USA.

Lund, V., Coleman, G., Gunnarsson, S., Appleby, M.C., karkinen, K. 2006. Animal welfare science-working at the interface between the natural and social sciences. Appl Anim Beh Sci 97:37-49.

Oliver, S. P., M. J. Lewis, B. E. Gillespie, H. H. Dowlen, E. C. Jaenicke, and R. K. Roberts. 2003. Prepartum antibiotic treatment of heifers: Milk production, milk quality and economic benefit. J. Dairy Sci. 86:1187-1193.

Seegers, H., Fourichon, C., Beaudeau, F. 2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet Res 24:475-491.

Zadoks, R.N., Allore, H.G., Barkema, H.W., Sampimon, O.C., Grohn, Y.T., Schukken, Y.H., 2001. Analysis of an outbreak of *Streptococcus uberis* mastitis. J. Dairy Sci. 84, 590–599.

Zadoks, R.N., Allore, H.G., Hagenaars, T.J., Barkema, H.W., Schukken, Y.H. 2002. A mathematical model of *Staphylococcus aureus* control in dairy herds. Epidemiol. Infect. 129:397-416.

Chapter 2

Economic effects of bovine mastitis and mastitis management: A review

T. Halasa[1,2], K. Huijps[1], O. Østerås[3] and H. Hogeveen[1,2]

[1] Department of Farm Animal Health and Reproduction, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.
[2] Business Economics Group, Wageningen University, Wageningen, The Netherlands.
[3] Department of Production Animal Clinical Sciences, Norwegian School of Veterinary Science, Oslo, Norway.

Published in Veterinary Quarterly 29 (2007) 18-31

Summary

Several studies have been published since 1990 on the economics of mastitis and mastitis management. However, hardly any of these studies has discussed the consistency of results with other studies. In the present paper, the economic factors associated with mastitis are explained, providing a framework for economic analysis. As a second step calculations of the costs of mastitis and the costs in relation to the benefits of mastitis management published since 1990 in peer-reviewed journals are extensively reviewed and analysed. The result shows a large variation in the calculated costs and benefits of mastitis and mastitis management between the different studies. Moreover, it is clear that important factors were ignored in some of the studies. The framework provided in this paper can provide a basis for analysis for future studies on the economics of mastitis and mastitis management.
Key words: Economics, Mastitis, Dairy cattle, Management.

INTRODUCTION

Mastitis is an endemic disease that is considered to be one of the most frequent and costly diseases in the dairy industry. Moreover, mastitis affects milk quality directly in the technical characteristics and the hygienic quality of the milk, and indirectly through the intrinsic milk quality (17). Management is considered to be one of the most effective means to control mastitis (42). Given the complex multi-factorial nature of mastitis, management consists of a wide range of activities, amongst others the treatment of the disease (clinical or subclinical form), dry cow therapy, prevention of transmission of infection (from cow to cow or through the environment) and improvement of the immune system. Mastitis management literature is quite abundant but less research has been published regarding the economics of mastitis and mastitis management (52).

Economic calculations vary between countries and even between regions within a country. Moreover, the results of these calculations change with time owing to changes in milk quality regulations (1) and changes in market circumstances (35) and, to complicate matters further, large variations between studies and several discrepancies between the estimated losses have been reported, albeit briefly (42). These variations and discrepancies are not only due to differences in methodology and differences between regions but also to the different levels of population used in the analyses. The consequence is that these variations, discrepancies and changes make it hard to reach a clear general conclusion about the economics of mastitis and mastitis management from the research that has been published. Nevertheless, a good review can provide an overall indication of the economic consequences and reveal the consistency of the different results. The last review on this topic, however, was published more than 15 years ago, covering the published literature up to 1990 (40). The objective of the present paper is to provide an extensive review of the calculations of the costs of mastitis and the benefits of mastitis management published since 1990. Moreover, an economic framework including the economic factors associated with mastitis and mastitis management is given, providing an explanation of these factors.

1. Consequences of mastitis: An economic framework

The economic consequences of mastitis (clinical or subclinical) are due to treatment, production losses, culling, changes in product quality and the risk of other diseases. The associated costs can be divided among the following factors:

- Milk production losses
- Drugs
- Discarded milk
- Veterinary services
- Labour
- Product quality
- Materials and investments
- Diagnostics
- Culling
- Other diseases.

Although the relative cost of these factors might differ between countries and between regions, the economic principles behind them are the same and will be explained below.

1.1 Milk production losses

In both clinical and subclinical mastitis there is a substantial loss in milk production. Production losses due to clinical mastitis have been estimated (13, 18, 19). Production losses due to subclinical mastitis are generally considered to be a direct log-linear relationship between somatic cell count (SCC) and test-day records (3, 33). However, St. Rose et al. (46) found that milk production does not improve after complete recovery of subclinical mastitis. Thus the assumed log-linear relationship might underestimate production losses due to subclinical mastitis.

The economic damage of lower milk production per cow depends on the structure of the farming business. Milk payment systems may differ (payment based on kilograms of milk or kilograms of milk components such as fat and protein). Moreover, the calculations of the economic damage due to decreased milk production differ between a quota system (as applied in the EU countries, Norway and Canada) and a non-quota system. In a dairy system where farmers do not face a milk quota, the production potential of a farm is the number of dairy cows present on that farm. The number of dairy cows might be limited by size of barn, available labour, available feed or available capital, but the milk that cows produce that can be delivered to the factory will be paid for at the market milk price. When milk production per cow is decreased by mastitis, less milk will be delivered to the factory and the net return of the farm will decrease. There might be some savings, because when cows are fed in relation to milk production the farmer might save on feed (concentrates) which will result in decreased costs (9).

In a quota system, calculation of economic damage for a decrease in milk production becomes much more complicated. The production potential of a farm, in most situations, is the quota and not the number of animals. Therefore, the returns of milk sales are

predetermined and the goal of the farmer is to produce milk within the quota as efficiently as possible. With decreased milk production a farmer has several options, depending on the legislation associated with the quota system:

- Milk more cows to fill the quota. In this case, economic damage is calculated as the additional cost of milking more cows. These costs however are not easy to estimate and consist of marginal costs for feed, veterinary service, labour and housing. Opportunity costs for these additional inputs should be assessed, and carefully, because these opportunity costs might differ from farm to farm.
- Increase the production of the cows (e.g., by using more concentrates) to fill the quota. In some farm situations, the milk production of the cows can be increased by application of a better (more expensive) feeding regime. Marginal costs are associated with the higher amount of (more expensive) feed which is necessary to achieve the production level demanded. In some cases (depending on the management capacities of the farmer), higher milk production per cow can lead to health disorders (51).
- Lease out milk quota to other farmers. In some quota systems, farmers can lease in or lease out milk quota relatively easily. This makes the quota system more flexible. When farmers do not fill their quota, the additional quota can be leased out to other farmers. When this is done because of mastitis and the associated milk production decrease, the returns from milk sales will be decreased. However savings might occur because less feed is needed (just as in the non-quota situation), and there will be new returns from leasing out milk quota.

1.2 Drugs

Drugs necessary to treat infected animals are a direct cause of economic damage, owing to their cost. The cost of drugs varies between countries, depending on the legislation and the infrastructure of the country,

1.3 Discarded milk

Economic damage due to discarded milk is comparable with that from decreased milk production. However, there is one difference: the discarded milk is actually produced by the cows, which means that feeding costs for that amount of milk have to be taken into account in the calculations. The economic damage of 100 kg of discarded milk is therefore larger than for 100 kg of decreased production. Although it is not advisable from a veterinary point of view, discarded milk is often fed to calves instead of milk replacer, thereby saving the cost of that milk replacer.

1.4 Veterinary services

Besides delivering drugs (in many countries), the veterinarian might have to spend time on diagnosis of a (clinical) mastitis case (29). Veterinary service may be mandatory for each (clinical) mastitis case, if required by national legislation, or is only provided upon request by the farmer.

1.5 Labour

Cost of labour is difficult to interpret. Opportunity costs of labour may differ from farm to farm. If the labour is external, then the cost of labour for the time that has been used to prevent mastitis is quite easy to calculate (hours x hourly wage). If the labour comes from the farmer's free time, the opportunity costs are zero. However, if because of mastitis the farmer spends less time on other management tasks, the opportunity costs are the decrease in income due to skipping these tasks.

1.6 Product quality

This factor includes meat and milk quality. Mastitis has no effects on the meat quality, but mastitis does influence the quality of milk (4, 16). Some of these changes cause less efficient processing of milk and might result in products with less valuable properties (26, 39). The associated economic damage is difficult to calculate and the direct effect of this economic damage for the individual dairy farmer is even more difficult to estimate. The only changes in milk quality that have a direct effect, and can be estimated, are the factors that are part of the milk payment system, for instance, bacterial count and somatic cell count. Bacterial count and/or somatic cell count do change with the mastitis status of a cow and therefore, in most countries, there is a regulatory limit (payment schemes or bonus systems) for bulk milk bacterial count and bulk tank somatic cell count (BTSCC). BTSCC can increase strongly due to a (subclinical) mastitis case (37), which will have economic consequences (28). Besides BTSCC and bacterial count, most milk payment schemes test for antibiotic residues. Although the mastitis in itself does not affect growth inhibition, the use of antibiotics in treatment of mastitis does increase the risk of penalties. Different countries and milk processors use different rules for antibiotic residues, but the economic consequences of antibiotic residues in the milk can be considerable (4).

1.7 Materials and investments

Mastitis management includes the use of materials and commodities that cost money. These materials can either be renewable (for instance disinfectants; and drugs could be seen as a specific type of renewable materials) or non-renewable (for instance a new milking parlour). The purchase of renewable materials has short term economic consequences and the costs can easily be calculated. The purchase of non-renewable materials has long-term consequences. Purchase costs have to be divided over various years by depreciation. Moreover, because capital is tied up by such purchases interest rates have to be calculated as well. Finally most non-renewable materials require maintenance and this also generates costs.

1.8 Diagnostics

Diagnostics costs that are relevant to mastitis must be included in the calculations, for instance costs of technicians and bacterial cultures (1, 55).

1.9 Other diseases

The factors described above (milk production losses, drugs, discarded milk, veterinary service, labour, product quality, materials and investments, diagnostics and culling) are the economic consequences of clinical and subclinical mastitis. Besides these direct costs, cows with mastitis are a constant source of infection due to the shedding of bacteria (47, 54). There might also be an association between mastitis and other cattle diseases (12, 23, 36). The causal relation, however, is difficult to determine. When the risk of other diseases is increased by mastitis, the economic damage of other disease cases attributable to mastitis can be seen as economic damage due to mastitis However, this damage is very hard to establish because the interactions between various diseases are hard to determine and they will not be further discussed in this paper. Perhaps this would be a good topic for further research.

1.10 Culling

Culling is a difficult factor to estimate since it is a result of other effects (except in the case of death from causes other than culling). Culling is a decision of the dairy farmer. A cow is culled when replacement is the optimal decision. Cows with mastitis have a higher risk of being culled (20, 45). The cost of premature replacement of animals due to mastitis is probably one of the largest areas of economic loss. However, it is very difficult to calculate precisely (12, 20, 24). When a cow is culled, there are direct costs that are the costs of rearing or buying a replacement animal (mostly heifers). Indirect costs are a decreased efficiency of milk production by the replacement animal, since the milk yield of multiparous cows is higher than that of primiparous cows. Moreover, the milk production of a heifer might be disappointing (heifers have relatively a high culling rate). On the other hand, there are returns of culling a cow that are mostly the price of meat. The costs of involuntary culling differ over time, depending on milk production, parity, lactation stage and reproductive status (20).

The basis of the economics of mastitis decision-making lies in the costs of cases of clinical and subclinical mastitis in relation to costs of management procedures. Decisions can be taken at 3 levels: quarter/cow level, farm level and region/country level. Quarter or cow level decisions are those that are related to an individual cow and are mainly treatment of clinical or subclinical mastitis or culling. However, treatment of an individual cow could also be considered as a farm level decision if it is meant to prevent more mastitis cases. Farm level decisions are those concerning the management and control of mastitis (35). The benefits of these measures lie in a lower incidence of clinical and subclinical mastitis and the improvement of milk quality, which might affect the level of milk payments (8).

Regional or country level decisions relate mainly to campaigns that include data collection from a whole region or country to investigate the benefits of mastitis control programme s (4, 14).

Quarter/cow, farm or region/country level decisions are all drastically affected by the prices of the above mentioned factors. Therefore these factors must be considered in management decisions related to mastitis within farms regardless of the level of the decision.

2. Economic consequences: A review

Published literature since 1990 and after the review of Schepers and Dijkhuizen (40) was reviewed and analysed using search by key words in Pubmed library[1] and the reference citation procedure in the ISI web of Knowledge[2]. Mastitis, economics, dairy and management were used as keywords. The reference citation procedure was used to search for articles that referred to older papers regarding mastitis economics. Currencies were all converted to Euro according to the currency exchange rates on 26th Jun 2006 and as mentioned in a footnote to Table 1 (German Mark was calculated according to the exchange rate on 1st January 2002, the date of introduction of the Euro).

2.1 Estimates of the cost of mastitis

Nine papers calculated the costs of mastitis and/or the cost of mastitis prevention (10, 15, 22, 23, 28, 30, 32, 38, 44, Table 1).

Kaneene and Hurd (22) reported costs of selected cattle diseases in Michigan, including clinical mastitis. They calculated the average monthly cost of clinical mastitis per cow based on number of cows at risk. Next, calculations were converted to average costs per cow per year. An economic analysis was carried out based on mastitis components weighted by their effect, then categorized as money spent and potential lost. The average cost of mastitis was €28 per cow per year and the average cost of mastitis prevention was €3.56 per cow per year, varying from €0 to €22.

Miller et al. (30) investigated the costs of diseases in 16 Ohio dairy farms, including mastitis. Different methods were used to estimate the costs depending on the records kept by producers. Estimates such as the value of labour differed between producers, reflecting their opportunity cost. Different mastitis-cost factors, including loss of body weight, were included in the calculation. Disease prevention costs included drugs, labour and veterinary service. They found that mastitis cost was €31 per cow per year and the prevention costs were €4 per cow per year.

Sischo et al. (44) described and evaluated the costs of clinical diseases and preventive measures on 43 California dairies. Costs were classified into costs of disease occurrence, costs of prevention and miscellaneous costs. They found that mastitis costs €22 per cow per year. Costs of mastitis prevention were €24 per cow per year. Approximately 80% of the prevention costs were due to the use of drugs to prevent clinical mastitis. In total the miscellaneous costs were €4 per cow per year.

Hillerton et al. (15) calculated the cost of summer mastitis in 95 herds in England. The calculated incidence was higher than 0.02. They found that summer mastitis, on average, costs Euro 279 per case per year. The greatest losses that occurred were due to the loss in milk production. A loss was reported of Euro 9.03 billion per year to the UK industry due to summer mastitis.

---

[1] NIH, 9000 Rockville Pike, Bethesda, Maryland 20892, USA.

[2] The Thomson Corporation, Stamford, CT, USA.

Table 1. Costs of mastitis and mastitis prevention (in Euro[1] per case or per average cow a year) as estimated in the peer-reviewed papers published since 1990. For each paper the total costs are given. Papers that provided insight in the underlying cost factors are also given (15, 27, 30, 32, 44).

Category	Hillerton (15)	Kossaibati (23)	McInerney (27)	Fourichon (10)	Kaneene (22)	Miller (30)	Miller[2] (32)	Reinsch (38)	Sischo (44)
Type of event	Summer mastitis	Clinical Mastitis	Subclinical mastitis	Mastitis Prevention	Clinical Mastitis	Clinical Mastitis	Mastitis and prevention	Treatment of mastitis	Clinical Mastitis
Analysis level	Case	Case	Case	Cow	Cow	Cow	Cow	Cow	Cow
Total costs	279	287	102	26	28	31	31	3	22
Cost Factors									
Loss in milk production	136		49			11[3]	11		8
Labour	28		-			1[4]	3		-
Treatment	6		-			6[5]	1		4[6]
Culling	103[4]		31			13	9		10
Death and disposal	-		-			-	1		-
Veterinarian	6		-			-	2		-
Milk quality	-		14			-	-		-
Materials and investments	-		8			-	4		-

[1] Currency exchange rate from US Dollar to Euro for papers (22, 30, 32, 44) was 0.7810, from UK Pound to Euro for papers (15, 23, 27) was 1.4520 and from German Mark to Euro for paper (38) was 0.5113.
[2] Costs of treatment were calculated per type of pathogen.
[3] Includes body weight losses.
[4] Includes costs of death and disposal.
[5] Includes costs of veterinary service.
[6] Includes costs of labour¸ body weight loss, veterinarian and death and disposal.

McInerney et al. (28) calculated the avoidable costs of subclinical mastitis in the UK. They defined subclinical mastitis as a quarter somatic cell count (SCC) exceeding 500,000 cells/ml together with the presence of pathogenic bacteria. The annual incidence was calculated assuming that subclinical mastitis lasted on average 0.6 years. Costs of subclinical mastitis were estimated to be Euro 102 per case per year.

Miller et al. (32) calculated the costs of clinical mastitis and mastitis prevention for different mastitis causative agents in 50 Ohio dairy herds. Costs were calculated based on Miller et al. (30) and using the marginal product value (MPV). Mastitis prevention contributed to 48% of the total costs of disease prevention. Costs of mastitis prevention were estimated to be Euro 12 per cow per year. The total costs incurred by producers were estimated to be €31 per cow per year. Furthermore, they calculated the loss due to *Escherichia coli* mastitis and found it to be responsible for the highest cost for a single factor in mastitis.

Reinsch and Dempfle (38) calculated the treatment costs of different diseases from 104 dairy herds in Upper Bavaria in Germany, using regression analysis. Clinical mastitis was defined as a disease trait. In total 691 mastitis observations from 88 herds were included. The cost of mastitis treatment was defined as cost of drugs and labour, which includes farmer, veterinary or external labour. However, these costs were not separated. The average cost of treatment per case of mastitis and per cow per year were Euro 20 and Euro 3, respectively.

Kossaibati et al. (23) studied the costs of the major production diseases in dairy herds in England, including clinical mastitis. The costs were categorised according to the severity of the mastitis case. The total costs were Euro 287 per average cow case per year.

Fourichon et al. (10) described health control costs in different dairy farming systems in western France. Mastitis was one major problem that was described in the study using data from 265 dairy herds. In general, health-control costs for multiparous cows were Euro 61 per cow per year, udder disorders contributed to 43.6% of these costs and mastitis contributed to 97% of the costs of udder disorders. In western France, health-control costs due to mastitis for multiparous cows are estimated to be Euro 26 per cow per year varying from Euro 19 to Euro 32 per cow per year for different types of farm.

2.2 Costs and benefits of mastitis management

2.2.1 Quarter / Cow level

Seven papers dealt with economics of mastitis management at quarter or cow level, five of them dealt with mastitis treatment (34, 43, 47, 48, 49), one papers dealt with the economic profit of dry cow treatment (DCT) (6), and one paper dealt with the economic profit of vaccination against clinical *E. coli* mastitis (7) (Table 2).

Van Eenennaam et al. (49) calculated the costs of three different treatments against clinical mastitis in two large dairy herds in California. Costs of therapy were added to the costs of the withheld milk. Costs varied from Euro 27 to Euro 43 per clinical mastitis case. Average costs were Euro 4 per cow per year leading to average benefits of Euro 2 per cow per year. The overall result was a loss of Euro 2 per cow per year.

Table 2. Costs and benefits of mastitis decisions at quarter/cow level (in Euro[1] per average cow per year). For each paper, the net result is given. Papers that provided insight in the cost and benefit factors are also given (7, 34, 43, 47, 48, 49).

	Berry (6)		DeGraves (7)		Oliver (34)		Shim (43)	
Type of decision	Dry cow Treatment		Vaccination		Treatment[2]		Treatment[3]	
	Costs	Benefits	Costs	Benefits	Costs	Benefits	Costs	Benefits
Net result	10		48		156		103	
Cost and benefit factors								
Milk production			-	22	-	168	-	71
Discarded milk			-	4	2	-	-	48
Labour			0.1	2	8	-	-	-
Treatment			3	9	-	-	16	-
Culling			-	7	-	-	-	-
Death and disposal			0.1	5	-	-	-	-
Veterinarian			-	2	-	-	-	-
Milk quality				-	-	-	-	-
Clinical mastitis			-	-	-	-	-	-
Materials and investments			-	-	0.1	-	-	-
Additional costs			-	-	2	-	-	-

Table 2 continued.

	Swinkels (47)		Swinkels (48)		Van Eenennaam (49)	
Type of decision	Treatment[4]		Treatment[5]		Treatment	
	Costs	Benefits	Costs	Benefits	Costs	Benefits
Net result	12		-19		-2	
Cost and benefit factors						
Milk production	0	-	0	-	-	-
Discarded milk	10	-	21	-	-	-
Labour	0	-	0	-	-	-
Treatment	27	-	27	-	4	-
Culling	-	28	-	17	-	-
Death and disposal	-	-	-	-	-	-
Veterinarian	-	-	-	-	-	-
Milk quality	0	-	0	-	-	-
Transmission		7		8	-	-
Clinical mastitis	-	14	-	12	-	2
Materials and Investments	-	-	-	-	-	-
Additional costs	-	-	8	-	-	-

[1] Currency exchange rate from US Dollar to Euro for papers (34, 43, 49) was 0.7810 and from UK Pound to Euro for papers (6) was 1.4520.
[2] Prepartum antibiotics treatment of heifers.
[3] Losses associated with antibiotic treatment were compared to losses associated with supportive treatment alone. Costs represent the loss associated with supportive treatment and benefits represent the decrease loss associated with antibiotic treatment. The overall result represents sum of benefits.
[4] Calculations were given for 3 days treatment of subclinical *S. aureus* mastitis.
[5] Calculations were given for 3 days treatment of subclinical *S. Dysagalactiae and S. uberis* mastitis.

Oliver et al. (34) quantified the economic consequences of prepartum antibiotic treatment of heifers until the subsequent lactation in Tennessee dairies based on two previous

studies that evaluated the prepartum antibiotic therapy of mastitis. They found that milk production and prevalence of clinical mastitis are significantly higher in treated groups compared to untreated cows. Moreover, antibiotic treatment would cost Euro 12 per heifer per year and would provide net benefits of Euro 156 per heifer per year.

Shim et al. (43) compared the milk production and the costs associated with antibiotic and supportive treatment for clinical mastitis in Illinois dairies. The effect of treatment on daily milk yield was obtained using regression. Treatment type was multiplied by the associated costs of treatment to obtain the total cost of treatment per lactation. Results showed that antibiotic treatment associated with supportive therapy is more cost-effective than supportive treatment alone. Antibiotic treatment would, on average, cost an extra Euro 16 per cow per year and would on average provide benefits of Euro 103 per cow per year, compared to the supportive treatment alone.

Swinkels et al. (47) calculated the economic benefits of antibiotic treatment of chronic subclinical *Streptococcus uberis* and *S. dysgalactiae* infections during lactation using partial budget analysis to compare 3 and 8 days treatment with no treatment. The deterministic model corrected for costs and probabilities, including the transmission rates of pathogens, different cure rates, duration of infection, recovery from new infections and persistency of infections. Treatment was usually not profitable but this depended mainly on the course of the disease (e.g. contagious mastitis), transmission rates of infection, days of treatment and percentage culled due to subclinical mastitis. In a follow-up study Swinkels et al. (48) used the same methodology to calculate the benefits of lactational treatment of subclinical *Staphylococcus aureus* mastitis. Net profit was calculated based on two treatment scenarios, 3 and 8 days treatment. The economic benefits were always negative. However, under specific farm circumstances, treatment could be cost-effective. Berry et al. (6) developed a decision tree model to compare the economic benefits of 3 different dry cow management strategies; treatment with antibiotics, teat seal and no treatment, under UK conditions. Probabilities of new infection, cure rate, culling rate and milk production losses were included. In cases of uninfected group or low infection, treatment with teat seal and antibiotics gave a benefit of Euro 9 (uninfected) and Euro 16 (low infection) per cow per year. In the case of the infected group, the benefits of antibiotic treatment ranged from Euro 3 to Euro 10 per cow per year, depending on cure rates for the pathogen involved.

DeGraves and Fetrow (7) used a partial budget analysis to estimate the profit from vaccinating dairy cattle against coliform mastitis with an *E. coli* J5 vaccine. A model was developed and a partial budget analysis based on literature knowledge was incorporated. Costs and benefits of vaccination 3 times were calculated. The average profit from vaccination was found to be Euro 48 per cow per year.

Table 3. Costs and benefits of mastitis decisions at farm level (in Euro per average cow per year). For each paper, the net result is given. Papers that provided insight in the cost and benefit factors are also given (8, 9, 11, 16, 21, 31, 52, 53).

	Dekkers (8)		Erskine (9)		Gill[2] (11)		Hoblet[2] (16)		Miller[2] (31)	
Type of decision	Management		Management		Management		Management		Management	
	Costs	Benefits	Costs	Benefits	Costs	Benefits	Costs	Benefits	Costs	Benefits
Net result	14		55[3]		26		-3		9	
Cost and benefit factors	-	-	-	79	-	-	-	-3	1	10
Milk production										
Discarded milk	-	-	8	-	-	-	-	-	-	-
Labour	-	-	-	-	-	-	-	-	-	-
Treatment	-	-	3	-	-	-	-	-	-	-
Culling	-	-	0	-	-	-	10	-	-	-
Death and disposal	-	-	-	-	-	-	-	-	-	-
Veterinarian	-	-	-	-	-	-	-	-	-	-
Milk quality	10	24	0	-	-	30	-	14	-	-
Clinical mastitis	-	-	-	-	-	-	-	-	-	-
Materials and investments	-	-	-	-	4	-	-	-	-	-
Additional costs	-	-	13	-	-	-	4	-	-	-

Table 3 continued.

	Yalcin (52)		Yalcin (53)		Zepeda (55)		Huijps (21)		Huijps (21)	
Type of decision	Management		Management		Management		Blanket vs. no therapy		Selective vs. no therapy	
	Costs	Benefits	Costs	Benefits	Costs	Benefits	Costs	Benefits	Costs	Benefits
Net result	183		56		9		2		4	
Cost and benefit factors										
Milk production	-	-	-	-			-	2	-	1
Discarded milk	-	-	-	-			-	-	-	-
Labour	24	-	10	-			1	-	1	0
Treatment	-	-	-	-			10	-	3	0
Culling	-	-	-	-			-	-	-	-
Death and disposal	-	-	-	-			-	-	-	-
Veterinarian	-	-	-	-			-	-	-	-
Milk quality	-	-	-	13			-	-	-	-
Clinical mastitis	-	-	-	43			-	11	-	7
Materials and investments	25	232	-	-			-	-	-	-
Additional costs	-	-	-	-			-	-	-	-

[1] Currency exchange rate from US Dollar to Euro for papers (9, 16, 31, 55) was 0.7810, from UK Pound to Euro for papers (52, 53) was 1.4520 and from Canadian Dollar to Euro for papers (8, 11) was 0.6938.
[2] Average costs and benefits values were extracted as much as possible based on the information from the original paper.
[3] Corrected for feed costs (uncorrected 110).

2.2.2 Farm level

Nine papers described the economic benefits of mastitis management on farm level. Eight of them evaluated the economic benefits of mastitis control strategies (9, 11, 16, 21, 31, 52, 53, 55) and one paper estimated the economic benefits of reducing bulk tank somatic cell count (BTSCC) under 500,000 cells/ml regardless of the control procedures that were used for this purpose (8) (Table 3).

Erskine et al. (9) investigated the effect of a herd mastitis management program on the prevalence of *Streptococcus agalactiae*, SCC, milk rolling herd average and butterfat production in 12 dairy herds in Pennsylvania. The difference in milk production between cows that had a SCC larger than 700,000 cells/ml and had no treatment and cows that were treated was used to calculate the benefits. 50% of the benefits were subtracted as feed cost for extra milk produced. Benefits to costs ratio ranged from 1.26:1 to 2.28:1 for treating all lactating cows and culture samples from all treated *Strep. agalactiae* cows, respectively.

Gill et al. (11) estimated various mastitis control strategies using their expected marginal costs and returns. A comparison between the marginal product value (MPV) and the marginal input cost (MIC) was used to calculate the benefits of the control strategies. A base value for a cow with an average SCC and a mean value for production characteristics were assumed. Thereafter the marginal effect of implementing a control strategy on SCC was calculated and the milk production per cow per day was recalculated with the new SCC. The MPV for a control strategy was then calculated by multiplying the difference between the two yields by the milk price. The MPV of milk production under milk pricing system varied from Euro -8 to Euro 59 per cow per year depending on the control strategy.

Miller and Bertlett (31) adjusted a statistical model from Bartlett et al. (3) to calculate the MPV of milk production, using specific control procedures. MPV was calculated by multiplying the average milk price by the marginal products (increased revenues) of using a specific control procedure. MPV ranged from Euro -7 to Euro 17 per cow per year. The low limit reflects the negative effect of summer non-lactating cow housing and the upper limit reflects the positive effect of quaternary ammonium teat dip.

Hoblet and Miller (16) studied the economic effects of bacteriological culturing and culling on the reduction of *Staph. aureus* mastitis and on maintenance of milk quality premium, using partial budget analysis in 3 Ohio dairy herds. Culling costs were calculated from the yearly culling rates. The first year of the study was compared to the last year to show the effect of bacteriological culturing on the benefits by the end of the study. Revenues from higher milk quality were calculated depending on differences of milk prices related to the SCC scores, and the increase in milk sold was added as additional revenues. Bacteriological culturing had a negative benefit for all herds.

Yalcin et al. (52) quantified the marginal net benefits of mastitis control strategies on herd average milk yield loss and BTSCC penalties using regression analysis in 623 Scottish dairy herds. Economic calculations were carried out using the marginal cost-benefit and frontier analysis. The marginal costs of mastitis control procedures were obtained from McInerney et al. (27). They found that post-milking teat disinfection, dry cow therapy, and

milking-machine testing would provide benefits of Euro 2, Euro 4 and Euro 2 per cow per year, respectively, per extra Euro 2 invested.

Yalcin and Stott (53) used a stochastic dynamic model for optimal replacement decisions for Scottish dairy cattle under the above mentioned mastitis control strategies. The model was formulated to predict the reduction of losses in milk production and somatic cell count penalties, using the different management strategies. The model followed each heifer over time and provided its expected net marginal value based on the margins of milk and calf sales over costs of feed, subclinical mastitis, involuntary culling and other fixed costs based on Yalcin et al. (52). They found that the marginal net returns of using the control procedures and their combination vary from Euro 6 to Euro 41 per cow per year. Moreover the reduction of involuntary culling by 50% would provide benefits of Euro 13 per cow per year.

Zepeda et al. (55) used a linear programming method to maximise profit of testing milk samples and estimate the economic benefits of control strategies that reduce the prevalence of *Staph. aureus* intramammary infection. Different scenarios of testing were examined. The economic analysis allowed for the variation in cure rates and costs associated with the applied therapies. Net profit ranged from Euro 1 to Euro 16 per cow per year.

Huijps and Hogeveen (21) compared the costs of three treatment strategies for dry cow therapy (DCT). Using a stochastic Monte Carlo model, the dynamics of intramammary infection around the dry period were simulated in order to predict the economic consequences. Costs were calculated per cow per year, and varied between Euro 11 to Euro 27 for blanket DCT, Euro 5 to Euro 29 for selective DCT and Euro 4 to Euro 43 for no DCT, taking into account the variation in parameters and pathogen-specific values.

Dekkers et al. (8) estimated the economic benefits of reducing BTSCC below 500,000 cells/ml in Ontario dairies. They developed a simulation model based on Schukken et al. (41) to determine the economic impact of herd average BTSCC on penalties. Economic profits were represented by the average and marginal values of reducing the average somatic cell score. The marginal value of reducing the population average somatic cell score by one unit from a mean of 3 is Euro 14 per cow per year and would cost Euro 10 per cow per year.

2.2.3 Region/Country level

Two papers estimated the economic effect of mastitis management at country level (4, 14) Table 4. Beck et al. (4) estimated the benefits of reduced mastitis in UK dairies using cost-benefit analysis. The profit was calculated by the gain that would be obtained from the reduction of clinical and subclinical mastitis. Benefits were assessed for two different scenarios, fixed and variable herd sizes, by adjusting the amount of concentrate depending on the scenario. Costs are presented in Table 4 as average cost per cow per year and benefits are presented as saved money due to the reduction of clinical and subclinical mastitis per cow per year, if the infection rate would be decreased by 5%.

Hall et al. (14) used the policy analysis matrix to assess the economic impact of herd health programmes on the societal and farm levels in Thailand. They found that implementing a veterinarian mastitis health control programme would provide profits of Euro 15 per cow per year.

DISCUSSION

Published research since 1990 shows a great impact of mastitis on the farming business. Despite the high economic damage of mastitis (Table 1), the most recent paper found on calculating the average cost of mastitis is almost 10 years old. Moreover, several mastitis factors were not presented. Bennett et al. (5) estimated the direct costs of endemic diseases of livestock in the UK using a spreadsheet model. Direct costs were calculated as loss in expected output of resource wastage due to mastitis, treatment costs incurred in trying to mitigate the effects of disease on production and/or the costs associated with specific disease prevention. There was no available information about the number of herds or the number of animals. They found that the average output loss or resource wastage due to mastitis was Euro 175,692,000 per year, the treatment costs were on average Euro 89,298 million per year and mastitis prevention costs were Euro 5,808,000 million per year. Weigler et al. (50) calculated production diseases-related expenditures and costs of disease components in 29 dairy herds in California. Mastitis was categorised as a part of udder disorders that contributed to a cost of Euro 266 per case of udder disorder per year (the annual costs per cow were Euro 39) and the costs of prevention were Euro 70. However, the percentage of mastitis in the udder disorders was not mentioned and thus a calculation of mastitis cost and/or cost of prevention can not be made.

Research related to mastitis management is abundant. However, only a limited amount of research deals with the economics of mastitis management strategies. Moreover, relatively few papers included the benefits of using combined management procedures which might lead to more benefits as shown by Yalcin et al. (52, 53). In some publications (1, 20, 25, 29, 45), results were presented in such a way that we could not transform them to data that could be compared to other studies. Allore and Erb (1) used stochastic discrete modelling to calculate the present value annual benefit (PVAB) of different mastitis control strategies (lactation therapy, dry cow therapy, prevention and vaccination). They found that revenues from milk pricing varied seriously depending on the milk pricing system and the pathogens involved in the intramammary infection. Moreover the avoidance of culling would return from Euro 1 to Euro 10 per cow per year. Mastitis control strategies were shown to improve the PVAB by amounts varying from Euro 21 to Euro 83. However, from the data presented in the paper, it was not possible to determine average costs or benefits per cow per year of those control strategies. Another method used in economic studies is dynamic programming which can be used to calculate optimal mastitis management (2). Using dynamic programming it is also possible to account for stochasticity, which is an important factor. Stott and Kennedy (45) used stochastic dynamic programming to obtain an optimal replacement policy for mastitic cows. They found that mastitis reduces the age of optimum replacement and mastitis treatment does not reduce milk production. Houben et al. (20) used a hierarchic Markov simulation process to model the optimal replacement decision of clinical mastitic cows. Culling was described as an effective measure, but no value was given. However, decisions were more frequent to keep or treat a cow rather than culling it. Nevertheless, it was not possible to calculate the costs and/or benefits of the factors included from Stott et al. (45)

and Houben et al. (20). Losinger (25) estimated the economic impacts attributable to an increase in BTSCC for US dairies using social welfare analysis. He estimated the effect of increased BTSCC on the milk production to perform the economic analysis. It was the only paper using supply and demand curves to calculate the economic losses. However, it was assumed that cost of mastitis would not be reflected on the producer, which allowed a high positive effect of the BTSCC reduction on the producer's surplus. McNab and Meek (29) used a cost benefit analysis to estimate the benefits of antibiotic dry cow treatment in Ontario dairies. Two scenarios were assumed, based on quota systems, for 297 herds, of which 143 herds had complete SCC data. Two different regression models were implemented based on the data and a mail questionnaire to evaluate the quality of milk for purposes of penalty and to estimate the effect of dry cow therapy on herd average kilogram milk production. The Cost-Benefit ratio ranged from 0.5:1 to 31:1 based on the methodology used to assess the benefits of a therapy. Nevertheless, it was not possible to extract an overall value from the paper.

Table 4. Costs and benefits of mastitis decisions at region/country level (in Euro per average cow per year). For both papers the net results and the underlying cost and benefit factors are given.

	Beck (4)		Hall[2] (14)	
Type of decision	Management		Management	
	Costs	Benefits	Costs	Benefits
Net result	32		15	
Cost and benefit factors				
Milk production	-	-	1	36
Discarded milk	-	-	-	-
Labour	-	-	2	-
Treatment	-	-	-	-
Culling	8	-	-	-
Death and Disposal	-	-	-	-
Veterinarian	-	-	1	-
Milk quality	0	-	-	-
Clinical mastitis	13	12	-	-
Subclinical mastitis	-	54	-	-
Materials	-	-	-	4[4]
Investments	13	-	4[3]	-
Additional costs	-	-	17[5]	-

[1] Currency exchange rate from UK Pound to Euro for paper (4) was 1.4520 and from Thailand Bath to Euro for paper (14) was 0.0210.
[2] Calculations are performed assuming a herd size of 15 cows. Calf sales were not included.
[3] Includes depreciation of buildings, equipment and cows.
[4] Includes benefits from sales of animals and manure.
[5] Includes costs of feed, artificial insemination, debt payments, miscellaneous and taxes.

The calculated costs and benefits vary between studies (Table 2). This variation is not just due to different data sources, regions, methodology and/or assumptions but also to the different levels (quarter, animal, farm and region) that are used to conduct the calculations. Moreover variations in the calculated costs associated with mastitis cost factors have been observed even within one country. Hillerton et al (15) assumed the cost of labour to be Euro 28 (per mastitis case in farm) but Kossaibati and Esslemont (23) assumed that this cost was Euro 1. Variations were also encountered in the benefits of mastitis treatments and/or

management procedures, e.g. Yalcin et al. (52) calculated total benefits of Euro 183 from using 3 different management procedures. However, for the same management procedures, but using different methodology, Yalcin and Stott (53) calculated total benefits of Euro 56. The use of different methodology would demand usage of different assumptions, which might have an influence on the results (2). Several mastitis cost factors were frequently ignored in the calculations and therefore all cost and benefit factors were mentioned in the tables to show the estimates that were used in each study to conduct the calculations.

Despite the fact that mastitis is caused by different pathogens that vary in their biological reactions in the body, and in consequence the damage they cause to the udder parenchyma, pathogen-specific calculations of the economic damage were very few (7, 9, 16, 47, 48). The need for such calculations could be of high importance and would facilitate the development and assessment of pathogen-specific control strategies.

The variations between studies do not allow a general conclusion about the cost and the cost benefits of mastitis management. Moreover, there might be large differences in the economic damage of mastitis between farms. Farms do differ in terms of mastitis incidence, pathogen involved and management. It might be very helpful to be able to conduct a farm specific economic analysis. This analysis would eliminate the inaccuracy that could occur due to the differences between farms. Moreover, it would provide more precise calculations which can be used to improve prediction of the economic benefits of management improvement and development.

CONCLUSION

There are large variations between studies in the calculations of the economic damage of mastitis and the benefits of mastitis management. Farm-specific and pathogen-specific calculations would improve the estimation of the economic damage of mastitis and the benefits of mastitis management. Results also showed that factors included in the calculations varied between studies. Moreover, in some studies, important factors were ignored. The framework provided in this paper can provide future studies on the economics of mastitis and mastitis management with a basis for analysis of the factors that should be considered.

References

Allore HG and Erb HN. Partial budget of the discounted annual benefits of mastitis control strategies. Journal of Dairy Science 1998; 81:2280-2292.

Allore HG and Erb HN. Approaches to modeling intramammary gland infections in cattle. Preventive Veterinary Medicine 1999; 39:279-293

Bartlett PC, Miller GY, Anderson CR and Kirk JH. Milk production and somatic cell count in Michigan dairy herds. Journal of Dairy Science 1990; 73:2794-2800.

Beek HS, Wise WS and Dodd FH. Costs benefit analysis of bovine mastitis in the UK. Journal of Dairy Research 1992; 59:449-460.

Bennett R, Christiansen K and Clifton-Hadley R. Preliminary estimates of the direct costs associated with endemic diseases of livestock in Great Britain. Preventive Veterinary Medicine 1999; 39:155-171.

Berry EA, Hogeveen H and Hillerton JE. Decision tree analysis to evaluate dry cow strategies under UK conditions. Journal of Dairy Research 2004; 71:409-418.

DeGraves FJ and Fetrow J. Partial budget analysis of vaccinating cattle against coliform mastitis with an *Escherichia coli* J5 vaccine. Journal of American Veterinary Medicine Association 1991; 199:451-455.

Dekkers JCM, Van Erp T and Schukken YH. Economic benefits of reducing somatic cell count under the milk quality program of Ontario. Journal of Dairy Science 1996; 79:396-401.

Erskine RJ and Eberhart RJ. Herd Benefit-to-Cost Ratio and Effects of a Bovine Mastitis Control Program That Includes Blitz Treatment of Streptococcus-Agalactiae. Journal of the American Veterinary Medical Association 1990; 196:1230-1235.

Fourichon C, Seegers H, Beaudeau F, Verfaille L and Bareille N. Health-control costs in dairy farming systems in western France. Livestock Production Science 2001; 68:141-156.

Gill R, Howard WH, Leslie KE and Lissemore K. Economics of Mastitis Control. Journal of Dairy Science 1990; 73:3340-3348.

Grohn YT, Rajala-Schultz PJ, Allore HG, Delorenzo MA, Hertl JA and Galligan DT. Optimizing replacement of dairy cows: modeling the effects of diseases. Preventive Veterinary Medicine 2003; 61:27 – 43.

Grohn YT, Wilson DJ, Gonzalez RN, Hertl JA, Schulte H, Bennett G and Schukken YH. Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. Journal of Dairy Science 2004; 87:3358-3374.

Hall DC, Ehui SK and Shapiro BI. Economic analysis of the impact of adopting herd health control programs on smallholder dairy farms in Central Thailand. Agricultural Economics 2004; 31:335-342.

Hillerton JE, West JGH and Shearn MFH. The Cost of Summer Mastitis. Veterinary Record 1992; 131:315-317.

Hoblet KH and Miller GY. Use of partial budget to determine the economic outcome of *Staphylococcus aureus* intramammary infection reduction strategies in three Ohio dairy herds. Journal of American Veterinary Medicine Association 1991; 6:714-720.

Hogeveen H and Lankveld JMG. Economics of milk quality - some starting points for discussion. In: Rasmussen, M.D. (editor), Proceedings of the workshop definition of normal and abnormal milk at the time of milking, Foulum, Denmark 2002; 81-89.

Hortet P and Seegers H. Loss in milk yield and related composition changes resulting from clinical mastitis in dairy cows. Preventive Veterinary Medicine 1998; 37:1-20.

Houben EHP, Dijkhuizen AA, Van Arendonk JAM and Huirne RBM. Short- and long-term production losses and repeatability of clinical mastitis in dairy cattle. Journal of Dairy Science 1993; 76:2561-2578.

Houben EHP, Huirne RBM, Dijkhuizen AA and Kristensen AR. Optimal replacement of mastitis cows determined by a hierarchic Markov process. Journal of Dairy Science 1994; 77:2975 – 2993.

Huijps K and Hogeveen H. Economic evaluation of dry cow therapy. Journal of Dairy Science 2007; 90:1225-1234.

Kaneene JB and Hurd HS. The National Animal Health Monitoring-System in Michigan: 3. Cost Estimates of Selected Dairy-Cattle Diseases. Preventive Veterinary Medicine 1990; 8:127-140.

Kossaibati MA and Esslemont RJ. The costs of production diseases in dairy herds in England. The Veterinary Journal 1997; 154:41-51.

Lehenbauer TW and Oltjen JW. Dairy cows culling strategies: making economic culling decisions. Journal of Dairy Science 1998; 81:264-271.

Losinger WC. Economic impacts of reduced milk production associated with an increase in bulk-tank somatic cell count on US dairies. Journal of the American Veterinary Medical Association 2005; 226:1652-1658.

Ma Y, Ryan C, Barbano DM, Galton DM, Rudan MA and Boor KJ. Effects of somatic cell count on quality and shelf-life of pasteurized fluid milk. Journal of Dairy Science 2000; 83:264-274.

McInerney JP, Howe KS and Schepers JA. 1990. A framework for the economic analysis of disease in farm livestock. Report of a Research Project (Ref. CSA 873) Funded by the MAFF, The University of Exeter, Agricultural Economics Unit. 87 pp.

McInerney JP, Howe KS and Schepers JA. A Framework for the Economic-Analysis of Disease in Farm Livestock. Preventive Veterinary Medicine 1992; 13:137-154.

McNab BW and Meek AH. A benefit cost analysis of dry-cow mastitis therapy in dairy cattle in Ontario. Canadian Veterinary Journal 1991; 32:347-353.

Miller GY and Dorn R. Costs of dairy cattle diseases to producers in Ohio. Preventive Veterinary Medicine 1990; 8:171-182.

Miller GY and Bertlett PC. Economic effects of mastitis prevention strategies for dairy producers. Journal of the American Veterinary Medical Association 1991; 198:227-231.

Miller GY, Bertlett PC, Lance SE, Anderson J and Heider LE. Costs of clinical mastitis and mastitis prevention in dairy herds. Journal of American Veterinary Medicine Association 1993a; 8:1230-1236.

Miller RH, Paape MJ, Fulton LA and Schultz MM. The relationship of somatic cell count to milk yield for Holstein heifers after first calving. Journal of Dairy Science 1993b; 76:728-733.

Oliver SP, Lewis MJ, Gillespie BE, Dowlen HH, Jaenicke EC and Roberts RK. Prepartum antibiotic treatment of heifers: Milk production, milk quality and economic benefit. Journal of Dairy Science 2003; 86:1187-1193.

Østerås O, Hogeveen H, Singh DK and Leslie KE. Economic consequences of mastitis. Bulletin of the International Dairy Federation 394/2005. IDF, Bruxelles, Belgium. 2005.

Peeler EJ, Otte MJ and Esslemont RJ. Inter-relationships of periparturient diseases in dairy cows. Veterinary Record 1994; 5:129-132.

Pyörälä S and Mattila T. Inflammatory changes during experimental bovine mastitis induced by *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus uberis*. Journal of Veterinary Medicine A 1987; 34:574-581.

Reinsch N and Dempfle L. Investigations on functional traits in Simmental: 1. Treatment costs for ten different diseases. Journal of Animal Breeding and Genetics 1997; 114:407-417.

Santos MV, Ma Y and Barbano DM. Effect of somatic cell count on proteolysis and lipolysis in pasteurized fluid milk during shelf-life storage. Journal of Dairy Science 2003; 86:2491-2503.

Schepers JA and Dijkhuizen AA. 1991. The economics of mastitis and mastitis control in dairy cattle: a critical analysis of estimates published since 1970. Preventive Veterinary Medicine 1991; 10:213-224.

Schukken YH, Weersink AJ, Leslie KE and Martin SW. Dynamics and regulation of bulk milk somatic cell counts. Canadian Journal of Veterinary Research 1993; 57:131.

Seegers H, Fourichon C and Beaudeau F. Production effects related to mastitis and mastitis economics in dairy cattle herds. Veterinary Research 2003; 24:475-491.

Shim EH, Shanks RD and Morin DE. Milk loss and treatment costs associated with two treatment protocols for clinical mastitis in dairy cows. Journal of Dairy Science 2004; 87: 2702-2708.

Sischo WM, Hird DW, Gardner IA, Utterback WW, Christiansen KH, Carpenter TE, Danaye-Elmi C and Herson B. Economics of disease occurrence and prevention on California of data collected for the National Animal Health Monitoring System, 1986-1987. Preventive Veterinary Medicine 1990; 8:141-156.

Stott AW and Kennedy JOS. The economics of culling dairy cows with clinical mastitis. Veterinary Record 1993; 133:495 – 499.

St.Rose SG, Swinkels JM, Kremer WDJ, Kruitwagen CLJJ and Zadoks RN. Effect of penethamate hydriodide treatment on bacteriological cure, somatic cell count and milk production of cows and quarters with chronic subclinical *Streptococcus uberis* or *Streptococcus dysgalactiae* infections. Journal of Dairy Research 2003; 70:387-394.

Swinkels JM, Rooijendijk JGA, Zadoks RN and Hogeveen H. Use of partial budgeting to determine the economic benefits of antibiotic treatment of chronic sub clinical mastitis caused by *Streptococcus uberis* or *Streptococcus dysgalactiae*. Journal of Dairy Research 2005a; 72:75-85.

Swinkels JM, Hogeveen H and Zadoks RN. A partial budget model to estimate economic benefits of lactational treatment of subclinical *Staphylococcus aureus* mastitis. Journal of Dairy Science 2005b; 88:4273-4287.

Van Eenennaam AL, Gardner IA, Holmes J, Perani L, Anderson RJ, Cullor JS and Guterbock WM. Financial analysis of alternative treatments for clinical mastitis associated with environmental pathogens. Journal of Dairy Science 1995; 87:2086-2095.

Weigler BJ, Hird DW, Sischo WM, Holmes JC, Danaye-Elmi C, Palmer CW and Utterback WW. Veterinary and non-veterinary costs of disease in 29 California dairies participating in the national animal health monitoring system from 1988 to 1989. Journal of the American Veterinary Medicine Association 1990: 196:1945-1949.

Windig JJ, Calus MPL, De Jong G and Veerkamp RF. The association between somatic cell count patterns and milk production prior to mastitis. Livestock Production Science 2005; 96:291-299.

Yalcin C, Stott AW, Logue DN and Gunn J. The economic impact of mastitis-control procedures used in Scottish dairy herds with high bulk-tank somatic cell count. Preventive Veterinary Medicine 1999; 41:135–149.

Yalcin C and Stott AW. Dynamic programming to investigate financial impacts of mastitis control decisions in milk production systems. Journal of Dairy Science 2000; 67:515-528.

Zadoks RN, Allore HG, Hagenaars TJ, Barkema HW and Schukken YH. A mathematical model of *Staphylococcus aureus* control in dairy herds. Epidemiology and Infection 2002; 129:397-416.

Zepeda L, Buelow KL, Nordlund KV, Thomas CB, Collins MT and Goodger WJ. Corrigendum to “A linear programming assessment of the profit from strategies to reduce the prevalence of *Staphylococcus aureus* mastitis” [Prev. Vet. Med. 33 (1998) 183-193]. Preventive Veterinary Medicine 2000; 44:61-71.

Chapter 3

Production Loss Due to New Subclinical Mastitis in Dutch Dairy Cows Estimated With a Test-Day Model

T. Halasa,*† M. Nielen,* A. P. W. De Roos,‡ R. Van Hoorne,‡ G. de Jong,‡ T. J. G. M. Lam,§† T. van Werven,*§ and H. Hogeveen*†

*****Department of Farm Animal Health and Reproduction, Utrecht University, P.O. Box 8015, 3584 CN Utrecht, The Netherlands

†Business Economics Group, Wageningen University, P.O. Box 8130, 6706 KN Wageningen, The Netherlands

‡NRS, P.O. Box 454, 6800 AL Arnhem, The Netherlands

§Dutch Udder Health Center at the Animal Health Service Ltd., P.O. Box 9, 7400 AA Deventer, The Netherlands

Published in Journal of Dairy Science 92 (2009) 599-606

ABSTRACT

Milk, fat, and protein loss due to a new subclinical mastitis case may be economically important. The objective was to estimate this loss. The loss was estimated based on test-day (TD) cow records collected over a 1-year period from 400 randomly selected Dutch dairy herds. After exclusion of records from cows with clinical mastitis, the dataset comprised 251,647 TD records from 43,462 lactations of 39,512 cows. The analysis was carried out using the random regression test-day modeling approach that predicts the cow production at each TD based on the actual production at all previous TDs. The definition of new subclinical mastitis was based on literature and assumed a new subclinical case if SCC > 100,000 cells/mL after a TD with SCC < 50,000 cells/mL. A second dataset was created by applying an adjustment to correct low SCC for the dilution effect when determining if the previous test day SCC was < 50,000 cells/mL. Thereafter the loss was estimated for records with SCC > 100,000 cells/mL. The production (milk, fat, or protein) losses were modeled as the difference between the actual and predicted production (milk, fat or protein) at the TD of new subclinical mastitis, for 4,382 cow records, 2,545 cow records after dilution correction. Primiparous cows were predicted to lose 0.31 (0.25-0.37) and 0.28 (0.20-0.35) kg/d milk at a SCC 200,000 cells/mL, for unadjusted and adjusted low SCC, respectively. For the same SCC increase, multiparous cows were predicted to lose 0.58 (0.54-0.62) and 0.50 (0.44-0.56) kg/d milk, respectively. Moreover, it was found that the higher the SCC increase >100,000 cells/mL, the higher the production losses. The estimated production losses were more precise than previously reported estimates.
***Key words*:** production loss, new case, subclinical mastitis, test-day model

INTRODUCTION

Subclinical mastitis affects milk quality and quantity causing great economic loss for producers (Swinkels et al., 2005; Halasa et al., 2007). Several studies estimated milk production loss due to subclinical mastitis (e.g. Hortet and Seegers, 1998; Koldeweij et al., 1999), but a wide range of estimates was reported (Seegers et al., 2003). This variation is not only caused by different populations or mastitis indicators (Hortet et al., 1999) but also by the use of different analytical approaches (Hortet and Seegers, 1998). Estimates of changes in milk composites are scarce and not appropriate for economic calculations (Seegers et al., 2003). Recently, a dilution effect due to high production and low SCC was quantified; suggesting an overestimation in SCC related production loss in earlier research (Green et al., 2006). Economically, it might be important to correct the estimated production loss for the dilution effect to be able to precisely quantify economic effects of subclinical mastitis. Precise estimation of milk production loss and milk composite changes due to a new subclinical mastitis case is important for good economic calculations in the light of treatment decisions. Moreover, reliable economic calculations are important to motivate farmers to adopt management practices.

Several approaches have been proposed to estimate milk production loss at herd level (e.g. De Graaf and Dwinger, 1996). They have focused on comparing the production of infected and uninfected cows or production before and after infection in the same animal (Hortet and Seegers, 1998; Rajala-Schultz et al., 1999). The introduced variation between animals using the first methodology would decrease the precision of the results. Random regression test-day modeling (RRTM) has been developed to analyze test-day (TD) records of dairy cattle for genetic evaluation (Jamrozik and Schaeffer, 1997). Besides fixed effects such as parity, lactation stage, and season, the RRTM includes genetic and non-genetic animal effects and herd-specific lactation curves (De Roos et al., 2004). De Roos and De Jong (2006) presented an RRTM to analyze TD milk urea, and used that model to extend lactation curves by using only the TD records up to a certain point in time. The predicted TD productions, later in lactation, were compared to actual TD productions, to evaluate whether a cow was producing more or less than initially predicted. Because such predictions are based on cow- and herd-specific lactation curves, they are very accurate, which makes this approach attractive for estimation of the effect of environmental factors, such as diseases on subsequent lactation production.

The aim of this research was to estimate milk production loss and its composites changes following a new subclinical mastitis case based on the RRTM.

MATERIALS AND METHODS

Data

For the purpose of improving udder health in The Netherlands, the Dutch Udder Health Center (UGCN, Deventer, The Netherlands) in cooperation with dairy herd improvement organizations (CR Delta and NRS, Arnhem, The Netherlands) collected cow production and clinical mastitis records. Initially, 600 farms were selected randomly from the Dutch dairy herd improvement association records. To be selected, farm size had to be at least 50 cows, age of the farmer had to be < 57 years, farmers had to fill in a questionnaire and agree to data collection. The data collection took place in 400 farms from 1 July 2004 to 30 June 2005 on the basis of a herd TD interval (**TDInt**) of 3-6 weeks. For the present study, if clinical mastitis occurred, TD records of the remainder of the lactation were excluded. Clinical records were based on farmer diagnosis of inflamed udder, abnormal milk color and/or presence of clots in the milk. The dataset consisted of 251,647 TD records from 43,462 lactations of 39,512 cows. The NRS provided cow identification number, herd identification number, lactation number, SCC $\times 10^3$ cells/mL, milk (kg), fat (g), and protein (g) production, calving date, and test date. Clinical mastitis dates were provided by the UGCN.

Definition of a New Subclinical Mastitis Case

The definition of a new case of subclinical mastitis was as follows: If at TD_{i-1} SCC < 50,000 cells/mL and at TD_i SCC > 100,000 cells/mL, a cow was considered to have a new subclinical mastitis case at TD_i, where TD_i is the record of the new subclinical mastitis TD

and TD_{i-1} is the previous TD. Cases were included only when both TD_{i-1} and TD_i were within the same lactation. Moreover, only lactations that started with a calving during the trial period were included in the definition of a new subclinical mastitis case. Only records of the first subclinical mastitis case were included in the analysis.

Dilution effect

Recently, it has been pointed out that low SCC has an inverse relationship with high milk production due to a dilution effect (Green et al., 2006). The dilution was apparent for low SCC (< 50,000 cells/mL) and high producing cows, which indicated that the high yield caused underestimation of the true concentration of the SCC. For SCC > 100,000 cells/mL, the inflammation caused high SCC and dilution was negligible. For the current analysis, the Lambda (λ) value (0.485) of Green et al. (2006) was used to correct for dilution effect for animals with crude SCC < 50,000 cells/mL and milk production > 10 kg/d as follows:

$$\text{Adjusted SCC} = \text{Crude SCC} + (-\lambda \times \text{Actual Milk Production}) \quad (1)$$

After adjustment, SCC at TD_{i-1} would increase relative to actual milk production above 10 kg/d. This means that the SCC of some cows that were selected to be free of subclinical mastitis with SCC < 50,000 cells/mL at TD_{i-1} actually exceeded this limit after SCC adjustment. Those cows were therefore excluded and the resulting smaller dataset was analyzed separately.

Dataset Construction Based on the RRTM

Predicted milk, fat, and protein production were calculated by the NRS (Arnhem, The Netherlands) based on the RRTM (De Roos et al., 2004; De Roos and De Jong, 2006). The model is based on a combined analysis of TD production (known means and variances), herd-specific regression curves, and standard lactation curves. Predictions of milk, fat, or protein production were provided for TD_i based on the production at all previous TDs, corrected for random genetic and fixed environmental effects, parity, DIM and other important effects. The RRTM is presented and explained in more detail in the Appendix.

The predicted production at TD_i represents the production of the cow assuming that the SCC and all other factors remained the same as for at TD_{i-1}. The difference between the actual and predicted production (kilograms milk, grams fat, or grams protein) at TD_i (ΔProd) would reflect the effect of SCC (cells/mL) increase, as a marker of new subclinical mastitis, on production.

$$\Delta\text{Prod} = \text{Actual Production at } TD_i - \text{Predicted Production at } TD_i \quad (2)$$

ΔProd represent the change in production (kilograms milk, grams fat, or grams protein) at the TD of new subclinical mastitis (TD_i). Only the first subclinical mastitis case per cow was considered in the analysis in order not to bias the results due to the effect of previous subclinical cases. Cows that started the lactation with SCC > 50,000 cells/mL were excluded, to be able to use predicted production based only on healthy TDs prior to the new case.

Statistical Analysis

ΔProd at TD_i was modeled using 'Proc Mixed' in SAS (SAS Institute, 2004). The models were fitted using the restricted maximum likelihood method, and a backward stepwise regression procedure for the change (Δ) in milk, fat or protein production, according to the following model equation:

$$Y_{hjkl} = \beta_0 + \beta_1 \times \text{LnSCC} + \beta_2 \times \text{Parity}_h + \beta_3 \times \text{TDInt}_j + \beta_4 \times \text{DIM}_k + \text{Herd}_l + e_{hjkl} \qquad (3)$$

Y_{hjkl} is the ΔProd at the new subclinical mastitis TD (TD_i) for each cow in herd l in parity h with a TD interval class j, and in DIM class k. β_o is the overall mean ΔProd at TD_i. β_1 is the regression coefficient of the natural logarithm of the SCC × 10^3 cells/mL (**LnSCC**), LnSCC is the fixed effect of LnSCC at TD_i on ΔProd. β_2 is the regression coefficient of the hth class of parity, Parity_h is the fixed effect of class h of parity (5 classes, parity = 1, 2, 3, 4, and ≥ 5) on ΔProd. β_3 is the regression coefficient of the jth class of TD interval, TDInt is the fixed effect of class j of the time interval between TD_{i-1} and TD_i (4 classes, 3, 4, 5, and ≥ 6 weeks interval). β_4 is the regression coefficient of the kth class of DIM, DIM_k is the fixed effect of class k of DIM (30 classes) at TD_i, Herd_l is the random effect of herd l, and e_{hjkl} is the residual error.

Separate models were run for each production parameter (milk, fat or protein). No correlation structure was fitted because only one record per cow existed in the model, which is the ΔProd corresponding to the new subclinical mastitis TD (TD_i). For the same reason a cow as a random effect was not included. The estimated loss from each model was assumed to exist in the interval around TD_i, from halfway between TD_{i-1} and TD_i to halfway between TD_i and TD_{i+1}, which is the TDInt of a specific herd. The fit of the models was examined using normality of the residuals, and homoscedasticity of the fitted values.

Table 1. Number, mean and standard deviation (s.d.) of test-day (TD) records of milk (kg/d), fat (g/d), and protein (g/d) production and the geometric mean and standard deviation of SCC[1] per parity for 1 year data on 400 Dutch dairy farms

Parity	Cows	TD Records	Milk (kg)		Fat (g)		Protein (g)		SCC[1]	
			Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
1	13,751	80,816	23.1	6.00	1,062	239	817	186	64.89	2.66
2	10,920	63,898	27.36	8.66	1,209	348	971	263	76.12	2.89
3	7,616	43,901	28.96	9.60	1,283	393	1,017	287	94.00	3.08
4	4,976	28,552	29.29	9.68	1,303	406	1,023	293	112.2	3.21
≥5	6,199	34,480	28.71	9.69	1,271	408	990	290	138.3	3.46

[1] Geometric mean of the crude SCC × 10^3 cells/mL.

The analysis was carried out on 2 subsets of the final dataset that includes records at TD_i; 1) all TD records at TD_i were included to represent the effect of new subclinical mastitis on production without adjustment of SCC at TD_{i-1} for dilution effect; 2) A subset of 1 where records with SCC at TD_{i-1} > 50,000 cells/ml after adjustment for the dilution effect were removed. The two subsets were analyzed separately according to equation 3 to show results when adjustment of SCC to dilution was considered, and when adjustment was ignored.

RESULTS

Descriptive Analysis

Number of cows, TD records, and distribution of milk production parameters per parity are shown in Table 1; TD records following clinical mastitis were excluded from that lactation. Of the total number of lactations, 13.3% of the heifers and cows had a first clinical mastitis case in lactation and 77% occurred during the first 60 days of the lactation. No information was available about second or more clinical cases per cow. Mean milk production for primiparous cows was 23.2 and a standard deviation 6 kg/d with a geometric mean SCC 65,000 cells/mL and a standard deviation 2,660 cells/mL. For multiparous cows mean, milk production was 28.3 kg/d and a standard deviation 9.2 kg/d with a geometric mean SCC 105,000 cells/mL and a standard deviation 3,230 cells/mL (Table 1). Primiparous cows comprised 31.6% of the whole study population. TDInt varied on cow level between 3 weeks (1.2% of the records), 4 weeks (69.9%), 5 weeks (15.2%), 6 weeks (10.6%), and > 6 weeks (3.1%) intervals.

Table 2. The intercept (β_o) and coefficient (β_1) of the natural logarithm of SCC $\times 10^3$ cells/mL (LnSCC) with the standard errors (s.e.) for the models of the change (Δ) of milk (kg), fat (g), and protein (g) production for primiparous and multiparous cows using unadjusted SCC or adjusted SCC values to selected healthy cows according to the definition[1] of new subclinical mastitis cases

	Unadjusted SCC to dilution effect (n = 4382)				Adjusted SCC to dilution effect (n = 2545)			
	Parity 1		Parity ≥ 2		Parity 1		Parity ≥ 2	
	β_o(s.e.)	β_1(s.e.)	β_o(s.e.)	β_1(s.e.)	β_o(s.e.)	β_1(s.e.)	β_o(s.e.)	β_1(s.e.)
Δ Milk	0.80	-0.21	1.59	-0.41	0.78	-0.20	1.62	-0.40
	(0.20)	(0.03)	(0.18)	(0.02)	(0.22)	(0.04)	(0.20)	(0.03)
Δ Fat	33.70	-7.40	62.24	-13.59	33.10	-7.20	54.62	-12.20
	(17.20)	(2.30)	(16.11)	(2.81)	(18.12)	(2.42)	(16.73)	(3.21)
Δ Protein	17.70	-5.10	44.80	-10.72	17.52	-4.85	39.45	-9.90
	(12.61)	(1.61)	(9.72)	(2.01)	(13.20)	(1.94)	(9.84)	(2.43)

[1] If at TD_{i-1} SCC < 50,000 cells/mL and at TD_i SCC > 100,000 cells/mL, a cow was considered to have a new subclinical mastitis case at TD_i, where TD_i is the record at the new subclinical mastitis TD and TD_{i-1} is the previous TD.

Milk, Fat and Protein Production Loss

The new subclinical mastitis cases were distributed throughout the lactation, where 31%, 37%, 22%, and 10% of the cases occurred in the first 100 DIM, between 100 and 200 DIM, between 200 and 300 DIM, and in > 300 DIM; respectively. There was no significant difference in production loss among different parities of multiparous cows, thus the results are presented for primiparous (parity = 1) and multiparous (parity ≥ 2) cows. The TDInt classes did not affect the change in milk, fat, or protein production significantly. For instance, when adjustment to dilution was not considered and for the classes 3, 4, 5 and ≥ 6 weeks intervals, and using the 3 weeks interval as the reference class, the coefficient and standard errors were 0.23 (0.19), 0.21 (0.17), 0.22 (0.18); respectively. Moreover, changing the reference class did

not change the insignificancy of TDInt classes toward the change in milk, fat, or protein production models. Similarly, none of the 30 classes of DIM were found to affect the change in milk, fat, or protein production significantly (lowest P-value = 0.65). Therefore, TDInt and DIM were not included in the reduced final models.

The number of new subclinical mastitis cases was 1372 and 3010 for primiparous and multiparous cows, respectively. The regression coefficient λ was found to be -0.491. After adjustment to dilution effect, the number of primiparous and multiparous cows that had adjusted SCC < 50,000 cells/mL was 989 and 1556, respectively. The median SCC before adjustment was 40,000 cells/mL, but after adjusting the SCC values according to equation (1) the median value (including cows that exceeded 50,000 cells/mL after adjustment) was 48,000 cells/mL.

Table 3. Estimates of predicted milk production loss (kg/d) together with the 95 % confidence interval at different high crude SCC $\times 10^3$ cells/mL levels for primiparous and multiparous cows using unadjusted SCC or adjusted SCC values to selected healthy cows according to the definition[1] of new subclinical mastitis cases

SCC	Unadjusted SCC to dilution effect (n = 4382)		Adjusted SCC to dilution effect (n = 2545)	
	Parity		Parity	
	1	≥2	1	≥2
100	0.17 (0.11-0.23)	0.30 (0.26-0.34)	0.14 (0.06-0.22)	0.22 (0.16-0.28)
200	0.31 (0.25-0.37)	0.58 (0.54-0.62)	0.28 (0.20-0.35)	0.50 (0.44-0.56)
300	0.40 (0.34-0.47)	0.75 (0.71-0.78)	0.36 (0.28-0.44)	0.66 (0.61-0.72)
400	0.46 (0.40-0.52)	0.87 (0.83-0.91)	0.42 (0.34-0.50)	0.78 (0.72-0.83)
500	0.51 (0.45-0.56)	0.96 (0.92-1.00)	0.46 (0.38-0.54)	0.87 (0.81-0.92)
600	0.55 (0.49-0.60)	1.03 (0.99-1.07)	0.50 (0.42-0.58)	0.94 (0.88-1.00)

[1] If at TD_{i-1} SCC < 50,000 cells/mL and at TD_i SCC > 100,000 cells/mL, a cow was considered to have a new subclinical mastitis case at TD_i, where TD_i is the record at the new subclinical mastitis TD and TD_{i-1} is the previous TD.

The parameter estimates are presented in Table 2 based on the final models as reduced from equation 3 for the two subsets with or without adjusted SCC. The prediction of each model represents the loss in production estimated for TD_i, so per day. In Table 3 the predicted loss in milk production is presented for different crude SCC values together with the 95 % confidence interval. For example, a primiparous and a multiparous cow with a SCC 200,000 cells/mL were predicted to lose 0.31 (0.25-0.37) and 0.58 (0.54-0.62) kg milk per day, respectively, when dilution effect was not considered to select healthy cows at TD_{i-1}. When the dilution effect was considered to select healthy cows at TD_{i-1}, a primiparous and a multiparous cow with a SCC 200,000 cells/mL were predicted to lose 0.28 (0.20-0.35) and 0.50 (0.44-0.56) kg milk per day, respectively. Table 3 shows also the dose-effect relationship between the increase of SCC and production loss. When SCC increase > 100,000 cells/mL, the production losses increase.

Fit of the Models

Figure 1a and b show the distribution of the standardized Pearson residuals of the models for two data sets, for Δ kilograms milk. A long tail exists on the right side, which might have caused disturbance of the normality. However, the normality of the residuals is acceptable in both situations. In Figure 2a and b the fitted values of the same models are shown. A slight clustering to the right side exists in both figures, but the variances seem to be equal in both sides of the plots reflecting homoscedastic values. Figures of Δ fat and Δ protein models were similar (results not shown).

DISCUSSION

The approach used in this study (RRTM) is based on within-animal comparison, which compares the actual versus the predicted production of a cow. Using this approach, the variability that is introduced because of differences between animals in different herds is eliminated. Studies that estimated production loss within cow by comparison of production before and after infection miss-estimated the loss, because of the effect of lactation stage on the cow-specific lactation curve as explained by Rajala-Schultz et al. (1999). Studies that estimated milk production loss due to subclinical mastitis showed low precision of the estimated loss (Hortet and Seegers, 1998). In two studies, the number of observations in the analysis was larger than the current study. Nevertheless, the current study estimated production loss more precisely, based on the reported standard errors in these two studies (Hortet et al., 1999; Koldeweij et al.; 1999).

The standardized Pearson residuals showed a slightly long tail to the right side (Figure 1a and b). Although this tail might have disturbed the normality, there is no dramatic deviation from normality. Normality tests could be very sensitive to residual values, which makes the graphical demonstration the reference test (Dohoo et al., 2003). Slight disturbance might have influenced the homoscedasticity because of more observations on the right side; otherwise the fitted values are quite homoscedastic (Figure 2a and b). Because the number of observation is different between the two presented analyses, comparison between model fits using the deviance or AIC would not be correct. Nevertheless, in both situations, the models appeared to fit the data adequately.

Fitting a specific LnSCC level in the models in Table 2 reveals the production loss at that level of LnSCC for milk, fat, and protein. Literature estimates of milk production loss for two-fold increase in crude SCC are 0.40 and 0.60 kg/day for primiparous and multiparous cows, respectively, as reviewed by Seegers et al. (2003), which is close to the estimates in this study (would be 0.38 and 0.46 kg/d for the same relationship). In this study, the clinical records were excluded from the analysis, consistent with Hortet et al. (1999) and Koldeweij et al. (1999). Reksen et al. (2007) in a recent pathogen-specific study estimated milk loss from cows with an intramammary infection. The study focused on comparing sparse and rich bacterial growth correcting for the clinical mastitis history of the cows. They found on average that primiparous and multiparous cows lose 0.30 and 0.66 kg milk per day corresponding with an increase of SCC to 200,000 cells/mL from the healthy level (< 50,000 cells/mL), which is close to the estimates of this study, but no estimates of precision in

relation to SCC were provided. Moreover, Reksen et al. (2007) did not estimate the fat and protein production loss.

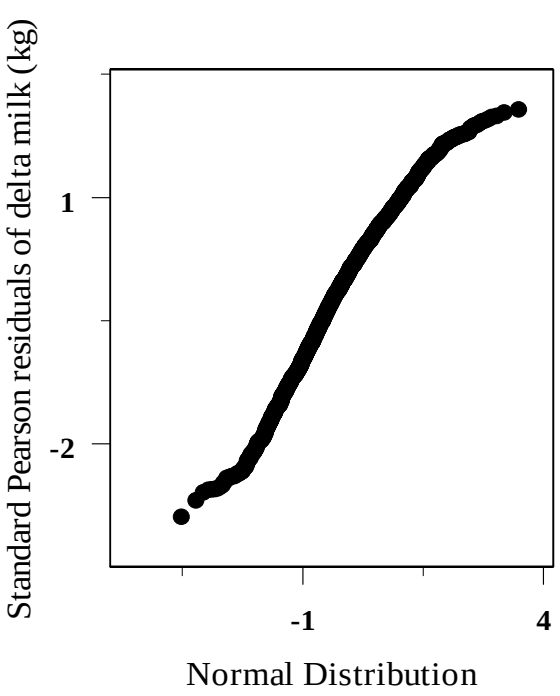


Figure 1a

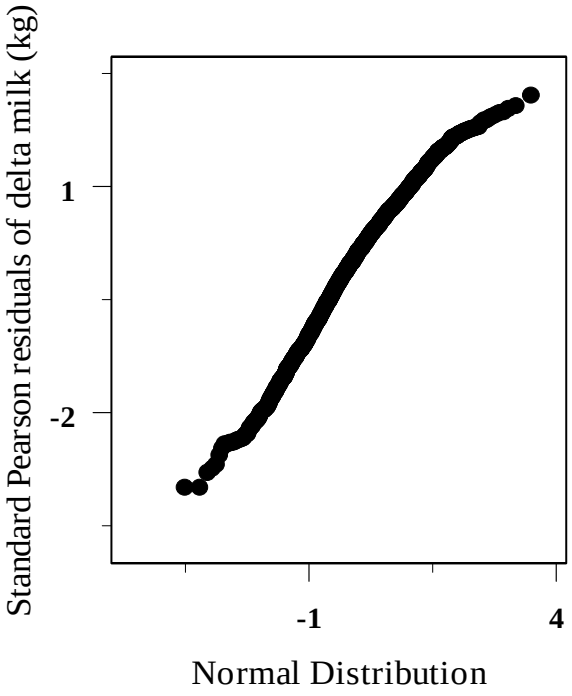


Figure 1b

Figure 1. Distribution of standard Pearson residuals of the change in milk (kg) production models at the new subclinical mastitis test-day (TD_i) based on new subclinical mastitis definition, which consider a case if SCC > 100,000 cells/mL at TD_i and was preceded by a test-day SCC < 50,000 cells/mL; a) unadjusted SCC values were used to select healthy cows at TD_{i-1}, records were 4382; b) only when dilution adjusted SCC values were used select healthy cows at TD_{i-1}, records were 2545.

There was no significant effect of DIM class and season of calving on the change of milk, fat, or protein production. This, most likely, was because predictions of milk, fat, or protein were already corrected for DIM and season of calving. TDInt classes did not affect the change in production significantly. This could indicate that the longer the TD interval the larger the total loss because we assume that the loss continues from halfway between TD_{i-1} and TD_i to halfway between TD_i and TD_{i+1}. The level of SCC increases seems the most determinant factor of the production loss (Table 3), as an indicator of the severity of inflammation (Schukken et al., 2003). The higher the SCC increase > 100,000 cells/mL, the higher the production loss (Table 3).

Fat and protein production were also affected negatively with a new case of subclinical mastitis. In many countries, farmers are paid for the fat and protein content of the milk, which makes fat and protein loss more economically important than the loss of kilograms milk. Primiparous and multiparous cows were estimated to lose around 6 and 10 g/d of fat, respectively, during a new subclinical mastitis with SCC 200,000 cells/mL (Table 2). The two subsets showed very similar predicted losses. At the same level of SCC increase, primiparous and multiparous cows are estimated to lose 9 and 13 g/d of protein, respectively. Previous research found fat and protein losses of 5 and 4 g/d, respectively (assuming a cow produces

25 kg milk per day) per two-fold increase in SCC, regardless of the parity of the cow and ignoring other risk factors (Hortet and Seegers, 1998). Koldeweij et al. (1999) found a protein loss of 42 and 67 g/d for a one-fold increase in $\log_{10}$ SCC for primiparous and multiparous cows, respectively, which is close to the estimates in the current study.

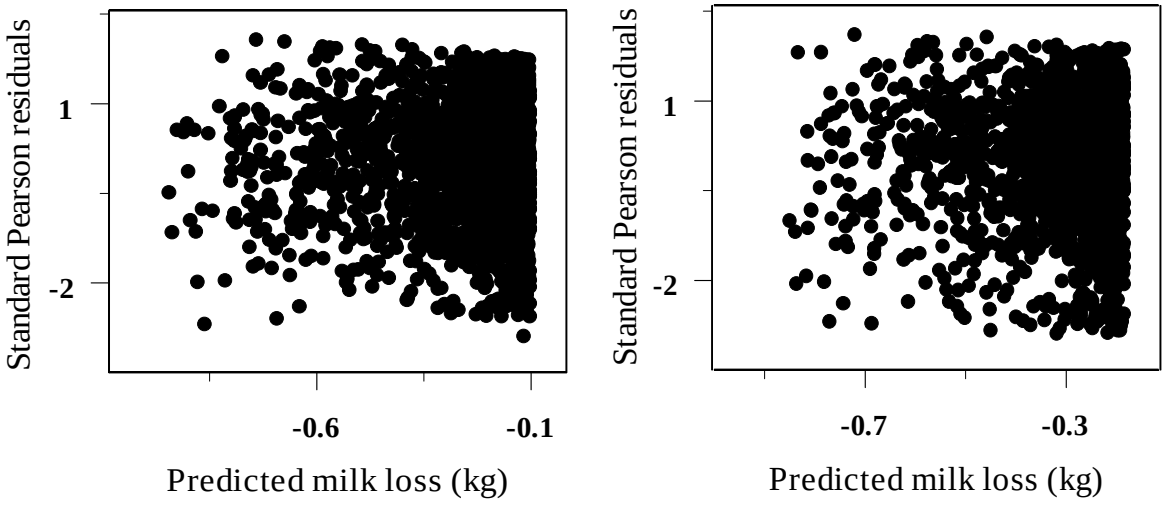


Figure 2a Figure 2b

Figure 2. Fitted values of the change in milk (kg) production models at the new subclinical mastitis test-day (TD_i) based on new subclinical mastitis definition, which consider a case if SCC > 100,000 cells/mL at TD_i and was preceded by a test-day SCC < 50,000 cells/mL; a) unadjusted SCC values were used to select healthy cows at TD_{i-1}, records were 4382; b) only when dilution adjusted SCC values were used select healthy cows at TD_{i-1}, records were 2545.

The estimated coefficient lambda in the current study was very close to the value (-0.485) found by Green et al. (2006). The coefficient means that for a kilogram higher milk yield than 10 kg/d, for cows that had SCC < 50,000 cells/mL, the SCC is underestimated by 0.491×10^3 cells/kg milk because of dilution. Therefore after adjustment, the SCC could be > 50,000 cells/mL at the TD_{i-1}. The predicted milk production loss based on SCC count adjusted to dilution effect was slightly lower than the predicted loss when unadjusted SCC was used (Table 3). However, both results were presented to allow economic calculations to further investigate the importance of considering dilution effect. Roughly milk production loss was 13 % less using the adjusted SCC compared to the crude SCC, which is in close agreement with Green et al. (2006) who found that production loss would be overestimated by 15 % for crude SCC.

A general debate about the definition of a healthy cow in relation to SCC level can be inferred from the literature. Hillerton (1999) considered an udder healthy if SCC < 100,000 cells/mL. Djabri et al. (2002) found that the average SCC for culture-negative quarters was 68,000 cells/mL. Seegers et al. (2003), Leitner et al. (2003), and Hamann (2005) considered an udder healthy if SCC < 50,000 cells/mL. We assumed a new subclinical mastitis case at a specific TD if the SCC > 100,000 cells/mL at that TD, which was preceded by healthy TDs,

where the SCC was always < 50,000 cells/mL. This seems consistent with recent literature on the definition of a healthy udder. The definition considered a subclinical case if a low SCC TD was followed by a high SCC TD within parity. This means by definition that a cow must initiate the lactation with a low SCC TD to be a new case of subclinical mastitis at the subsequent TD. This selection bias might have caused an underestimation of the loss because cows that start the lactation with high SCC TD were excluded. The analysis included only the first subclinical mastitis case per cow as indication of new subclinical mastitis. This was imposed because we wanted to calculate the change in production based on the RRTM prediction of a healthy udder only.

CONCLUSION

Random regression test-day modeling is a useful method to estimate effects of a disease on production. There was a significant loss in milk, fat, and protein production of dairy cows with new subclinical mastitis and the predicted production losses were more precise than earlier studies. The magnitude of losses was mainly determined by the SCC elevation of the new subclinical mastitis. The predicted losses were slightly lower when the SCC dilution effect was considered in the definition of healthy cows.

Acknowledgement

The authors acknowledge the Dutch Udder Health Center (UGCN), CR-Delta and the NRS for providing the necessary data, Bart van den Borne, Jan van den Broek, and Hans Vernooij for assistance with the data analysis.

REFERENCES

De Graaf, T., and R. H. Dwinger. 1996. Estimation of milk production losses due to sub-clinical mastitis in dairy cattle in Costa Rica. Prev. Vet. Med. 26:215–222.

De Roos, A. P. W., A. G. F. Harbers, and G. De Jong. 2004. Random herd curves in a test-day for milk fat and protein production of dairy cattle in the Netherlands. J. Dairy Sci. 87:2693–2701.

De Roos, A. P. W., and G. De Jong. 2006. Genetic parameters of test-day milk urea in Dutch dairy cattle. In Proc. 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte, MG, Brazil.

Djabri, B., N. Bareille, F. Beaudeau, and H. Seegers. 2002. Quarter milk somatic cell count in infected dairy cows: a meta-analysis. Vet. Res. 33:335–357.

Dohoo, I. R., S. W. Martin, and H. Stryhn. 2003. Veterinary Epidemiological Research. Atlantic Veterinary College Inc., Charlottetown, Prince Edward Island, Canada.

Green, L. E., Y. H. Schukken, and M. J. Green. 2006. On distinguishing cause and consequence: Do high somatic cell counts lead to lower milk yield or does high milk yield lead to lower somatic cell count. Prev. Vet. Med. 76:74–89.

Halasa, T., K. Huijps, O. Østerås, and H. Hogeveen. 2007. Economic effects of bovine mastitis and mastitis management: a review. Vet. Q. 29:18–31.

Hamann, J. 2005. Diagnosis of mastitis and indicators of milk quality. Page 82 in Proc. 4th International Mastitis Seminar, IDF, Mastitis in Dairy Production, Current Knowledge and Future Solutions. Wageningen Academic Publisher, Wageningen, The Netherlands.

Hillerton, J. E. 1999. Redefining mastitis based on somatic cell count. IDF Bulletin 345:4–6.

Hortet, P., and H. Seegers. 1998. Calculated milk production losses associated with elevated somatic cell counts in dairy cows: a review and a critical discussion. Vet. Res. 29:497–510.

Hortet, P., F. Beaudeau, H. Seegers, and C. Fourichon. 1999. Reduction in milk yield associated with somatic cell counts up to 600 000 cell/mL in French Holstein cows without clinical mastitis. Livest. Prod. Sci. 61:33–42.

Jamrozik, J., and L. R. Schaeffer. 1997. Estimates of genetic parameters for a test day model with random regression for yield traits of first lactation Holsteins. J. Dairy Sci. 80:762–770.

Koldeweij, E., U. Emanuelson, and L. Janson. 1999. Relation of milk production loss to milk somatic cell count. Acta Vet. Scand. 40:47–56.

Leitner, G., R. Eligulashvily, O. Krifucks, and A. Saran. 2003. Immune cell differentiation in mammary gland tissues and milk of cows chronically infected with *Staphylococcus aureus*. J. Vet. Med. B 50:45–52.

Rajala-Schultz, P. J., Y. T. Gröhn, C. E. McCulloch, and C. L. Guard. 1999. Effects of clinical mastitis on milk yield in dairy cows. J. Dairy Sci. 82:1213–1220.

Reksen, O., L. Sølverød, and O. Østerås. 2007. Relationships between milk culture results and milk yield in Norwegian Dairy cattle. J. Dairy Sci. 90:4670-4678.

SAS Institute. 2004. User's Guide. SAS Institute, Inc., Cary, NC, USA.

Schepers, A. J., T. J. G. M. Lam, Y. H. Schukken, J. B. M. Wilmink, and W. J. A. Hanekamp. 1997. Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters. J. Dairy Sci. 80:1833–1840.

Schukken, Y. H., D. J. Wilson, F. Welcome, L. Garrison-Tikfsky, and R. Gonzalez. 2003. Monitoring udder health and milk quality using somatic cell count. Vet. Res. 34:579–596.

Seegers, H., C. Fourichon, and F. Beaudeau. 2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet. Res. 24:475–491.

Swinkels, J. M., H. Hogeveen, and R. N. Zadoks. 2005. A partial budget model to estimate economic benefits of lactational treatment of subclinical *Staphylococcus aureus* mastitis. J. Dairy Sci. 88:4273–4287.

Whist, A.C., and O. Østerås. 2007. Association between somatic cell counts at calving or prior to dry off and clinical mastitis in the remaining or subsequent lactation. J. Dairy Sci. 74:66-73.

APPENDIX

The RRTM used data of approximately 1000 herds in the Netherlands to predict the milk, fat, and protein production for the study data set.
The model included fixed, random, and random regression effects as follows:

Effects	Variables included
Fixed	Parity ×Days in milk
	Parity × Age at calving × Month of calving × Rolling 3-years of calving × Lactation stage
	Parity × Age at calving × Rolling year of calving
	Parity × Stage of pregnancy × Lactation stage
	Parity × Length of dry period × Lactation stage
	Parity × % heterosis × Lactation stage
	Parity × % recombination × Lactation stage
	Year × Week of test
Random	Herd × Test date
Random	Herd × Rolling year of test (lactation curves for parity (1, 2, and ≥ 3)
regression	Additive genetic effect for cow (lactation curves for parity (1, 2, and ≥ 3)
	Common permanent environment of cow (lactation curves for parity (1, 2, and ≥ 3)
	Lactation specific permanent environment of cow (lactation curves for parity (3, 4, 5, and higher parities)

Differences between cows are described by the fixed and the additive genetic effects in the model. The random effect describes the day to day variation within a herd. The random regression effect of herd × rolling year of test describes herd specific lactation curves that both model differences in level of production between herds as well as differences in shape of the lactation curve between herds. The lactation curves of individual cows are described by an additive genetic effect and two non-genetic effects (the common and lactation specific permanent environment effects). The cow's genetic and permanent environmental effects are also random regression effects, so they describe not only differences in level of production in each lactation, but also in shape of the lactation curve (persistency).

The accuracy of prediction is high because:

- Large number of cows in the population is used to estimate the fixed effects, so fixed effects are described in very great detail and high accuracy.

- Records from herd mates that are not in the current trial are used to estimate the herd test date effect, i.e. the day to day variation and/or seasonal patterns within the herd.

- The additive genetic effect of the cow is used to predict production and level of production, shape of the lactation curve and progress in production across lactations.

- Additive genetic effects of cows are estimated accurately because most cows have sires with many progeny and heritabilities for milk production traits are high.

- By taking into account the permanent environmental effect of the cow, the level and shape of the lactation curve in lactations previous to the current trial is taken into account in the prediction.

- The herd and cow effects are modeled with random regression effects, so variances and correlations within and across lactations are taken into account.

Chapter 4

Stochastic bio-economic model of bovine intramammary infection

T. Halasa[a,b,*], M. Nielen[a], R.B.M. Huirne[b], H. Hogeveen[a,b]

[a] Department of Farm Animal Health and Reproduction, Utrecht University, P.O. Box 8015, 3584 CL Utrecht, The Netherlands

[b] Business Economics Group, Wageningen University, P.O. Box 8130, 6706 KN Wageningen, The Netherlands

Accepted for publication in Livestock Science

Abstract

Although the dynamics of transmission play an important role in the occurrence of intramammary infection (IMI), they have not been considered in previous models used to estimate the cost of IMI. The bio-economic model described includes within-herd dynamics of pathogen-specific IMI. The model simulated *Staphylococcus aureus, Streptococcus uberis, Streptococcus dysgalactiae,* and *Escherichia coli* IMI stochastically and estimated the cost of these IMI in a herd of 100 dairy cows in a situation where a quota is applied to milk production. A Reed-Frost model was used for *Staph. aureus, Strep. uberis,* and *Strep. dysgalactiae* IMI and a Greenwood model for *E. coli* IMI. Economic analysis was conducted per pathogen for clinical and subclinical IMI. The parameters used in the model were based on the literature and were deemed credible and valid. Median annual incidence of clinical and subclinical IMI for all pathogens varied considerably. This variation was greatest for *Staph. aureus* IMI. The annual incidence of IMI in a herd of 100 dairy cows caused by *Staph. aureus* varied between 0 and 88 cases, with a median of 5 cases and the 5th and 95th percentiles of 0 to 36 for clinical IMI, and a median of 7 cases with the 5th and 95th percentiles of 0 to 52 for subclinical IMI. In consequence, the average total annual net costs also varied widely for *Staph. aureus* IMI. Clinical IMI costs were € 1375, with the 5th and 95th percentiles of 0 to 4716 and subclinical IMI costs were € 1219, with the 5th and 95th percentiles of 0 to 4030. The average annual net cost due to the 4 simulated pathogens combined was € 4896 and varied from € 915 to € 11,287 in a herd of 100 dairy cows. The bio-economic model developed for this study will be utilized as a tool to investigate the economic impact of management of pathogen-specific IMI.
Keywords: Bio-economic model; Dynamic; Intramammary infection; Stochastic; Dairy cows.

INTRODUCTION

Bio-economic modeling can be defined as the integration of economic models with the dynamics of biological processes such as diseases. Such simulation models have been developed for bovine mastitis (Allore and Erb, 1999; Østergaard et al., 2005) to assess the economic impact on diseases of changes in management in farming systems.

Pathogens that cause intramammary infection (IMI) through cow-to-cow contact, such as *Staphylococcus aureus* can be described using a Reed-Frost model (Becker, 1989; Zadoks et al., 2001a). The Reed-Frost model assumes that the probability of infection of a susceptible cow is dependent on the number of infected cows in the herd to which it is exposed (Becker, 1989; Zadoks et al., 2001a). The dynamics of the spread of pathogen-specific IMI between cows can be represented by the transmission rate parameter (Becker, 1989). Modeling of pathogen-specific IMI transmission, allows proper representation of IMI over time (Østergaard et al., 2005). In the case of environmental IMI, such as *Escherichia coli*, a Greenwood model can be used to describe the infection process (Becker, 1989). This model assumes that, once the infectious agent is present in a population, the probability of infection is independent of the number of IMI cows. In this case, prevalence or incidence of the disease

is most commonly used to represent the probability of new IMI per unit of time (Becker, 1989).

Previous bio-economic models used the herd-level prevalence of pathogen-specific IMI, adjusted for IMI risk factors, as the probability of IMI per unit of time (Allore and Erb, 1998; Seegers et al., 2000; Østergaard et al., 2005). The main constraint of these models was the use of prevalence as a basic input to model transmission instead of a pathogen-specific transmission rate parameter. Transmission drives the spread of contagious IMI between cows and this influences the variation of herd-level total cost. Insight in dynamics of contagious IMI is an important prerequisite to evaluate treatment decisions especially treatment of subclinical IMI, which could limit the spread of these IMI between cows (Zadoks et al., 2002).

Allore and Erb (1999) proposed a basic transition state model that consisted of susceptible, infectious clinical and infectious subclinical states. The probability of infection in the proposed model depended on the number of infected animals, the transmission rate of pathogen-specific IMI and the total number of dairy cows in the herd. However, the model was not implemented because of the absence of estimates of transmission rates of IMI pathogens at that time. Later research provided estimates of transmission rates of pathogen-specific IMI, which now make it possible to implement a dynamic bio-economic model of IMI. Such a model could provide more accurate estimation of the variation of pathogen-specific IMI cost in dairy herds (Swinkels et al., 2005a; Steeneveld et al., 2007). Consequently, this is expected to provide a more accurate estimation of the cost-effectiveness of pathogen-specific IMI control procedures than previously published estimates (Swinkels et al., 2005b).

This paper presents a stochastic and dynamic bio-economic model to calculate the cost of pathogen-specific IMI in a herd of 100 dairy cows. The infection dynamics were modeled on the basis of pathogen-specific transmission rate parameters.

MATERIALS AND METHODS

A pathogen-specific discrete-event model was developed to simulate the dynamics of IMI during a single quota year. The stochastic model simulates the dynamics of IMI in a herd, caused by *Staph. aureus*, *Strep. uberis*, *Strep. dysgalactiae*, or *E. coli*. As suggested by Allore and Erb (1999), a Reed-Frost SIS representation was used to model the dynamics of IMI caused by *Staph. aureus*, *Strep. uberis*, and *Strep. dysgalactiae*. A Greenwood model was developed to study the dynamics of *E. coli* IMI (Becker, 1989). The model was written in Mathematica 6.0 (Wolfram Research, 2007) and each time interval in the model was 2 weeks. This 2 week time period was chosen because a clinical IMI would be considered as a new IMI when it appeared 2 weeks later than the previous clinical IMI (Grohn et al., 2004). Moreover, a 2 week time step was convenient to estimate persistent effects in the course of a lactation, such as milk production loss due to clinical IMI. Additionally, the bulk tank somatic cell count is usually reported every 2 weeks, which facilitate calculating penalties.

Modeling IMI dynamics

The IMI model defined one of two states for every cow per 2 week time period. A cow was either free of IMI and considered susceptible, or infected (Figure 1). The presence or absence of IMI depended on the probability of obtaining IMI as calculated at each time period. Each IMI cow was considered to be infectious (the shaded area in Figure 1) as long as it did not recover bacteriologically. After recovery, a cow became susceptible again (Zadoks et al., 2001b). Clinical IMIs were assumed to have been treated with antibiotics and either changed status within the same time period to bacteriological recovery, or persisted as subclinical IMI until the next time period, on the basis of a pathogen-specific IMI recovery probability (Table 1).

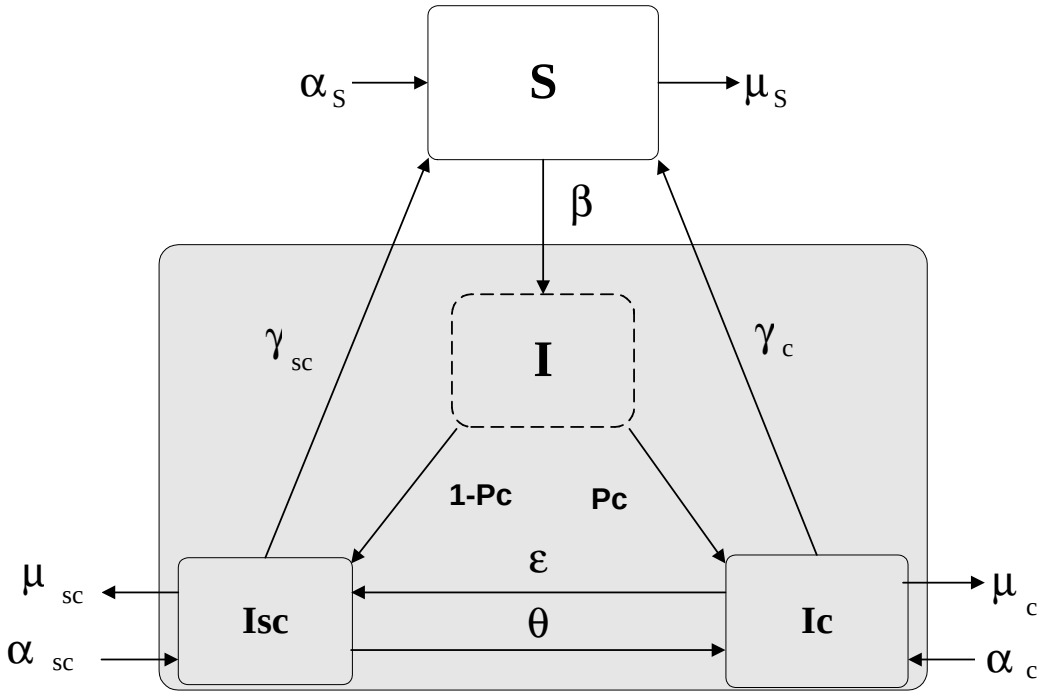


Figure 1. Outline of transmission model of pathogen-specific IMI using the pathogen-specific parameters of transmission rate (β), recovery rate from clinical IMI (γ_c) and subclinical IMI (γ_{sc}), flare up rate (θ), remission rate (ε), culling rate (μ) from the susceptible population (μ_S), from the clinical IMI population (μ_c), from the subclinical IMI population (μ_{sc}), replacement rate (α), of cows culled and to compensate loss of milk production, introduced to the susceptible population (α_S), to the clinical IMI population (α_c), to the subclinical IMI population (α_{sc}), and the proportions of animals that develop clinical IMI (Pc) or subclinical IMI (1-Pc), with IMI (I), subclinical IMI (Isc), clinical IMI (Ic) and susceptible (S) states. The model represents *Staph. aureus*, *Strep. uberis*, *Strep. dysgalactiae*, and *E. coli* IMI. For *E. coli* IMI the cumulative incidence per 14 cow-days at risk was used to replace the transmission rate parameter.

The dynamics of IMI were defined by various parameters. These were the transmission rate (β), recovery rate of clinical IMI (γ_c) and subclinical IMI (γ_{sc}), flare up rate (θ), and remission rate (ε) (Figure 1). The proportion of animals that developed clinical IMI (Pc) or subclinical IMI (1-Pc) per pathogen was determined on the basis of data from the literature cited in Table 1. Default values for these parameters are presented in Table 1, together with the reference. The herd level parameters were also dynamic and were represented by the rate of culling (μ) from the susceptible population (μ_S), from the clinical IMI population (μ_c), and from the subclinical IMI population (μ_{sc}), as well as the rate of replacements (α), of cows

culled and to compensate loss of milk production, introduced to the susceptible population (α_S). Any replacement cow can, in fact, be susceptible (α_S), clinical (α_c), or subclinical (α_{sc}) IMI. The transmission rate parameters were re-calculated from the original studies (cited in Table 1) per 14 cow-days at risk in a steady state.

Each susceptible cow during each time period was at risk of becoming a new IMI cow. This was determined in 2 steps during each time period to prevent underestimation of *E. coli* IMI. Firstly, the susceptible cow was at risk to become a new *E. coli* IMI cow. Secondly, if the susceptible cow escaped becoming a new *E. coli* IMI, it was at risk of becoming a new IMI cow with *Staph. aureus*, *Strep. uberis*, or *Strep. dysgalactiae*. Details of assigning new IMI cows are explained below.

Whether susceptible cow *i* became an *E. coli* IMI during time period *t* was determined on the basis of the following equation

$$IMI_{ECi}(t) = Binomial[1, P_{EC}] \quad (1)$$

Where IMI_{ECi} (t) is the *E. coli* IMI status of cow *i* during time period *t* with *t* = 14 cow-days at risk. The status was determined using a binomial function with P_{EC} as the probability of a susceptible cow *i* becoming a new *E. coli* IMI cow, this probability being based on the cumulative incidence of *E. coli* per 14 cow-days at risk. The probability was similar for all susceptible cows.

The probability that a susceptible cow would not escape IMI caused by *Staph. aureus*, *Strep. uberis*, or *Strep. dysgalactiae* differed from one time period to the next. The overall probability of becoming a new IMI cow caused by any of these 3 pathogens during time period *t* (PI (*t*)) was calculated on the basis of the following equation

$$PI(t) = 1 - Exp(-(\frac{\beta_{SA} I_{SA(t-1)}}{N_{t-1}} + \frac{\beta_{SU} I_{SU(t-1)}}{N_{t-1}} + \frac{\beta_{SD} I_{SD(t-1)}}{N_{t-1}})) \quad (2)$$

β_{SA}, β_{SU}, and β_{SD} were the transmission rate parameters of *Staph. aureus* (SA), *Strep. uberis* (SU), and *Strep. dysgalactiae* (SD), respectively. $I_{SA(t-1)}$, $I_{SU(t-1)}$, and $I_{SD(t-1)}$ were, respectively, the number of infectious cows with *Staph. aureus*, *Strep. uberis* and *Strep. dysgalactiae* during the previous 2 week time period (*t*-1), and N was the total number of lactating cows in the herd during the previous time period (*t*-1).

Each specific susceptible cow *i* could be assigned to be a new IMI cow with *Staph. aureus*, *Strep. uberis*, or *Strep. dysgalactiae* during time period *t* according to the following equation

$$IMI_i(t) = Binomial[1, PI(t)] \quad (3)$$

Where IMI_i (*t*) is the IMI status of cow *i* during time period *t*, which depended on the probability that a susceptible cow *i* would become a new IMI cow during time period *t* (PI (*t*)) using a binomial function.

Table 1. The transmission rate parameter (β), recovery rate (γ) from clinical IMI (γ_c), from subclinical IMI (γ_{sc}), flare up rate (θ), remission rate (ε), proportion of infections that develop clinical IMI (PC) and subclinical IMI (1-PC) per 14 days together with the lower and upper limits (for the sensitivity analysis) as obtained from the corresponding reference(s).

Pathogens and Parameters	Rate Values[1]	Lower limit	Upper limit	Reference
Staph. aureus				
β	0.25			Zadoks et al. (2002)
γ_c	0.35	0.14	0.50	Sol et al. (2000)
γ_{sc}	0.10	0.06	0.68	Zadoks et al. (2002); Swinkels et al. (2005b)[5]
				Swinkels et al. (2005b)
θ	0.12	0.08	0.13	
ε	1- γ_c	-[2]	-	Swinkels et al. (2005b)
PC	0.17	0.12	0.23	
Strep. uberis				
β	0.21			Zadoks et al. (2001a)
γ_c	0.83	0.80	0.86	Zadoks et al. (2001b)
γ_{sc}	0.11	0.05	0.34	Swinkels et al. (2005a)
θ	0.10	0.09	0.18	Swinkels et al. (2005a)
ε	1- γ_c	-	-	
PC	0.32	0.15	0.48	Zadoks et al. (2003)
Strept. dysgalactiae				
β	0.21			Zadoks et al. (2001a)
γ_c	0.67	0.50	0.83	McDougall (1998)
γ_{sc}	0.13	0.05	0.28	Swinkels et al. (2005a)
θ	0.10	0.08	0.15	Swinkels et al. (2005a)
ε	1- γ_c	-	-	
PC	0.51	0.45	0.60	Swinkels et al. (2005a)
E. coli				
CI[3]	0.002			Barkema et al. (1998)
γ_c	0.80	0.70	1.00	Golodetz (1983); Hill et al. (1978)[4]
γ_{sc}	0.90	0.8	1.00	Hogan et al. (1994)[4]
θ	0.05	0.01	0.10	Döpfer et al. (1999)[4]
ε	1- γ_c	-	-	
PC	0.85	0.80	1.00	Hogan et al. (2003)[4]

[1] Values per 14 cow-days at risk.
[2] Value was equal to the 1- γ_c of the opposite γ_c limit.
[3] Cumulative incidence per 14 cow-days at risk.
[4] Approximation based on the reference.
[5] Calculations based on the average estimate from both references

For each susceptible cow that was determined to be a new IMI cow in a particular time period *t* (equation 3), the probability of becoming specifically either a *Staph. aureus*, or *Strep. uberis* or *Strep. dysgalactiae* new IMI cow, was based on the proportion of each of these pathogens in the herd during the previous time period (*t*-1) in a discrete function. Thus, the probability of a new IMI cow becoming a specific *Staph. aureus* new IMI cow during time period *t* ($P_{SA}(t)$) was:

$$P_{SA}(t) = \frac{\beta_{SA} I_{SA(t-1)}}{\beta_{SA} I_{SA(t-1)} + \beta_{SU} I_{SU(t-1)} + \beta_{SD} I_{SD(t-1)}} \quad (4)$$

The probabilities of becoming new *Strep. uberis* or *Strep. dysgalactiae* IMI cows were calculated similarly, with different numerators, but the same denominator. Abbreviations used for parameters are the same as in equation 2.

The approach via equations 2, 3, and 4 to modeling IMI was assumed to represent a valid structure of contact between cows, and offered the possibility of modeling pathogen-specific IMI transmission, while providing for the inclusion of competition between pathogens in both endemic and epidemic situations (Bremermann and Thieme, 1989; Pugliese, 2002). Because *E. coli* IMI was based on a Greenwood model, new *E. coli* IMI was assumed to be independent of the other contagious IMI. The model assumed that a cow could only be infected by one IMI pathogen at a time.

Herd dynamics

Modeling cows, milk production and quota

In the current paper, an initial herd with 100 dairy cows was generated. A specific parity number, calving season and lactation length were assigned to each cow, based on different random distributions. Parity was based on a multidiscrete distribution with the probability of being parity 1, 31%; parity 2, 25%; and parity 3 or higher, 44%. Calving season was based on a uniform distribution to represent seasons through spring to winter. Lactational length was based on the probability of conception after insemination using a geometric distribution with probability of conception = 0.62 (Østergaard et al., 2005). The geometric distribution would indicate the number of inseminations before conception. The number of extra time periods before conception was calculated based on the number of extra inseminations before conception. A lactation stage of 0 to a maximum of 36 stages, representing the periods in lactation for each cow (14 days per stage), was based on the calving season and the lactational length using a uniform distribution, where 0 denotes the dry period. The length of the dry period was set at 8 weeks. A lactational milk yield (LMY_i) was randomly assigned to each *i* cow based on normal distribution (8500, 1500) kg milk (NRS, 2005), and adjusted to the parity and calving season of the cow (Van Arendonk, 1985). The characteristics of the simulated herd represent typical Dutch figures of dairy cows (NRS, 2005). The distribution of parities in the initial herd is subjected to changes during the simulated year according to culling and replacement as explained in the next section.

The milk yield of each individual cow *i* during the lactation stage *LS* was calculated by drawing the lactation curve of each cow, and then partitioning this curve over the different lactation stages of each cow according to equation 5.

$$AMY_i(LS) = \begin{cases} 0, if; Cull = 1 \\ 0, if; LS = DP \\ \dfrac{LMY_i \times a \times (LS_i)^b \times e^{-c \times LS_i}}{14 \times \sum_{LS=1}^{22} (a \times (LS_i)^b \times e^{-c \times LS_i})} - PL_i, if; Cull = 0 \\ i = 1, \ldots, R_t \\ LS = 1, \ldots, DP \\ t = 1, \ldots, 26 \end{cases} \quad (5)$$

Where AMY_i (LS) is the actual milk yield of cow i during the lactation stage LS, from stage 1 to the next dry period (DP) of that cow. The slope of the lactation curve during the different lactation stages of cow i was represented by a, b and c, or Wood's constants (Wood, 1976). Cull is whether the cow was culled, R_t is the total number of lactating cows including the replacement heifers during time period t, and 14 represents the number of days per lactation stage LS. PL_i is the milk production loss of cow i in a case of clinical or subclinical IMI as explained below.

Milk production loss and carry over effects due to clinical IMI were calculated as described by Grohn et al. (2004). In this study, the milk production loss was modeled in proportion to the milk production of the cows to correct for the high producing cows in the reference study. On average, the production of cows in the reference study was 15% higher than the production of cows in the current model, and hence the production loss was reduced by 15%. Because the infectious status of the cow in the current model was known and because it was believed that the production loss prior to a clinical IMI was due to a subclinical IMI, production loss before the clinical IMI was not considered, but was assumed to be captured by subclinical IMI losses. This was carried out similarly to Østergaard et al. (2005) to prevent overestimation of the production loss. A categorical variable, indicating the time period after clinical IMI onset was generated and updated at each time period in the model. This history variable was used to include the carry over loss due to the clinical IMI per pathogen based on the time since clinical IMI occurrence. When a cow had a clinical IMI for the second time before the carry over period of the previous clinical IMI was finished, the loss was assumed to be due to the new clinical IMI as also found by Bar et al. (2007). The milk withdrawal period during and after the treatment was set at 6 days, and during this period the milk was assumed to be discarded (Huijps and Hogeveen, 2007).

Milk production loss due to subclinical IMI was calculated in the same way as by Halasa et al. (2009). When a cow had subclinical IMI after a clinical IMI, in order not to overestimate the loss, only carry over effects due to the clinical IMI were considered in calculating the production loss (Seegers et al., 2003).

The quota situation in the herd during the simulated year was modeled with an approach similar to that of Houben et al. (1994) and Seegers et al. (2000). A total actual milk yield (TAMY) during time period t in the simulated year was calculated for all lactating cows in the herd during each time period t, based on the following equation:

$$TAMY(t) = \begin{cases} \sum_{i=1}^{R} AMY_i(t) \\ t = 1, \ldots, 26 \end{cases} \quad (6)$$

The AMY_i (t) represented the actual milk production of cow i during time period t from equation 5, R was the total number of lactating cows including the replacement heifers during time period t.

A quota was set to be the sum of all cow-specific LMY assigned to the herd at the start, to be attained by the end of the quota year. An expected milk yield (EMY) was calculated per time period for all cows at the start, without the effects of culling and IMI on production (see equation 5), but only as a technical variable to represent the individual milk production. The total EMY (TEMY) during time period t was calculated as the sum of the EMY in a similar way to equation 6. The TEMY would represent the amount of milk that should be produced in each time period t to reach the full quota by the end of the year in question.

The amount of milk that was deficient during time period t (from what the cows should have produced in that time period to fulfill the quota by the end of the quota year) was calculated as TAMY minus TEMY. This variable represented the quota deficiency during each time period t. The quota deficiency was accumulated over the time periods to represent the cumulative number of kilograms milk deficient over time in relation to the quota by the end of the quota year.

Modeling replacement and culling

During the simulated year, replacement and culling could occur at each time period. The introduction of fresh heifers was based solely on the cumulative quota deficiency. When the cumulative quota deficiency at time period t was more than the production of an average cow in the herd, a new heifer entered the process in the next time period with an average production of 23 kg/day and a 97% probability of being susceptible versus a 3% probability of being an IMI cow by any of the 4 IMI pathogens (Zadoks et al., 2002). Owing to the absence of pathogen-specific probability of IMI for the replacement heifers, an equal chance of infection with the different IMI pathogens was assumed. In this model, the farmer was assumed to introduce heifers to compensate the decreased milk production due to IMI and culling. It was also assumed that the replacement heifer would be available within 2 weeks.

Culling was included in the model as culling due to clinical IMI (including risk of death) or subclinical IMI adjusted to production level, due to conception failure or to other reasons. The probability of a primiparous clinical IMI cow being culled was 0.031 (0.01-0.08) per 2 weeks, and 0.067 (0.04-0.12) for a multiparous clinical IMI cow (Houben et al., 1994). Subclinical IMI cows had a probability of being culled of 0.02 (0.01-0.05) per 2 weeks (Beck et al., 1992). The limits in parenthesis represent a realistic variation based on the original studies. When an IMI cow produced less than the average of its fellow members of the herd, probabilities of culling due to clinical and subclinical IMI were increased by 50%. When the cow was an IMI cow for more than one time period, the IMI cow was subjected to the same clinical and subclinical IMI probabilities of culling for each IMI time period, consistent with

recent findings of Bar et al. (2008). The probability of culling due to other reasons was assumed to be 0.01 per 2 weeks (Hadley et al., 2006).

Modeling bulk tank somatic cell count

Healthy cows were assigned a natural logarithmic score of somatic cell count × 10^3 from a normal distribution (N~3.34, 0.43) based on Halasa et al. (2009), resulting in healthy cows always having somatic cell count < 50 × 10^3 cells/ml. Subclinical IMI cows had a score (N~5.58, 0.84) based on Halasa et al. (2009). Subclinical IMI cows would thus on average have a somatic cell count of 265 × 10^3 cells/ml, but with a large variability. Because the effects of different pathogens on somatic cell count of subclinical IMI cows differ only slightly from each other (Reksen et al., 2008), the same distribution was used to represent the effect of all pathogens modeled. The natural logarithmic score of SCC for clinical IMI cows, excluding the withdrawal period, was set at 6.40 based on De Haas et al. (2002).

The bulk tank somatic cell count (BTSCC) and the geometric mean of BTSCC (GBTSCC) were modeled during each time period *t* according to the following equations:

$$BTSCC(t) = \begin{cases} \dfrac{\sum_{i=1}^{R} (Exp(SCS_i(t)) \times AMY_i)}{TAMY(t)/R} \\ i = 1,...,R \\ t = 1,...,26 \end{cases} \quad (7)$$

$$GBTSCC(t) = \begin{cases} Exp\left(\dfrac{\sum_{t-2}^{t} Log(BTSCC(t))}{3}\right) \\ t = 3,...,26 \end{cases} \quad (8)$$

Where SCS_i is the somatic cell score, which represent the natural logarithm of somatic cell count × 10^3 of cow *i* during time period *t*, AMY_i is the actual milk yield of cow *i* during time period *t*, TAMY is the total actual milk yield during time period *t*, and *R* is the total number of lactating cows including the replacement heifers during time period *t*.

Modeling economic effects

The economic losses due to pathogen-specific IMI were calculated in a quota year in accordance with previous recommendations (Halasa et al., 2007), and Dutch market circumstances were used by way of illustration. Default values of prices included in the model are presented in Table 2 along with the references cited in the footnote to the table.

Annual net cost of milk yield loss, feed, and penalty due to high BTSCC

In a quota situation, the production potential of the farm is the quota and not the number of animals. This means that decreased production due to IMI can be compensated by milking extra cows or changing the feed regime so that cows produce more kilograms of milk (Halasa et al., 2007).

The cost of milk yield loss due to pathogen-specific IMI was calculated according to the following equation:

$$CMYL(P) = \sum_{t=1}^{26} \sum_{i=1}^{R} \left(\frac{PL_{Pti}}{\Pr H_P}\right) \times (PH + FC - RFC + OC_P) \quad (9)$$

Where CMYL(*P*) is the cost of milk yield loss due to IMI caused by pathogen *p*, *Staph. aureus*, *Strep. uberis*, *Strep. dysgalactiae* and *E. coli*, PL*pti* is the milk production loss of cow *i* caused by pathogen *p* during time period *t*, PH is the price of a heifer depreciated over 3 years to replace the milk production loss caused by pathogen *p*, PrH_P is the milk production of the replacement heifer, and FC is the feed cost necessary to maintain the replacement heifer during that lactation, RFC is the reduction in feed cost that might occur because IMI cows will produce less and therefore would be fed less. The amount of silage was assumed to be fixed in the herd and sufficient to satisfy a production of up to 23 kg per cow per day (CVB, 2005). The changes in necessary energy were assumed to be covered with additional concentrate. The OC_P is other costs due to IMI caused by pathogen *P* including extra labour and housing for the replacement heifer, which were assumed to be 0 because it was assumed that labor and space were available for the replacement heifers. The CMYL(*P*) was calculated separately for clinical and subclinical IMI. Because the production loss was calculated at each time period in the model corresponding to the infection status of the cows present, as soon as a cow was culled the production loss and milk production of the culled cow were forced to 0 (see equation 5).

Table 2. Default values of costs, prices and lower and upper limits for the sensitivity analysis of parameters used in the economic calculations.

Economic parameters	Value	Lower Limit	Upper Limit
Price concentrate, €/kg	0.25[1]	0.21	0.28
Price antibiotics, €/case	40[2]	30	50
Price of new heifer, €	1000[1]	900	1200
Veterinary service, €/case	7.5[2]	6	20
Labour hourly wage, €	18[2]	16	22
Time to treat clinical case, hours			
Penalty for high BTSCC, €/kg	2[2]	1.5	2.5
	0.045[3]	-	-

[1] LIE (2007)
[2] Huijps and Hogeveen (2007)
[3] Dutch Dairy Board (2006)

A penalty of 0.045 €/kg milk had to be applied to every shipment with a GBTSCC > 400,000 cells/ml (equation 8). Because the BTSCC resulted from SCC of all animals and included all pathogens, the penalty was not calculated separately for each pathogen-specific IMI.

Annual net cost of medication, veterinary service and labour

Clinical IMI was assumed to be treated for 3 days (Huijps et al., 2008). The cost of medication was calculated as the number of clinical IMI cases per year multiplied by the price of the medication per case. The cost of veterinary service was calculated as the number of

clinical IMI cases per year multiplied by the price of the veterinary consultancy per case taking in to account that not all clinical IMI cases are visited by a veterinarian (Huijps et al., 2008). The cost of labour was calculated as the number of clinical IMI cases per year multiplied by the time spent per case and the hourly wage of labour. Values and prices are mentioned in Table 2.

Annual net cost of culling

The net cost of culling an IMI cow consists of the loss of future returns from the culled cow, the cost of purchasing or raising new heifer, the revenue from selling the carcass, and the future returns of the new heifer, which is usually higher than the culled cow. The RPO value was used because it takes into account these costs (Houben et al., 1994).The cost of culling varies depending on the characteristics of the culled cow and the replacement heifer (Houben et al., 1994). The sum of retention pay off (RPO) values of the culled cows per pathogen IMI represented the net cost of culling per pathogen IMI. The RPO values depended on the parity, lactation stage, and pregnancy status of the culled cows based on Houben et al. (1994) and updated to recent prices (Huijps and Hogeveen, 2007). Production was not taken into account in calculating the RPO value, because it had already been considered in milk production loss (equation 9) and in an increased probability of culling. This would prevent overestimating pathogen-specific IMI culling cost.

Annual net cost of IMI

The total annual net cost of IMI was calculated per pathogen for clinical and subclinical IMI separately. The total annual net cost of clinical IMI was calculated as the sum of the cost of milk yield loss due to clinical IMI, cost of culling, cost of veterinary service, and cost of labour. The total annual net cost of subclinical IMI was calculated as the sum of the cost of milk yield loss due to subclinical IMI, cost of culling, and penalty.

A combined annual net cost of IMI was calculated as the sum of total annual net cost due to the 4 IMI pathogens modeled.

An average cost, with the 5^{th} and 95^{th} percentiles, was calculated for each of the cost factors, for the total annual net cost of clinical and subclinical IMI per pathogen, separately, the total annual net cost of IMI per pathogen, and the combined annual net cost of IMI.

The cost of IMI was also expressed per IMI case, which was calculated by dividing the total annual net cost of clinical or subclinical IMI per pathogen by the number of clinical or subclinical IMI cases caused by that pathogen per iteration. The mean, and 5^{th} and 95^{th} percentiles, were then calculated ranked over all iterations per pathogen.

Validation

Because data were not available to validate the model, several methods were used to test internal validity (Sørensen, 1990; Law, 2007). 1) Rationalism method: Several scenarios of inputs were used and compared to the output to check the consistency and the credibility of the model output. 2) Tracing method: Individual animals generated in the model were followed over the different time periods, and then the consistency of the output was verified.

3) Face validity method: An expert in IMI management in bovine dairies was consulted for feedback on the assumptions and the credibility of the model and the model output.

Model run and sensitivity analysis

Initially, the model was run for 4 years until a stable prevalence of infection (endemic state) was reached. This prevalence was used to assign infection states to all cows at start of the simulated quota year for economic analysis in a separate model run, in which the cows had similar characteristics among the different iterations at the start, but the IMI status of the cows was different. This was carried out to conduct comparisons in the herd IMI dynamics between iterations correctly, to represent the variability in the costs of IMI caused by the variability in the dynamics of pathogen-specific IMI. The characteristics of the cows would differ over time in the different iterations as indicated previously. The output of the simulated quota year was used to calculate the cost of pathogen-specific IMI in the default situation. The model run consisted of 1000 iterations.

A sensitivity analysis was conducted on input parameters for the dynamics of infection, for economics, and for production parameters to assess the impact of input values on the combined annual net cost compared to the default situation. The sensitivity analysis was also carried out for every herd and cow level parameter that was not stochastic. The lower and upper limit values of the parameters presented in Tables 1 and 2 were obtained from the literature and used for sensitivity analysis and the outcome were compared to the default situation. Values used in sensitivity analysis for culling were based on confidence limits mentioned in section 2.2.2. The transmission parameter β was varied by using half the default value (0.5D), and 2 times the default value (2D) for each pathogen, separately, while holding the transmission rate of other pathogens constant at default value. The lower and upper limits for the production loss (per pathogen) due to clinical IMI were based on the 95 % confidence limits from Grohn et al. (2004) and adjusted to the high producing cows in that study. When sensitivity analysis was carried out on one parameter, the other parameters were retained at default values. The results of sensitivity analysis on each parameter were compared to the results of the model outcome in the default situation to assess the impact of each parameter on the combined annual net cost of IMI.

RESULTS

The different scenarios in the rationalism and tracing back methods provided results as expected. The model was confirmed by the expert to represent credible IMI dynamics in bovine dairies.

Descriptive data

At start of the simulated quota year, 30% of the assigned cows were primiparous, producing 23 kg of milk per day, but varying between 18 and 27 kg per day. Multiparous cows produced on average 27 kg (22 to 34 kg) of milk per day. Peak production was reached at 60-80 days post-partum and the average number of extra inseminations per cow was 0.71.

This led to an average lactation length of 339 days and a calving interval of 399 days. On average, replacement rate was 25% per year for reasons other than IMI, and varied between 18 and 31%. The replacement rate was similar to the culling rate for reasons other than IMI. When IMI effect was included, replacement rate was on average 32 %, and varied between 22 and 43% per year. The herd annual milk production was on average 832,000 kg and varied from 821,000 to 849,000 kg of milk. The herd annual milk withdrawal was on average 4,555 kg and the 5^{th} and 95^{th} percentiles were 1,017 and 10,800 kg, respectively. The annual bulk tank somatic cell count was on average 61×10^3 cells/ml and the 5^{th} and 95^{th} percentiles were 36×10^3 and 118×10^3 cells/ml, respectively. The herd geometric mean somatic cell count was 32×10^3 cells/ml and the 5^{th} and 95^{th} percentiles were 14×10^3 and 80×10^3 cells/ml, respectively.

Dynamics of infection

The incidence of new IMI varied considerably. The median incidence of IMI per year differed between the pathogens involved in the IMI process (Table 3). The median annual incidence of clinical *Staph. aureus* IMI was 5 cases, for *Strep. uberis* 2 cases, for *Strep. dysgalactiae* 2 cases and 5 cases of *E. coli* IMI. Many of these clinical cases started as subclinical IMI followed by clinical flare ups (Table 3). For instance, the median annual incidence of *Staph. aureus* flare up cases was 3. Figure 2 shows the variation of the number of clinical IMI within herd as simulated by the model. It shows the nonlinear effect of transmission dynamics, particularly for *Staph. aureus*. However, *E. coli* increased linearly.

Subclinical *Staph. aureus* IMI had a median annual incidence of 7 cases and varied between 0 and 52 (Table 3). Median annual incidences of subclinical *Strep. uberis* and *Strep. dysgalactiae* IMI were 2 (0 to 17) and 1 (0 to 9) cases, respectively (Table 3). The median incidence of subclinical *E. coli* was 1 case per year, but could reach up to 3 cases per year (Table 3). In general, 5 cows were culled per year due to IMI, mainly *Staph. aureus* IMI (Table 3).

Table 3. Median incidence of new pathogen-specific IMI in a herd of 100 dairy cows per year as produced by the model together with the 5^{th} and 95^{th} percentiles, as ranked 4 times

	Staph. aureus	*Strep. uberis*	*Strep. dysgalactiae*	*E. coli*
Clinical IMI	5 (0-36)	2 (0-14)	2 (0-13)	5 (2-10)
Subclinical IMI	7 (0-52)	2 (0-17)	1 (0-9)	1 (0-3)
Flare ups	3 (0-18)	1 (0-7)	1 (0-5)	0 (0-1)
Remission	4 (0-25)	0 (0-3)	0 (0-3)	0 (0-2)
Culling due to:				
Clinical IMI	1 (0-6)	0.5 (0-3)	0.5 (0-3)	0.5 (0-2)
Subclinical IMI	2 (0-9)	0.5 (0-3)	0 (0-2)	0 (0)

Economic calculations

The net cost of IMI and the IMI cost factors for the different pathogens are presented in Table 4. The average combined annual net cost of IMI caused by the 4 simulated IMI pathogens was € 4,896. The cost varied considerably, with 5^{th} and 95^{th} percentiles of € 915

and 11,287, respectively (Figure 3). The total annual net cost of clinical IMI was on average € 3,136, varying from 802 to 10,101 (Table 4). The average total annual net cost of clinical IMI caused by *Staph. aureus* was € 1,375, of which culling costs were on average € 529 per year. Cost of clinical *Staph. aureus* IMI varied greatly, and reached up to € 4,716 under the same management. Calculating the total annual net cost to a cost per new case, *Staph. aureus* clinical IMI costs € 194 per case, varying between € 114 and € 307 per case.

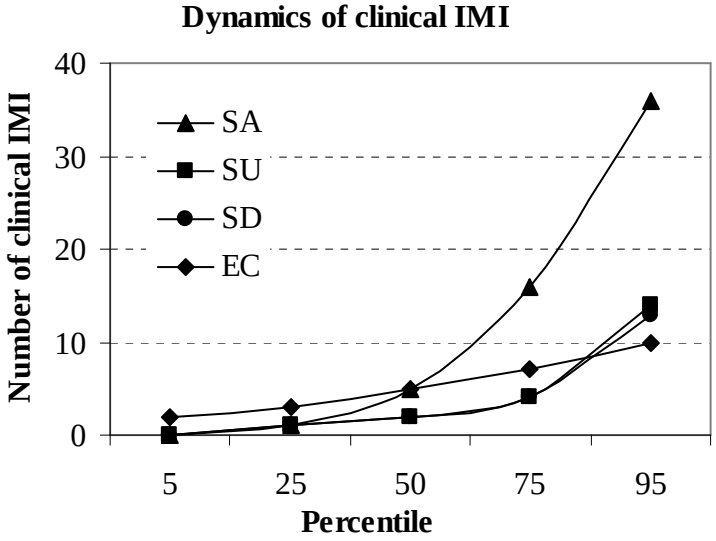


Figure 2. The 5th, 25th, 50th, 75th, and 95th percentiles of the number of clinical intramammary infections (IMI) caused by *Staph. aureus* (SA), *Strep. uberis* (SU), *Strep. dysgalactiae* (SD), and *E. coli* (EC) in a herd of 100 dairy cows, as ranked 4 times over all iterations.

The average total annual net costs of clinical IMI due to *Strep. uberis* and *Strep. dysgalactiae* were € 484 and € 466, respectively (Table 4). The average net cost per case was € 180 (110 to 317) and € 168 (101 to 310), respectively. The average total annual net cost of *E. coli* clinical IMI was € 811, and per case € 189 (120 to 328), mainly due to culling, and medication (Table 4).

The cost of subclinical IMI varied, depending on the pathogen involved in the infection. For *Staph. aureus*, the average total annual net cost was € 1,219 (Table 4). Calculating the cost per case, a new case of *Staph. aureus* subclinical IMI costs € 112 on average, but varied greatly between € 0 and € 225. For *Strep. uberis*, the average total annual net cost of subclinical IMI was € 306, and for *Strep. dysgalactiae* € 208 (Table 4). Converting the cost to an average net cost per subclinical case of *Strep. uberis* and *Strep. dysgalactiae* IMI, the cost was € 107 (5 to 222) and € 121 (5 to 310), respectively. A subclinical *E. coli* IMI case would cost only € 27 on average, but varied between € 2 and € 211. For all pathogens, the most important cost factor of subclinical IMI was culling (Table 4).

Sensitivity analysis

Results of the sensitivity analysis showed that the pathogen-specific transmission rate (β) was the most influential parameter on the total annual net cost per pathogen (Figure 4). In case of *E. coli* IMI, it was the incidence of infection which was the most influential

parameter. Increasing the transmission rate resulted in a non-linear increase in the total annual net cost due to *Staph. aureus*, *Strep. uberis* and *Strep. dysgalactiae* IMI. In case of *Staph. aureus* IMI, an increase above the default value might lead to a drastic loss. A similar relationship was observed in case of *Streptococcus* IMI, but with a lesser degree of loss. In case of *E. coli* IMI, an increase in the incidence of infection caused a linear increase in loss (Figure 4) that was due to the Greenwood model approach.

Table 4. Pathogen-specific average total annual net cost and cost factors (€) of clinical IMI (CIMI) and subclinical IMI (ScIMI) in a herd of 100 dairy cows together with the 5th and 95th percentiles, as ranked 4 times

Cost factors	*Staph. aureus*	*Strep. uberis*	*Strep. dysgalactiae*	*E. coli*
Total	2594 (0-8,395)	790 (0-3,281)	674 (0-2,266)	838 (200-1,713)
Cost of CIMI	1375 (0-4,716)	484 (0-1,850)	466 (0-1,598)	811 (199-1,664)
Milk loss[1]	273 (0-1,033)	75 (0-318)	74 (0-339)	147 (50-262)
Medication	399 (0-1,440)	142 (0-560)	138 (0-520)	227 (80-400)
Vet. service	75 (0-270)	26 (0-105)	26 (0-98)	43 (15-75)
Labor	359 (0-1,296)	128 (0-504)	124 (0-468)	204 (72-360)
Culling	529 (0-2,000)	185 (0-1,015)	175 (0-1,001)	310 (139-1,200)
Saved cost	260 (0-996)	72 (0-309)	71 (0-187)	120 (42-247)
Cost of ScIMI	1219 (0-4,030)	306 (0-1,510)	208 (0-710)	27 (0-48)
Milk loss[1]	69 (0-242)	23 (0-103)	17 (0-82)	3 (0-4)
Culling	1215 (0-4,012)	303 (0-1,505)	206 (0-691)	25 (0-45)
Penalties	0	0	0	0
Saved cost	65 (0-230)	20 (0-99)	15 (0-69)	1 (0-2)

[1] Calculated as the cost of replacement heifers to compensate milk production loss

The results of sensitivity analysis for parameters that affected the combined annual net cost of IMI by more than 10% are presented in Table 5. The probability of recovery from subclinical IMI drastically affected the combined annual net cost. A low probability of recovery (worst case) leads to an average combined annual net cost of € 8,124. When the probability of recovery was high (best case) the average combined annual net cost was € 1,930. In the best case of subclinical IMI recovery, clinical IMI contributed 90 % of the average combined annual net cost (Table 5).

DISCUSSION

Often real data for validating models are not obtained because of the difficulty of monitoring processes such as IMI and the high associated costs. This makes methods such as sensitivity analysis, rationalism, tracing, and face validation necessary alternatives (Sørensen, 1990). These methods increase confidence in the simulation models and make advice based on them acceptable (Sørensen, 1990). For our model, the extraordinary high price associated with measuring the transmission rates in the field and the large number of input parameters make it difficult to obtain farm data to validate the dynamics of IMI and the model as whole. Nevertheless, the methods that were used confirmed its credibility and validity. Barkema et al. (1998) found that the annual incidence of clinical IMI per herd of 100 dairy cows caused by *Staph. aureus*, *Strep. uberis*, *Strep. dysgalactiae* and *E. coli* IMI were respectively 5, 3, 2, and

5 cases. The incidences are very close to the output of the median number of clinical IMI per pathogen as simulated by the model (Table 3). Barkema et al. (1998) also found that 5 cows were culled per year because of clinical IMI and high SCC (representing subclinical IMI), which was again similar to our finding of 5 culled cases due to clinical and subclinical IMI (Table 3). It was not possible to compare our findings with other field data because those data were collected at quarter level.

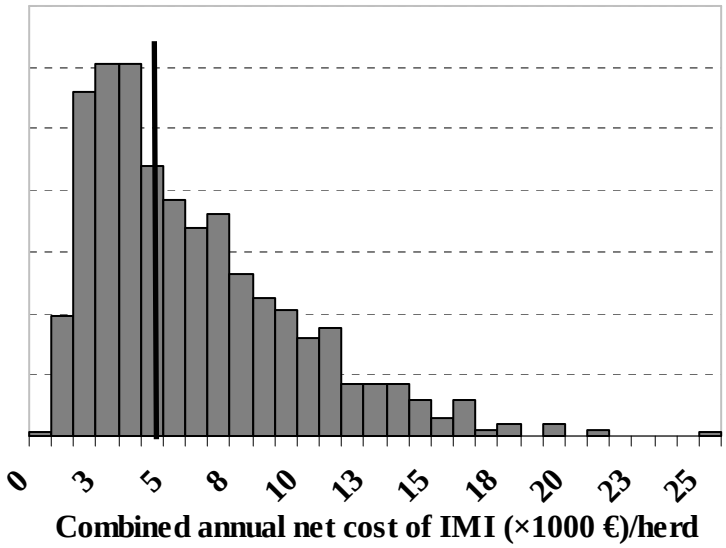


Figure 3. The distribution of the combined annual net cost (€) of intramammary infection (IMI), which is the sum of the total annual net cost of IMI caused by *Staph. aureus*, *Strep. uberis*, *Strep. dysgalactiae*, and *E. coli* IMI in a herd of 100 dairy cows. The heavy vertical line stands for the average value.

The average total annual net cost of clinical IMI was € 3,136. Per case of clinical IMI, the average net cost was € 185, but varied between € 124 and € 331 per case. This cost is slightly higher than the average estimation of € 155 per case of clinical IMI in the literature (Halasa et al., 2007). In the current study, the average net cost of a subclinical IMI case was € 96 (10 to 265), which is very close to the average literature finding of € 102 per case (Halasa et al., 2007). Seegers et al. (2000) and Østergaard et al. (2005) in bio-economic models estimated the average annual impact of IMI to be € 87 and € 146 per cow present. Our estimation would be € 49. Our model simulated only the 4 main IMI pathogens in the Netherlands, while more IMI pathogens were simulated in the other models. Therefore, the annual incidence of IMI, in the current study, was lower than that in those studies, leading to lower estimated costs. In a recent study (Huijps et al., 2008), a relatively straightforward approach was used to provide a tool to calculate the loss due to IMI in Dutch dairy herds. They found that the average net cost of clinical IMI was € 210 per case, and the average net cost of subclinical IMI was € 106 per case, which is close to our estimates. Bar et al. (2008) found that generic clinical IMI cost was $ 179 per case, which would be approximately 30 % lower than our estimation. Prices of medication, labor, and culled clinical IMI cases were lower than the prices used in our study. Moreover, they assumed that a veterinarian would not visit the clinical cases. In contrast, in our study we assumed that a veterinarian could visit some cases and therefore the costs were included (Huijps et al., 2008). If comparisons are to

be made between costs in countries using different currencies, fluctuations in exchange rate need to be taken into account, in addition to the costs due to farm systems.

The average net cost per case for clinical and subclinical IMI cases for all simulated pathogens varied considerably (see section 2.3). This reflects the dynamics of infection and management within the herd. For instance, a case could occur with the cow being a high producing cow: the cost of loss of milk production, discarded milk, possible treatment, and culling would be high (high RPO value). On the other hand, if the cow was a low producing cow the cost in such a case would be lower. This is important because actions such as treatment should be ranked on the basis of the complete set of attributes of the IMI cow and not only its IMI status.

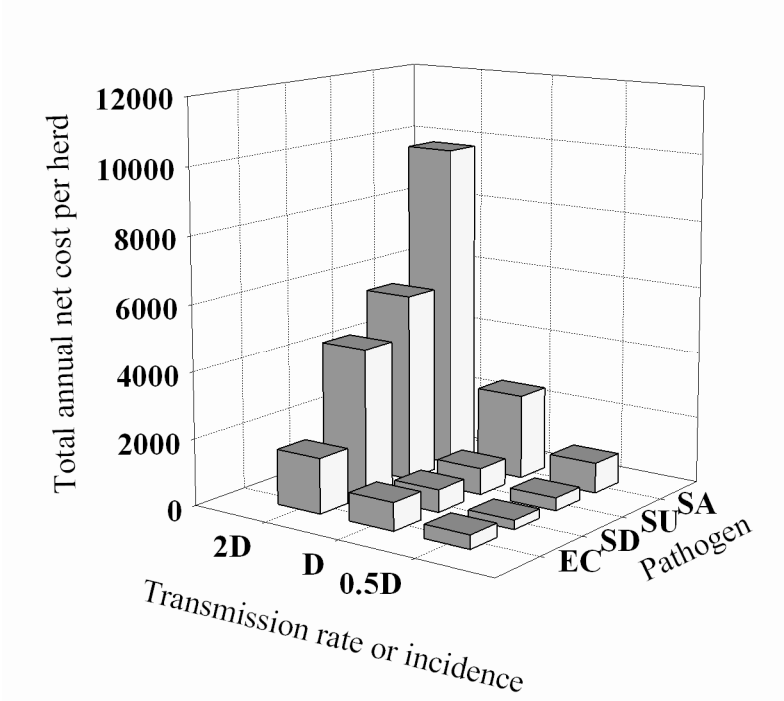


Figure 4. Sensitivity analysis conducted on the total annual net cost of IMI (€) per pathogen, in a herd of 100 dairy cows, for *Staph. aureus* (SA), *Strep. uberis* (SU), and *Strep. dysgalactiae* (SD) with half default (0.5D), default (D), and 2 times default (2D) values of transmission parameters (β) and the cumulative incidence values for *E. coli* (EC) IMI, examined separately per pathogen.

The values of transmission rate parameters used in this model were calculated in the original papers from farms with specific IMI management. However, the variation in the number of infections was high, as shown in Figure 2. This would lead to a large variation in the combined annual net cost of IMI caused by the 4 pathogens (Figure 3). In practice this implies that the net cost of IMI might differ greatly between years, despite the fact that the same IMI management has been applied in the herd. Figure 4 implies that it may not be cost-effective to attempt to reduce transmission rate by 50 %. Nevertheless, it might be cost-effective to decrease a high transmission rate or, in other words, to invest to prevent a fast spread of infection in a herd.

The rate of new IMI in recovered quarters was found to be higher than that in un-infected quarters. At cow level, the history of IMI did not affect the rate of new IMI (Zadoks et al., 2001b), and therefore the recovered state was not modeled separately. Instead, the

transmission rate parameters were re-calculated from the original studies (cited in Table 1) weighted by the number of new infections originating in the uninfected and recovered state, or by using the geometric mean when the infection history of the new infection quarters was not mentioned in those studies. This would preserve the balance of the rate of new IMI in each IMI state.

The sensitivity analysis showed the importance of the transmission rate parameter as one of the main determinants of spread of pathogen-specific IMI between cows. A high transmission rate can lead to large losses (Figure 4). Only 2 studies have estimated the transmission rate of *Staph. aureus*, with few farms included, leading to high variation within and between the studies. Lam (1996) found that the transmission rate varied between 0.0028 and 0.006 per quarter-day at risk in a steady state depending on management. Zadoks et al. (2002) found it to vary between 0.007 and 0.052 per quarter-day at risk for farms with similar IMI management. One study estimated the transmission rate due to *Strep. uberis*, based on 2 phases of IMI, also with a high variation between the 2 estimates (Zadoks et al., 2001a). The current model is the closest approach to reality compared with other bio-economic models in the sense that it considers the transmission of pathogen-specific IMI between cows mechanistically. Still, the accuracy of the model outcomes is dependent on the accuracy of the pathogen-specific IMI transmission rate parameter. Given the high uncertainty of the transmission rate parameters, improving IMI simulation and consequently the precision of IMI cost will require improving estimates of the pathogen-specific IMI transmission parameter from field data.

Table 5. The average combined annual net cost of IMI caused by *Staph. aureus*, *Strep. uberis*, *Strep. dysgalactiae*, and *E. coli* IMI in a herd of 100 dairy cows in € and the (percentage) loss due to clinical IMI in the sensitivity analysis conducted on the parameters included in the model.

Parameters in sensitivity analysis	Combined annual net cost of IMI per herd (€)	
	Best case	Worst case
Recovery of clinical IMI (γ_c)	3109[2] (72)	6192[1] (65)
Recovery of subclinical IMI (γ_{sc})	1930[2] (90)	8124[1] (63)
Culling clinical IMI (μ_c)	3926[1] (86)	5202[2] (89)
Culling subclinical IMI (μ_{sc})	4108[1] (91)	5802[2] (80)
Replacement heifer is a subclinical IMI (α_{sc})	4622[1] (82)	5572[2] (74)
Milk yield loss due to clinical IMI	4661[1] (71)	5436[2] (83)

[1] Based on the lower limit value in the sensitivity analysis
[2] Based on the upper limit value in the sensitivity analysis

The second most influential parameter was the bacteriological recovery rate from subclinical IMI (γ_{sc}) (Table 5). Subclinical IMI cows are a constant source of infection leading to higher annual IMI costs when the recovery from subclinical IMI is low. In 2 studies (Swinkels et al., 2005a,b), it was indicated that the treatment of subclinical IMI might be a good way to decrease the net cost of IMI. They recommended the use of stochastic and dynamic modeling to estimate this indirect effect of treatment properly, and this was possible in the current model.

The risk of clinical IMI was found to be highly associated with cow factors such as parity and lactation stage (Steeneveld et al., 2008). The proposed model would be able to include such effects by adjusting equation 2 per class of each of the cow factors. However, because the transmission parameters were presented as average values from the whole herd in the original field studies and were not stratified per class of any of the cow factors; it was not possible to include or to predict the effect of cow factors on the transmission dynamics of IMI and consequently on the cost of IMI. To include the effect of cow factors, transmission parameters should be estimated in field studies per class of each factor, to be considered in future models. This could improve the estimation of IMI cost and the resulting variability due to cow- and herd- level risk factors.

In this model, the milk yield of cows was modeled using Wood's lactation curve. This curve has been criticized, mainly because the high autocorrelation between the parameters would lead to over parameterization leading to incorrect prediction of the actual production (Dhanoa, 1981; Yadava et al., 1977). The high correlation between the parameters in Wood's curve is actually due to the fact that models that were used to test Wood's curve were fitted to the average weekly milk yield pooled over lactation and farms rather than individual observations (Barta, 1986). Although Wood's curve explained less than the polynomial function method of the variation between the $\log_{10}$ milk yields, it was able to explain 97 % of the variation (Barta, 1986). This, and its ease of use, made Wood's curve an attractive choice, making it possible to allocate computer power to modeling the biological process of IMI rather than to extended parameters of the lactation curve.

The choice of discrete-event simulation modeling restricted the ability of the model to solve for optimum solutions (Allore and Erb, 1999). However, the choice of this modeling method was aimed at modeling the complex process of pathogen-specific IMI using less computer power, and thus modeling a trade off between the possibilities of optimization and the level of complexity of the underlying biological processes. In the model, culling was based on fixed probabilities and replacement was not restricted, which might not be truly consistent with real life. However, these decisions vary largely between herds and are consequently hard to model to represent a country situation. The culling and replacement decisions could be adjusted in the current model to represent farm-specific situations. The model assumed a cow to be an IMI cow by only one pathogen simultaneously. Although uncommon, a cow could be infected by more than one pathogen simultaneously. The effect of this assumption on the cost of IMI is hard to predict, because the number of susceptible and IMI cows would consequently change. The bulk tank somatic cell count was lower than the average Dutch figures (NRS, 2005). This was because only the 4 main pathogens were simulated leading to lower incidence of IMI. Additionally, healthy cows were assumed to always have somatic cell count $< 50 \times 10^3$ cells/ml, which was necessary to be consistent with recent literature about the somatic cell count of healthy cows (Seegers et al, 2003; Halasa et al., 2009). The consequence could have been an underestimation of the penalty payment.

The model described in this paper can be an important tool to estimate the cost of pathogen-specific IMI in bovine dairy herds. In future, by changing input parameters, it will be possible to estimate the cost-effectiveness of pathogen-specific IMI management, taking

into account the dynamics of pathogen-specific IMI and the uncertainty that is highly associated, with it. The model has the capacity to permit evaluation of pathogen-specific IMI management, taking into account the existence of other IMI types in the herd.

CONCLUSIONS

The bio-economic model of IMI described is a credible and a valid way to calculate the economic costs of pathogen-specific IMI in bovine dairy herds. The annual incidence of *Staph. aureus* IMI varied between 0 and 88 cases. For *Strep. uberis* and *Strep. dysgalactiae* the annual incidence varied between 0 and 31 and 0 and 21 cases, respectively. The annual incidence of *E. coli* IMI varied between 2 and 13 cases.

On average, the total annual net cost of IMI in a herd of 100 dairy cows for *Staph. aureus* was € 2,594, for *Strep. uberis* € 790, for *Strep. dysgalactiae* € 674, and *E. coli* € 838, but costs varied greatly. The most important factor contributing to the total annual net cost of IMI was culling. The total net cost was most sensitive to the transmission rate parameter and the recovery rate from subclinical IMI.

Acknowledgements

The authors acknowledge Hans Heesterbeek, Paul Dobbelaar, Annet Velthuis, Don Klinkenberg, Wilma Steeneveld, and Tine van Werven for assistance in model development, both technically and practically.

References

Allore, H.G., Erb, H.N., 1998. Partial budget of the discounted annual benefits of mastitis control strategies. J. Dairy Sci. 81, 2280-2292.

Allore, H.G., Erb, H.N., 1999. Approaches to modeling intramammary gland infections in cattle. Prev. Vet. Med. 39, 279-293.

Bar, D., Grohn, Y.T., Bennett, G., Gonzalez, R.N., Hertl, J.A., Schulte, H.F., Tauer, L.W., Welcome, F.L., Schukken, Y.H., 2007. Effect of repeated episodes of generic clinical mastitis on milk yield in dairy cows. J. Dairy Sci. 90, 4643–4653.

Bar, D., Tauer, L.W, Bennett, G., Gonzalez, R.N., Hertl, J.A., Schukken, Y.H., Schulte, H.F., Welcome, F.L., Grohn, Y.T., 2008. The cost of generic clinical mastitis in dairy cows as estimated by using dynamic programming. J. Dairy Sci. 91, 2205-2214.

Barkema, H.W., Schukken, Y.H., Lam, T.J., Beiboor, M.L., Wilmink, H., Benedictus, G., Brand, A., 1998. Incidence of clinical mastitis in dairy herds grouped in three categories by bulk tank somatic cell counts. J. Dairy Sci. 81, 411-419.

Barta, T.R., 1986. Comparison of two mathematical models in fitting lactation curves for pureline and crossline dairy cows. Can. J. Anim. Sci. 66, 405-414.

Beck, H.S., Wise, W.S., Dodd, F.H., 1992. Cost benefit analysis of bovine mastitis in the UK. J. Dairy Res. 59, 449-460.

Becker, N.G., 1989. Analysis of infectious disease data. Chapman and Hall, London, UK.

Bremermann, H.J., Thieme, H.R., 1989. A competitive exclusion principle for pathogen virulence. J. Math. Biol. 27, 179-190.

CVB, 2005. Veevoedertabel, Gegevens over chemische samen voederwaarde van voedermiddelen, Lelystad. The Netherlands.

De Haas, Y., Barkema, H.W., Veerkamp, R.F., 2002. The effect of pathogen-specific clinical mastitis on the lactation curve for somatic cell count. J. Dairy Sci. 85, 1314–1323.

Dhanoa, M.S., 1981. A note on an alternative form of the lactation model of Wood. Anim. Prod. 32, 349-351.

Döpfer, D., Barkema, H.W., Lam, T.J., Schukken, Y.H., Gaastra, W., 1999. Recurrent clinical mastitis caused by *Escherichia coli* in dairy cows. J. Dairy Sci. 82, 80-5.

Dutch Dairy Board, 2006. Statistical annual report 2006. Louis Braillelaan, Zoetermeer, The Netherlands.

Golodetz, C.L., 1983. Prognosis of cow with severe clinical coliform mastitis. Vet. Rec. 23, 402-403.

Grohn, Y.T., Wilson, D.J., Gonzalez, R.N., Hertl, J., Schulte, H., Bennett, G., Schukken, Y.H., 2004. Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. J. Dairy Sci. 87, 3358–3374.

Hadley, G.L., Wolf, C.A., Harsh, S.B., 2006. Dairy cattle culling patterns, explanations, and implications. J. Dairy Sci. 89, 2286-2296.

Halasa, T., Huijps, K., Østerås, O., Hogeveen, H., 2007. Economic effects of bovine mastitis and mastitis management: A review. Vet. Q. 29, 18–31.

Halasa, T., Nielen, M., De Roos, A.P.W., Van Hoorne, R., de Jong, G., Lam, T.J.G.M., van Werven, T., Hogeveen, H., 2009. Production loss due to new subclinical mastitis in Dutch dairy cows estimated with a test-day model. J. Dairy Sci. 92:599-606.

Hill, A.W., Shears, A.L., Hibbitt, K.G., 1978. The elimination of serum-resistant *Escherichia coli* from experimentally infected single mammary glands of healthy cows. Res. Vet. Sci. 25, 89-93.

Hogan, J.S., Smith, K.L., Todhunter, D.A., Schoenberger, P.S., 1994. Therapy of experimentally induced Coliform mastitis with a *Propionibacterium acnes* product. J. Dairy Sci. 77, 462-7.

Hogan, J., Smith, K. L., 2003. Coliform mastitis. Vet. Res. 34, 507-519.

Houben, E.H.P., Huirne, R.B.M., Dijkhuizen, A.A., Kristensen, A.R., 1994. Optimal replacement of mastitis cows determined by a hierarchic Markov process. J. Dairy Sci. 77, 2975 – 2993.

Huijps, K., Hogeveen, H., 2007. Economic evaluation of dry cow therapy. J. Dairy Sci. 90, 1225-1234.

Huijps, K., Lam, T.J.G.M., Hogeveen, H., 2008. Costs of mastitis: facts and perception. J. Dairy Res. 75, 113-120.

Lam, T.J.G.M., 1996. Dynamics of bovine mastitis: A field study in low somatic cell count herds. PhD Dissertation, Utrecht University, The Netherlands.

Law, A.M., 2007. Simulation modeling and analysis, McGraw-Hill international, New York, USA.

LIE, Institute of agricultural economics, 2007. Agricultural prices in 2007, Wageningen University, Wageningen, The Netherlands.

McDougall, S., 1998. Efficacy of two antibiotic treatments in curing clinical and subclinical mastitis in lactating dairy cows. New Zeal. Vet. J. 46, 226–232.

NRS, 2005. Annual statistics (2005). CR-Delta, Arnhem, The Netherlands.

Østergaard, S., Chagunda, M.G.G., Friggens, N.C., Bennedsgaard, T.W., Klaas, I.C., 2005. A stochastic model simulating pathogen-specific mastitis control in a dairy herd. J. Dairy Sci. 88, 4243–4257.

Pugliese, A., 2002. On the evolutionary coexistence of parasite strains. Math. Biosci. 177-178, 355-375.

Reksen, O., Sølverød, L., Østerås, O., 2008. Relationships Between Milk Culture Results and Composite Milk Somatic Cell Counts in Norwegian Dairy Cattle. J. Dairy Sci. 91, 3102–3113.

Seegers, H., Fourichon, C., Sørensen, J.T., Hortet, P., Billon, D., 2000. Modelling the cost-benefits of prevention and control of mastitis in dairy herds. Pages 55–61 in Proc. Symp. Economic Modelling of Animal Health and Farm Management. Wageningen University, Wageningen, The Netherlands.

Seegers, H., Fourichon, C., Beaudeau, F., 2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet. Res. 34, 475–491.

Sol, J., Sampimon, O.C., Barkema, H.W., Schukken, Y.H., 2000. Factors associated with cure after therapy of clinical mastitis caused by *Staphylococcus aureus*. J. Dairy Sci. 83, 278–284.

Sørensen, J.T., 1990. Validation of livestock herd simulation models: A review. Livest. Prod. Sci. 26, 79–90.

Steeneveld, W., Swinkels, J., Hogeveen, H., 2007. Stochastic modelling to assess economic effects of treatment of chronic subclinical mastitis caused by *Streptococcus uberis*. J. Dairy Res. 74, 459-467.

Steeneveld, W., Hogeveen, H., Barkema, H. W., Van den Broek, J., Huirne, R. B. M., 2008. The Influence of Cow Factors on the Incidence of Clinical Mastitis in Dairy Cows. J. Dairy Sci. 91, 1391–1402

Swinkels, J.M., Rooijendijk, J.G.A., Zadoks, R.N., Hogeveen, H., 2005a. Use of partial budgeting to determine the economic benefits of antibiotic treatment of chronic subclinical mastitis caused by *Streptococcus uberis* or *Streptococcus dysgalactiae*. J. Dairy Res. 72, 75-85.

Swinkels, J.M., Hogeveen, H., Zadoks, R.N., 2005b. A partial budget model to estimate economic benefits of lactational treatment of subclinical *Staphylococcus aureus* mastitis. J. Dairy Sci. 88, 4273-4287.

Van Arendonk, J., 1985. Studies on the replacement polices in dairy cattle. PhD dissertation, Wageningen University, Wageningen, The Netherlands.

Wolfram Research Europe Ltd., 2007. Blenheim Office Park, Long Hanborough, Oxfordshire, UK.

Wood, P.D.P., 1976. Algebraic models of the lactation curves for milk, fat and protein production with estimates of seasonal variation. Anim. Prod. 22, 35-40.

Yadava, M.C., Katpatal, B.G., Kaushik, S.N., 1977. Study of lactation curves in Hariana and its Friesian crosses. Indian J. Anim. Sci. 47, 607-609.

Zadoks, R.N., Allore, H.G., Barkema, H.W., Sampimon, O.C., Grohn, Y.T., Schukken, Y.H., 2001a. Analysis of an outbreak of *Streptococcus uberis* mastitis. J. Dairy Sci. 84, 590–599.

Zadoks, R.N., Allore, H.G., Barkema, H.W., Sampimon, O.C., Wellenberg, G.J., Grohn, Y.T., Schukken, Y.H., 2001b. Cow- and Quarter-Level Risk Factors for *Streptococcus uberis* and *Staphylococcus aureus* Mastitis. J. Dairy Sci. 84, 2649–2663.

Zadoks, R.N., Allore, H.G., Hagenaars, T.J., Barkema, H.W., Schukken, Y.H., 2002. A mathematical model of *Staphylococcus aureus* control in dairy herds. Epidemiol. Infect. 129, 397-416.

Zadoks, R.N., Gillespie, B.E., Barkema, H.W., Sampimon, O.C., Oliver, S.P., Schukken, Y.H., 2003. Clinical, epidemiological and molecular characteristics of *Streptococcus uberis* infections in dairy herds. Epidemiol. Infect. 130, 335–349.

Chapter 5

Meta-Analysis of Dry Cow Management for Dairy Cattle. Part 1. Protection Against New Intramammary Infections

T. Halasa,‡[1] O. Østerås,† H. Hogeveen,‡ T. van Werven,‡ M. Nielen‡

‡ Department of Farm Animal Health and Reproduction, Utrecht University, P.O. Box 80151, 3584 CN Utrecht, the Netherlands

† Department of Production Animal Clinical Sciences, Norwegian School of Veterinary Science, P.O. Box 8146 Dep., N-0033 Oslo, Norway

Accepted for publication in Journal of Dairy Science

ABSTRACT

The objective of this study was to estimate the preventive effect of various dry cow management measures against quarter new intramammary infections (**IMI**) during the dry period up to 21 d post-calving. Moreover, the potential publication bias was assessed in the studies selected for this analysis. The intervention measures were blanket dry cow therapy (**BDCT**), selective DCT (**SDCT**), Cloxacillin compared to other DCT products, and teat sealant. A meta-analysis relative risk (**RR**) was calculated per intervention and pathogen group when enough studies were available from the 33 selected studies. Results of the meta-analyses were examined using publication bias tests. BDCT showed significant protection against new IMI caused by *Streptococcus* spp., the pooled RR was 0.39 (0.30-0.51), but no protection was observed against coliform new IMI; the pooled RR was 0.95 (0.81-1.10). After correction for publication bias, it became doubtful whether DCT is protective against new *Staphylococcus* spp. IMI. Cloxacillin showed similar protection against new quarter IMI compared to other DCT products with a pooled RR of 1.09 (0.94-1.25). SDCT showed higher protection against new IMI compared to no DCT; pooled RR was 0.51 (0.30-0.86). However, BDCT showed more protection when compared to SDCT; pooled RR was 0.55 (0.37-0.80), but the inference about whether BDCT is superior to SDCT was dependent on whether the selection criteria for SDCT was at cow or quarter level. Internal teat sealant showed a significant protection against new IMI during the dry period with a pooled RR of 0.39 (0.18-0.82). Publication bias should be taken into account when attempts are made to review literature in a meta-analysis. **Key words: Mastitis, Dairy Cattle, Management, Dry Period, Meta Analysis**

INTRODUCTION

While risk of new IMI for the ‘environmental’ pathogens is high in early and late dry period (**DP**), the risk for the ‘contagious’ pathogens such *Staphylococcus aureus* and *Streptococcus agalactiae* is lower in the DP than at other times (Bradley and Green, 2004). Different methods have been attempted to control new IMI during the DP. Several DCT products were tested and compared to each other to optimize protection (Parkinson et al., 2000). Emerging antibiotic bacterial resistance combined with economic incentives led to selective DCT (**SDCT**) based on cow characteristics such as SCC approaching dry off or clinical IMI history (Morris et al., 1978), or both.

An alternative to DCT is the use of an internal or external teat sealer (TS) that is meant to prevent pathogen access to the mammary glands (Meaney, 1976). Teat sealer application has been recommended by the National Mastitis Council together with DCT in some cases to provide higher protection against new IMI (Godden et al., 2003).

The importance and the protective effect of DP management on new IMI are well recognized (Bradley and Green, 2004). However, large variations in the protective effect and the risk of new IMI can be encountered when comparing the different studies (Bradley and Green, 2004). Moreover, different interventions are expected to provide different degrees of protection. For calculations of the economic effects and subsequent support of decisions, it is

essential to quantify an estimate of the risk of new IMI using the different DP interventions. Only one study quantified summary risks of new IMI related to the DP intervention (Robert et al., 2006). However, this study focused only on quantifying the effect of DCT with and without teat sealers. The protective effect post-calving was not estimated, which might be important because some long acting antibiotics are expected to limit new IMI around calving and early lactation (Pearson and Wright, 1969). More important, the potential publication bias in the studies reviewed was not assessed. Because studies that result in large and interesting treatment effects are more likely to be published than studies that show relatively small or no treatment effects, the outcome could be a biased body of research (Rothstein et al., 2005). Therefore, it is important to address and discuss this potential bias to properly draw conclusions on the preventive effect of DCT and other interventions. Meta-analysis is a statistical analysis of a large collection of analysis results from individual studies for the purpose of integrating the findings, which would facilitate drawing conclusions based on the available information (Dohoo et al., 2003). The technique is powerful in the sense that it considers study attributes, such as study precision and weight that properly, but in some cases, the diversity among studies demands caution while drawing conclusions (Dohoo et al., 2003).

The objectives of the present study are to: 1) provide a summary quantification of the protective effect of different DP interventions on the risk of new IMI during the DP up to 21 d post-calving, based on meta-analysis of existing peer reviewed literature, and 2) address and discuss the potential bias in the existing peer reviewed literature.

MATERIALS AND METHODS

Papers selected for analysis

A search was conducted on literature related to the DP intervention published between 1930 and the beginning of 2008. The search was carried out using two methods: 1) search by key words in Pubmed (the National Library of Medicine, Bethesda, USA) using the following key words in different combinations (*DP, transitional period, prepartum, peripartum, post partum, new intramammary infection, mastitis, pathogen, cattle, cow, dairy, management, control, udder health, dry off, therapy, treatment*), and 2) reference citation procedure in the ISI web of knowledge (The Thompson Cooperation, Philadelphia, USA) where a search was conducted for studies that cited older studies.

Papers included in the meta-analysis had to: 1) be original research papers published in peer reviewed journals; 2) report the number or the rate of new IMI at quarter level in at least 2 groups (treated and control group) and the total number of quarters in each group; 3) only papers that had both pre dry off and post-calving milk sampling at quarter level could be included, as by definition these were the only studies that could provide the new IMI data; 4) report the outcome of a new data set or new protocol. When several studies were published based on the same data, the most detailed study was used. Further details of the studies are provided in subsequent sections.

A total of 33 studies fitted the above criteria for inclusion in the meta-analyses. When studies reported the outcome of one or several protocols, Roman numerals were added to

distinguish protocols. Both negative control, i.e. those which included placebo or untreated cows or quarters, and positive control designs were included in the analyses.

Two formats for randomization and treatment were observed in the selected studies: 1) The whole udder was assigned to either the treatment or control group (between cow comparison), or 2) one or more quarters of the udder were assigned to the treatment group and the other quarters were assigned to the control group (within cow comparison).

Because different studies differed in the design and the observational units, discrepancies were expected that could influence the validity of the meta-analysis. Data related to the study design, level of analysis and dry cow management were recorded from the original studies and presented in Table 1, Appendices 1, 2, 3, and the descriptive results.

Management Groups Involved in the Meta-analyses

Blanket DCT (**BDCT**) was compared to no DCT, based on studies that reported the incidence or number of new quarter IMI during DP up to 21 d post-calving as a cumulative incidence. The 21 d post-calving was used as a cut off value because most studies took milk samples up to 21 d post-calving. Moreover, studies that used longer intervals could actually be partly reflecting the effects of early lactation management, and hence tend to nullify any effects of DCT which presumably was not having effect that far from calving.

Studies that compared Cloxacillin to other DCT products and reported the number or incidence of new quarter IMI during the DP were included in the analysis. In those studies, sampling at a single time point just after calving was used to define presence of a new IMI. Cloxacillin was included as the treatment group and the other DCT product as the control group. There was not enough data to conduct comparisons between other antibiotics, and hence only Cloxacillin was used for comparison to other DCT products.

The SDCT comparison was based on studies that measured the rate or number of new quarter IMI in the SDCT compared to no DCT during DP up to 21 d post-calving as a cumulative incidence. The analysis was carried out separately for quarter or cow level treatment and reported as a combined overall effect. A separate meta-analysis was carried out for studies that compared SDCT to BDCT as a positive control group.

Studies that investigated the protective effect of TS using negative or positive control groups were analyzed together. The studies reported the number or incidence of new quarter IMI during DP up to 21 d post-calving as a cumulative incidence in the treatment and control group.

A comparison was also conducted on supplementations that enhance the immune system to better protect against new IMI during the DP. These supplementations were vitamins, minerals and J5 vaccine. Another comparison was also conducted on the teat dipping during the DP as it could protect from new IMI during the DP. These 2 analyses were conducted to complete the message of the current study, in which all interventions that could protect against new IMI during the DP were reviewed in meta-analyses. The papers of these 2 analyses are not summarized nor referred to, but the outcomes and the reasons that impeded complete results presentation are discussed briefly.

Table 1. Description of each study involved in the meta-analyses in alphabetical order.

Study	Year	Origin	Herd type[1]	Fund-ing[2]	Study type[3]	ST[4]	PS[5]	Sample Herd/Cow	FP P[6]	D[7]
Berry and Hillerton-I*	2002a	UK	I	I	FT	S	16	2/236	1	0
Berry and Hillerton-II	2002a	UK	C	I	FT	S	49	2/54	1	0
Berry and Hillerton	2002b	UK	B	B	FT	S	20	7/401	1	0
Berry and Hillerton	2007	UK	I	I	FT	S	0	1/283	7	1
Bratlie	1973	NO	C	Na[b]	FT	S	Na	48/746	4	0
Browning et al.	1994	AU	C	C	FT	S	13	12/1044	1	0
Cummins and McCaskey	1987	US	I	I	E	D	42	1/90	10[c]	1
Davidson et al.	1994	CA	C	B	FT	S	12	7/84	7	0
Dingwell et al.	2002	CA	C	C	FT	S	Na	24/235	9	0
Eberhart et al.-I	1972	US	I	I	E	S	32	5/165	17[c]	0
Eberhart et al.-II	1972	US	I	I	E	S	13	5/165	17[c]	0
Funk et al.	1982	US	C	C	FT	D	20	141/3987	10	1
Godden[a] et al.	2003	US	C	C	FT	D	32	2/437	8	1
Harmon et al.	1986	US	I	I	E	S	29	1/156	7	0
Hassan et al.	1999	AU	C	I	FT	S	20	3/150	7[c]	0
Heald et al.	1977	US	C	C	FT	D	28	34/273	10	1
Hogan et al.	1994	US	C	I	FT	D	24	4/185	1	0
Langley et al.	1971	IE	C	Na	FT	S	Na	21/630	14	0
Meaney[a]	1976	IE	I	I	E	S	0	1/14	1	0
Meaney[a] and Mash	1977	IE	C	I	FT	S	14	2/140	1	0
Natzke et al.	1975	US	C	I	FT	D	23	1/800	10	1
Østerås et al.	1994	NO	C	C	FT	S	38	288/684	1	0
Pankey et al.	1982a	NZ	C	C	FT	D	26	7/214	21	1
Pankey et al.	1982b	NZ	C	C	FT	D	40	6/330	21	1
Pearson[a] and Wright-I	1969	IE	B	I	FT	S	37	60/146	21[c]	0
Person[a] and Wright-II	1969	IE	B	I	FT	S	50	1/49	4	0
Postle and Natzke	1974	US	C	I	FT	S	Na	Na/678	14	1
Rindsig[a] et al.	1978	US	I	I	E	S	10	1/232	14[c]	1
Robinson et al.	1988	UK	C	Na	FT	S	46	6/930	1	0
Schukken et al.	1993	NL	I	I	FT	D	88	1/68	1	0
Smith et al.	1967	ZA	I	I	FT	S	22	35/888	7	1
Soback et al.	1990	IL	C	I	FT	S	23	1/225	21	0
Swanson[a]	1979	US	C	I	FT	D	22	75/1318	10	1
Tarabla and Canavesio	2003	AR	C	I	FT	D	0	1/44	7	0
Williamson et al.	1995	NZ	B	I	FT	D	10	4/371	4	0
Ziv et al.	1981	IL	C	C	FT	S	6	14/1253	14	0

[*] The Roman numerals represent protocols within a study. [a] Within cow comparison was conducted (format 1). The other studies included between cow comparison (format 2). [b] Not available. [c] Collected extra post-calving sample. [1] Type of herd where the study was conducted: Institutional and research herds (I), commercial (C) or both herd types (B). [2] Source of funding: Institutional and governmental (I), commercial (C) or both (B). [3] Study type, field trial (FT) or experiment (E). [4] Samples type used to confirm diagnosis: single (S), or double (D). [5] Prevalence (%) of intramammary infections at dry off. [6] Follow up period post-calving in days. [7] Definition of new intramammary infection at calving or post-calving considered only new intramammary infections from healthy quarters at dry off (0) or from all quarters at dry off (1).

Milk and Secretion Samples for Bacterial Culturing. Most studies collected milk samples at dry off, and at calving or within few days after calving to diagnose IMI during the DP. Some studies collected 1 extra milk sample (up to 21 d after the calving sample) to examine the effects post-calving (Table 1). In 21 studies two or more consecutive single samples were collected to diagnose IMI, while a duplicate sample was collected in the other studies (Table 1).

Definition of a New Quarter IMI in the Meta-analysis

A quarter was considered as having a new IMI when a pathogen was isolated in the calving or post-calving samples from a quarter that was free of pathogen at the previous sampling or had a different pathogen (or pathogens) in the dry off sample. The majority of the studies defined a new IMI at calving or post-calving only when a pathogen was isolated from a previously healthy quarter (Table 1).

Re-calculating the Incidence of New Quarter IMI in the Meta-analysis

The majority of studies defined a new IMI in healthy quarters at dry off, and hence the incidence of new quarter IMI was recalculated for all studies based on the number of healthy quarters at dry off being the number of quarters at risk for new IMI (Appendix 3).

Meta-analysis Procedure

Outcome Parameters. The relative risk (**RR**) of new IMI was calculated as the incidence of new quarter IMI in the treatment group divided by the incidence of new quarter IMI in the control group per intervention and were pooled over studies in separate meta-analyses using a commercial analytical package (Comprehensive Meta-analysis (CMA), 2008): The pooled RR was calculated per intervention as an overall effect (all pathogens together), and per pathogen group (*Staphylococcus*, *Streptococcus*, and coliform) separately. In studies that included more than one protocol, a combined effect was calculated per study (CMA, 2008). Because studies were conducted by different people, in different areas, and times, which create heterogeneous population of studies, a random effect model was used to estimate the pooled RR. The pooled risk differences were similarly estimated. Forest plots were used to provide illustration of the calculated RR per study as well as the overall pooled effect of all studies in the last line of the plot. The forest plot is a graphical presentation of the results, which displays the point estimate and confidence interval of the effect observed in each study along with the summary estimate and its confidence interval (Dohoo et al., 2003).

Meta-Regression. A weighted meta-regression was conducted in an attempt to explain the variation between studies. Factors were selected for inclusion in the model based on expert assessment of likely factors, which were the factors presented in Table 1 (except sample size). Additionally, a factor Gram was defined to discriminate between studies that isolated only Gram positive bacteria and studies that isolated Gram positive and negative bacteria. All above factors were regressed against the RR results of each study and weighted by the inverse variance (Dohoo et al., 2003). The variables were first tested in univariable models and then combined in one multivariable model using a backward stepwise regression

method. A liberal P-value < 0.3 was chosen for the variable to be included in a combined multivariable model. In cases of a significant association between the explanatory variable and the dependant variable (RR per study) with P-value < 0.05 in the combined multivariable model, the variable was believed to explain the heterogeneity significantly. A meta-analysis was carried out only when at least 4 studies were available (Robert et al., 2006).

Publication Bias. The publication bias was assessed using so called funnel plots. A funnel plot is a plot of a measure of study size (standard error) on the vertical axis as a function of effect size on the horizontal axis. Large studies appear towards the top of the graph, and tend to cluster near the mean effect value. Smaller studies appear toward the bottom of the graph, and will be dispersed across a range of values. Methods included Duval and Tweedie's fill and trim method (Duval and Tweedie, 2000), Begg and Mazumdar's rank correlation test (Begg and Mazumdar, 1994), and Egger's regression test (Egger et al., 1997). When significant publication bias and change on the estimated pooled RR were detected, the number of studies necessary to reverse the overall pooled effect was calculated using Orwin's fail-safe N method (Orwin, 1983). The study influence was also examined using the one study removed method (Dohoo et al., 2003). When significant publication bias was deemed to exist, the pooled RR was presented based on the Duval and Tweedie's fill and trim method estimation after correcting for the bias. The interpretation of each of the above mentioned tests is provided in the results and the discussion sections. It is important to mention that these methods are hypothetical and based on statistical theory. They do not necessarily prove existence of bias, but they do indicate the potential to bias existence based on a statistical technique that mainly relate the effect size to the study size (Thornton and Lee, 2000). Further information about the strong and weak points of the publication bias methods are mentioned in the discussion section and are discussed in details by Thornton and Lee (2000).

RESULTS

Descriptive Results

Herds, Cows, and Funds. Eight studies had been conducted in institutional or research herds, 21 in commercial herds and 4 in both research and commercial herds (Table 1). Funding of 19 studies had been obtained from institutional and governmental funds, 9 from commercial funds, 2 from both institutional and commercial company funds, and the source of funding was unknown in 3 studies (Table 1). The number of herds varied from 1 herd, mainly in experimental studies, to 141 herds in field trials, the number of cows per study varied from 14 cows (also in an experiment) to 3987 in field trials (Table 1).

Management of Dry and Lactating Cows. In nearly all studies dry cows were separated from lactating cows. In some studies dry cows were kept in separate stalls from a few days before calving until calving or a few days post-calving and then moved to the dairy herd. Generally dry cows were housed on pasture, in free stalls or closed barn, and lactating cows were housed in free stalls or on pasture. The bedding mentioned was frequently sand, sawdust or straw.

Experimental Design. All studies involved in this analysis were randomized field trials (28 studies) or experiments (5 studies) (Table 1). Although the inclusion criteria varied between studies, frequently the infection status at dry off was used to include or exclude cows (Appendix 1). The majority of the studies had a negative control group, but some had positive controls (Appendix 2) and the majority of studies applied a between cow comparison.

Table 2. Pooled relative risk (RR[1]) together with the 95% confidence interval as estimated based on the corresponding studies in the meta-analysis for the different dry cow interventions for all pathogens (overall) and per pathogen group. Interventions were: Blanket dry cow therapy (BDCT), Cloxacillin vs. other dry cow therapy (DCT) products, selective DCT (SDCT), and teat sealant (TS). Foot notes are added to discriminate values presented after adjustment to publication bias when it significantly existed.

Intervention and pathogen group	Studies included in each meta-analysis to estimate the pooled RR	Pooled RR[1] (95% confidence)
BDCT vs. no DCT		
Overall	(Cummins, 1987; Dingwell, 2002; Eberhart, 1972; Funk, 1982; Harmon, 1986; Hassan, 1999; Heald, 1977; Hogan, 1994; Langley, 1971; Natzke, 1975; Pankey, 1982a,b; Pearson, 1969; Postle, 1974; Schukken, 1993; Smith, 1967; Soback, 1990; Swanson, 1979; Tarabla, 2003; Williamson, 1995)	0.61 (0.53-0.71)
Staphylococcus spp.	(Cummins, 1987; Eberhart, 1972; Funk, 1982; Harmon, 1986; Hassan, 1999; Heald, 1977; Hogan, 1994; Langley, 1971; Pankey, 1982a,b; Pearson, 1969; Postle, 1974; Schukken, 1993; Smith, 1967; Soback, 1990; Swanson, 1979; Tarabla, 2003; Williamson, 1995)	0.76[a] (0.54-1.07)
Streptococcus spp.	(Cummins, 1987; Eberhart, 1972; Funk, 1982; Harmon, 1986; Hassan, 1999; Heald, 1977; Hogan, 1994; Pankey, 1982a,b; Pearson, 1969; Postle, 1974; Smith, 1967; Swanson, 1979; Tarabla, 2003)	0.39 (0.30-0.51)
Coliform	(Cummins, 1987; Eberhart, 1972; Funk, 1982; Harmon, 1986; Hassan, 1999; Hogan, 1994; Postle, 1974)	0.95 (0.81-1.10)
Clox vs. other DCT		
Overall	(Davidson, 1994; Funk, 1982; Langley, 1971; Meaney, 1977; Ziv, 1981)	1.09 (0.94-1.25)
Staphylococcus spp.	(Davidson, 1994; Funk, 1982; Langley, 1971; Ziv, 1981)	1.12 (0.98-1.27)
SDCT vs. no DCT	(Berry, 2002a; Bratlie, 1973; Hassan, 1999; Østerås, 1994; Williamson, 1995)	0.51 (0.30-0.86)
SDTC vs. BDCT	(Browning, 1994; Hassan, 1999; Rindsig, 1978, Robinson, 1988; Williamson, 1995)	1.83 (1.24-2.71)
TS vs. no TS or TS+DCT vs. DCT Overall	(Berry, 2002b; Berry, 2007; Godden, 2003; Meaney, 1976)	0.39 (0.18-0.82)

[a] The pooled RR and the 95% confidence interval are presented based on Duval and Tweedie's (2000) fill and trim method owing to the existence of significant publication bias. [1] Incidence of new quarter IMI in treated group divided by the incidence of new quarter IMI in the control group. RR < 1, reduce risk of IMI; RR = 1, no protection; RR > 1, increase risk of IMI.

Bacteriological Culturing Procedure. The procedure of the National Mastitis Council was mainly followed in most studies (Hogan et al., 1999). Other studies used modified procedures based on this source. Nevertheless, in all studies sample handling from the farm until processing was similar especially storage, freezing and processing of the samples. Milk samples were obtained by proper cleaning of the teat and after discarding the first 3 to 4 squirts of milk. In most studies, samples were stored at -20˚ C until processed.

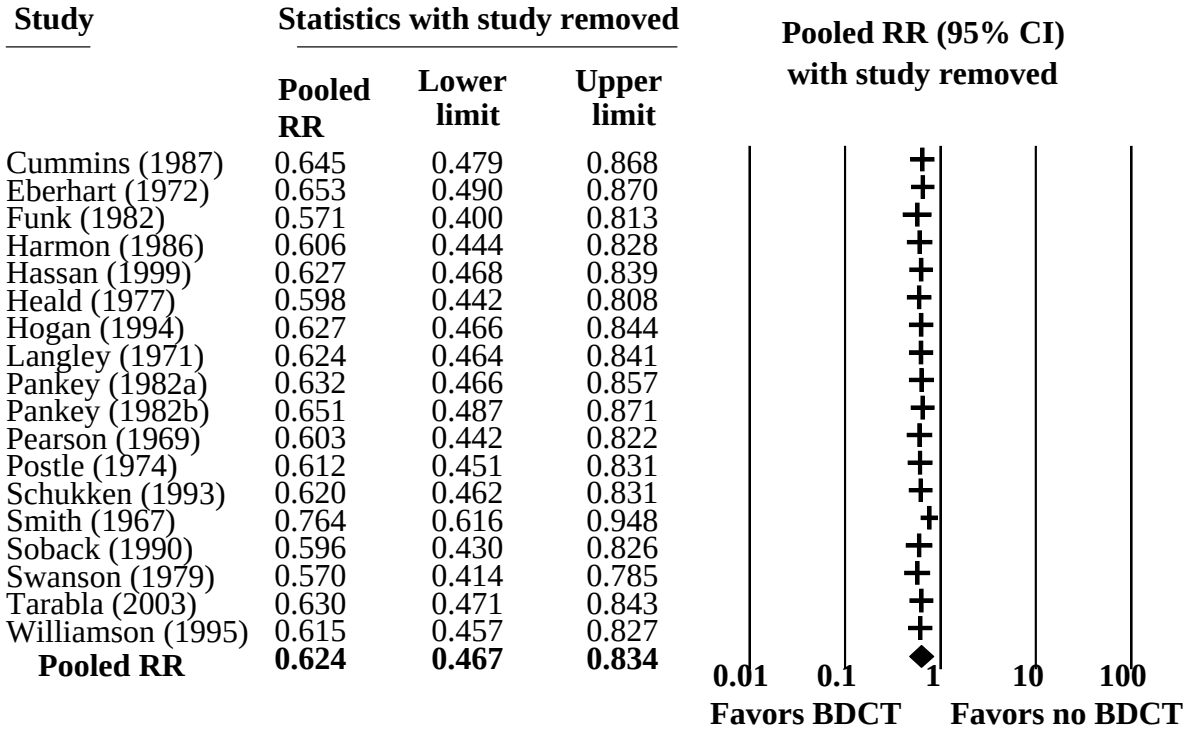


Figure 1. Forest plot of the change in the pooled relative risk (RR) of new *Staphylococcus* spp. quarter intramammary infections (IMI) during the dry period up to 21 d post-calving (RR = incidence of new *Staphylococcus* spp. quarter IMI in the blanket dry cow therapy (BDCT) group divided by the incidence in the untreated control group) together with the 95% confidence interval (CI), when the corresponding study was removed.

Interventions

BDCT vs. no DCT (n = 18 studies). The treatment was based on intramammary infusion of the antibiotic being tested in all quarters and all studies except two (Soback et al., 1990; Tarabla and Canavesio, 2003) where systemic DCT was applied intramuscularly.

Cloxacillin vs. other DCT Product (n = 5 studies). In the studies involved in the analysis, Cloxacillin and the other product were applied by the intramammary route.

SDCT vs. no DCT and SDCT vs. BDCT (n = 5 studies in each). Treatment was carried out in the SDCT group based on the unit of selection. Cow level selection treatment resulted in treating all quarters of the whole udder. At quarter level, only quarters that had elevated SCC or IMI were treated. Other quarters within the same udder were left untreated. When the control group was no DCT, no antibiotic was infused (negative control), but when

BDCT was the control group (positive control), all quarters of the udder were infused with the same antibiotic that was used in the SDCT group at quarter level only.

TS vs. no TS and TS + BDCT vs. BDCT (n = 4 studies). Studies used internal TS, which was applied after proper hygiene of the teat and after DCT in case of the positive control group studies.

Meta-analysis Results

BDCT vs. no DCT. In the separate univariable models, Gram (Gram positive vs. Gram positive and negative), year of publication (due to suspicions of bacterial antibiotic resistance over time), and the continent where the study originated from were selected to be included in the multivariable model (P-values < 0.3). None of the variables found to be associated at the Univariate level were found to be associated in the final (multivariable) model.

Overall, BDCT was protective against new IMI compared to untreated controls (Table 2). However, the protection varied between pathogens (Table 2). When *Staphylococcus* spp. were involved and before adjusting for publication bias DCT quarters had 0.62 (0.47-0.83) times less risk of new IMI than untreated quarters. Applying the one study removed method showed that removing any of the studies did not alter the random pooled RR significantly (Figure 1). The Begg and Mazumdar rank correlation test suggested that there was no correlation between the study size and effect (P-value = 0.33). However, the regression test of Egger contradicted Begg and Mazumdar's rank correlation test, suggesting a significant association between study size and effect size (Intercept = -1.73 with 16 degrees of freedom, one tailed P-value = 0.01). The fill and trim method of Duval and Tweedie indicated no missing studies on the left-hand side of the funnel plot, but 7 studies (black spots) were missing on the right-hand side to reach complete symmetry (Figure 2). This figure indicates that if publication bias did not exist and complete symmetry was reached by including the missing studies, the preventive effect would shift to the null effect. Because significant publication bias was indicated (Figure 2), the number of studies necessary to move the pooled RR above 1 was calculated using Orwin's fail-safe N method. According to Orwin's fail-safe N method and when the mean RR in the missing studies was assumed to be 1.10, the number of necessary studies was 17, but when the mean RR in the missing studies was assumed to be 1.20 the number of necessary studies was only 8. This number of studies is quite low suggesting that the protective effect of DCT against *Staphylococcus* spp. new IMI during the DP up to 21 d post-calving might be truly insignificant.

When meta-analysis was carried out for the *Streptococcus* spp. group, DCT provided high protection against *Streptococcus* spp. (Table 2). All publication bias tests indicated an absence of significant potential bias.

The protection of DCT against coliforms was insignificant (Table 2). All publication bias tests indicated an absence of potential bias.

Cloxacillin vs. other DCT Products. In general, other DCT showed similar protective effect from new IMI during DP compared to Cloxacillin (Table 2). Removing one study altered the effect, which is expected when a small number of studies is used (results not

shown). Duval and Tweedie's fill and trim method suggested that 2 studies are missing in the right-hand side, which would support the finding that Cloxacillin provides similar protection compared to other DCT (results not shown). Egger's regression test supported the presence of publication bias (P-value = 0.01). Correcting for publication bias would only confirm the insignificant difference between Cloxacillin and other DCT in protection against new IMI during the DP.

There was no significant difference between Cloxacillin and other DCT against new *Staphylococcus* spp. quarter IMI (Table 2). All publication bias tests indicated an absence of significant publication bias. The analysis was not carried out for other pathogen groups, because the number of studies available was less than 4.

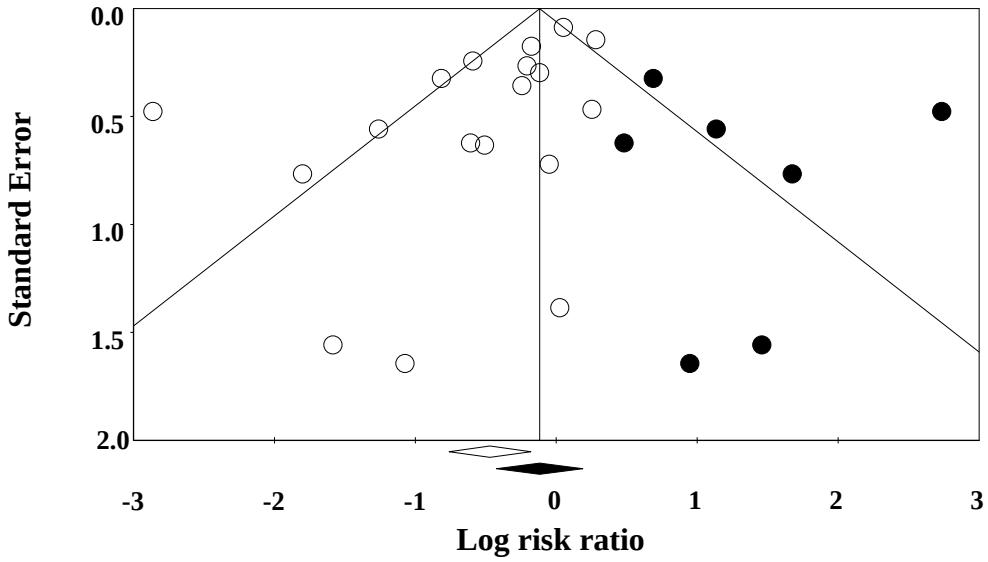


Figure 2. Funnel plot of the pooled relative risk (RR) of studies (empty circles) involved in the protective effect of dry cow therapy against new *Staphylococcus* spp. quarter intramammary infections (IMI) during the dry period up to 21 d post-calving (RR = incidence of new *Staphylococcus* spp. quarter IMI in the blanket dry cow therapy (BDCT) group divided by the incidence in the untreated control group). The dark spots are the potential missing studies according to Duval and Tweedie's (2000) fill and trim method (if they had existed, the pooled relative risk would have changed toward the null effect; the black diamond under the null effect). The light circles are the 18 studies involved.

SDCT vs. no DCT or SDCT vs. BDCT. SDCT provided significant protection against new quarter IMI, and protection was slightly higher when selection and treatment were carried out at cow level (Figure 3).

When SDCT was compared to BDCT, BDCT showed higher protection than SDCT (Figure 4). Nonetheless, there was no significant difference in protection from new quarter IMI between SDCT and BDCT when the selection unit was the cow, with the whole udder treated (Figure 4). When the SDCT selection unit was the quarter, BDCT provided better protection from new quarter IMI (on population level) than SDCT (Figure 4).

In both SDCT comparison analyses the one study removed test showed that some studies had significant influence on the pooled RR, which is expected when a small number of

studies is available (results not shown). Owing to the limited number of studies, the potential publication bias in relation to grouping was not further checked. Analysis was not conducted per pathogen group either, owing to the limited number of studies.

TS vs. no TS or TS + BDCT vs. BDCT. The only study with the positive control group gave similar protection compared to the other studies (P-value > 0.05) and therefore, a combined RR based on all studies was presented. In general, TS injected quarters have 0.39 (0.18-0.82) times less risk of new IMI than non TS injected quarters (Table 2). None of the publication bias tests indicated the existence of bias (results not shown). Analysis was not conducted per pathogen group, owing to the limited number of studies.

DISCUSSION

In order to limit the effect of prevalence at dry off on the incidence of new IMI during the DP and post-calving, and because it is the clearest indication of new IMI, the incidence of new IMI in the treatment and control groups was calculated considering only the number of healthy quarters at dry off to be the number of quarters at risk. Because infected quarters at start of the DP might recover and become infected again during the DP (re-infection), the re-calculation of the incidence might have estimated the true incidence wrongly in both groups. However, because all studies involved were randomized, the RR would not be affected because the re-calculation of the incidence was similar for the treatment and control group within study. In contrast, the pooled risk difference might have been slightly over-estimated, but the results of the risk difference showed the same patterns of the RR results. Risk differences results are not presented owing to space restriction, but are available from the first author. The calculated incidence assumed the uninfected glands at dry off to be the only quarters at risk. This might restrict the generalization of DCT efficacy on population level, because infected glands might recover and also contract new IMI during the DP. However, because the majority of the studies involved healthy quarters at dry off as quarters to be at risk of new IMI, the analysis was conducted accordingly. Thus conclusions of the current meta-analysis should be interpreted carefully. The companion paper provides a comprehensive discussion about the efficacy of DCT during the DP based on the current and the companion study (Halasa et al., unpublished data).

DCT was the largest group of studies, but results were heterogeneous indicating unexplained risk factors for the efficacy of DCT and that perhaps production system dependant decision about efficacy of DCT may be important to consider. Several attempts were made to restrict the heterogeneity. Inclusion only of studies with a negative control group, or only of studies that reported new quarter IMI, or only of studies conducted on dairy cows (excluding primiparous and beef cattle), or only randomized studies; none of these improved the homogeneity. Including only peer-reviewed papers in English language could have been a source of heterogeneity. Language might be a source of bias, because non-English speaking researchers might publish there positive results in English language journals to have more publicity, they would on the other hand publish there negative results in there native language (Gregoire et al., 1995). Although attempts using meta-regression with several

potential variables were made to explain the heterogeneity, none of them was able to explain it significantly. A possible source of heterogeneity could be the length of the DP, the longer the DP, the higher the chance of new IMI due to lower concentration of antibiotics around calving (Rindsig et al., 1978). However, because this information was not available in most studies and the length of the DP could differ considerably between cows within a study, it was not possible to test the effect of length of the DP on heterogeneity in the meta-regression.

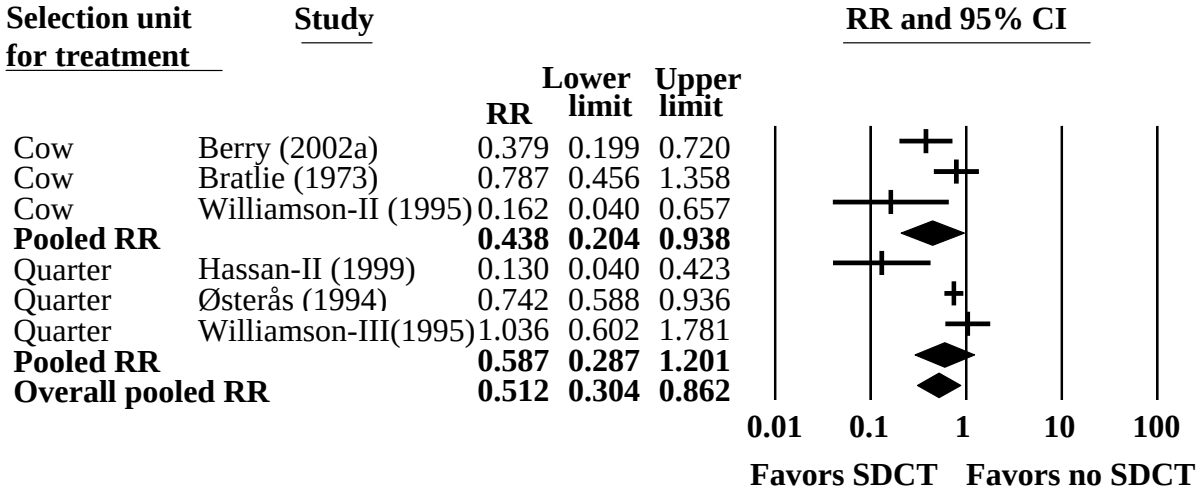


Figure 3. Forest plot of the relative risk (RR) of new quarter intramammary infections (IMI) during the dry period up to 21 d post-calving (RR = incidence of new quarter IMI in the selective dry cow therapy (SDCT) group divided by the incidence in the untreated control group) per study, and the pooled RR per SDCT selection unit and as an overall pooled RR effect together with the 95% confidence interval (CI) in the 5 studies involved.

The protective effect of DCT against coliform new IMI was not significantly higher than for untreated quarters (Table 2). Publication bias did not influence the result. In a previous meta-analysis (Robert et al., 2006), DCT did not significantly protect from new coliform IMI during the DP either. This was explained by the fact that coliform new IMI occurs late in the DP when DCT might not provide protection against new IMI any longer owing to low concentration (Robert et al., 2006). Another explanation of the apparent lack of efficacy is that the spectrum of activity of many of the DCT products does not cover Gram negative pathogens (Bradley and Green, 2001).

On the basis of a random effect model, initially BDCT provided significant protection against *Staphylococcus* spp. new quarter IMI. However, publication bias tests indicated the presence of significant bias. Adding 7 studies to the right-hand side of the funnel plot (Figure 2), led to nullifying the effect and suggested that DCT does not protect significantly against *Staphylococcus* spp. new IMI. Although large and small studies are present on both sides of the plot, 3 large studies including the largest study suggested that DCT does not protect against *Staphylococcus* spp. new IMI (Figure 2). The fact that the inclusion criterion of many studies that showed significant protection against *Staphylococcus* spp. new IMI was not mentioned (Appendix 1), suggested possible lower quality of the study design in those

studies. Moreover, the 7 missing studies might have been conducted, but not published in peer reviewed journals because they showed no protective effect. The fact that 2 of these studies would be small and another 2 studies relatively small (Figure 2), would support the speculation that they were not published (Dohoo et al., 2003). The reason for not publishing the data could be that those studies were sponsored by commercial companies and it would not be commercially beneficial to publish such data (Thornton and Lee, 2000). Another reason could be that the results might have not been interesting enough for publication. Many peer reviewed journals do not publish results if they are not striking or interesting enough or fail to show significant differences between treatment effects (Ferguson, 2007). Moreover, researchers themselves might not attempt to publish their research when the results contradict previous expectations (Ferguson, 2007). Egger's (1997) regression test was reported to be powerful in detecting publication bias for heterogeneous data such as ours (Peters et al., 2006). Orwin's (1983) fail-safe N method showed that adding a few studies would completely reverse the pooled RR and move it above 1. The disadvantage of the fail-safe N method is that it calculates the number of studies that are necessary to alter the effect, but does not calculate the number of studies that are needed to reach null effect as indicated by Rothstein (2008). Therefore, in this case, fewer studies are expected to make the difference insignificant, depending on the average RR in those studies.

In a previous meta-analysis (Robert et al., 2006), DCT quarters had 0.75 (0.61-0.91) RR of new coagulase-positive *Staphylococcus* spp. IMI during the DP, but not significantly less risk in the case of coagulase-negative *Staphylococcus*. However, the potential publication bias was not further investigated in that study. A separate analysis for coagulase-positive *Staphylococcus* in the current study showed the same trends as for the *Staphylococcus* group as a whole, indicating that asymmetry existed because of the missing studies (results not shown). Moreover, because sub-therapeutic doses were tested in 4 studies (Appendix 2) and might have affected the symmetry around the pooled RR, a separate subset was analyzed by removing protocols with sub- therapeutic doses. Still, Duval and Tweedie's (2000) fill and trim method suggested adding 5 studies; mainly small studies to the right-hand side of the plot to reach complete symmetry, which would lead to nullifying the protective effect against new *Staphylococcus* spp. IMI during the dry period up to 21 d post-calving (results not shown).

The procedure followed in the meta-analysis of Robert et al. (2006) to calculate the pooled RR was apparently assuming one true pooled RR (fixed effect model). Because of the heterogeneous nature of the studies selected, this assumption was violated. Therefore, the pooled RR should have been calculated assuming a distribution of true RRs (random effect model), in which weighting would be applied differently as indicated by Borenstein et al. (2007) and as applied in recent research (Duffield et al., 2008). Because different assumptions are involved for the different models of calculating the pooled RR, the pooled RR would not be precise, and in some cases it might actually fall out of the confidence limit of the true pooled RR (Borenstein et al., 2007). In the current study, the nature of the studies selected was taken into account and proper models and assumptions were considered.

Several studies found that many *Staphylococcus* spp. new IMI occur late in the DP and in early lactation (Soback et al., 1990; Hassan et al., 1999). Soback et al. (1990) indicated that

the failure of DCT to prevent new *Staphylococcus aureus* IMI during the DP could have occurred owing to the failure of the DCT to cure existing infections at dry off. Hogan et al. (1994) showed that DCT was not successful in preventing new *Staphylococcus aureus* IMI during the DP. They suggested that because *Staphylococcus aureus* is part of a normal flora of the teat skin and in late DP the teat canal would open and the bacteria would have access causing new IMI. Rindsig et al. (1978) observed that the longer the DP, the higher the chance of new *Staphylococcus* spp. IMI, and reasoned that the decreased DCT concentration provided less prophylactic effect. Analysis of data from a recent large field trial in Norway indicated that neither SDCT nor BDCT protected significantly from new quarter *Staphylococcus aureus* IMI during the DP and early lactation (A.C. Whist, unpublished data). When the publication bias tests indicate a potential bias, it might not be ultimately true. However, the methods are useful, because they indicate a potential bias, and hence enhance thinking of possible explanations that should be based on rational and biological reasoning. In our case and for all the rational and biological explanations mentioned above, it was deemed that publication bias could have truly existed in the peer reviewed literature, and hence making it difficult to conclude upon the protective effect of BDCT against *Staphylococcus* spp. new quarter IMI during the DP up to 21 d post-calving. On the other hand, the pooled RR was < 1, which economically might be good enough to pay off the cost of DCT, but economic analysis is still to be conducted.

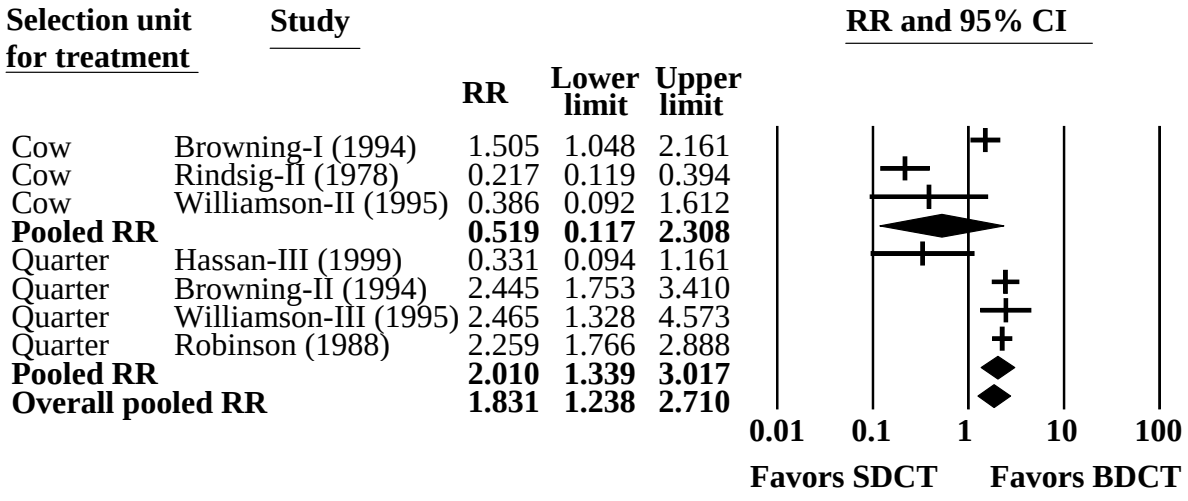


Figure 4. Forest plot of the relative risk (RR) of new quarter intramammary infections (IMI) during the dry period up to 21 d post-calving (RR = incidence of new quarter IMI in the selective dry cow therapy (SDCT) group divided by the incidence in the blanket dry cow therapy (BDCT) group) per study, and the pooled RR per SDCT selection unit and as an overall pooled RR together with the 95% confidence interval (CI) in the 5 studies involved.

The preventive effect of SDCT was significantly higher than not treating quarters (Table 2). When SDCT was compared to BDCT, and when selection was carried out at cow level for SDCT, there was no significant difference between SDCT and BDCT. However, BDCT showed higher protection when SDCT selection was carried out at quarter level (Figure 4). This might be explained because an infected quarter in a cow could have a higher

chance of infecting other healthy quarters in the same cow during the DP than infecting healthy quarters in other cows in the DP (Buddle et al., 1987). Treatment at cow level could or could not cure this infected quarter but at least would prevent the infection of other healthy quarters. Another possible reason is a misclassification error whereby some glands within a cow are incorrectly left untreated due, for example, to intermittent shedding of bacteria. Nonetheless, the low number of studies per stratum precluded meta-analysis per stratum and therefore the results were presented as a stratified overall effect.

Generally, internal TS provided a significant protection against new IMI (Table 2). Although large studies do exist, only 4 studies were found to fit our criteria, and therefore more studies could be necessary to draw conclusions properly on the efficacy of TS.

A meta-analysis was conducted on studies that challenged the effect of external supplementation to enhance the immune system to protect against new IMI during the DP. The supplementations did not provide significant protection against new IMI during the DP. Similarly, teat dipping did not provide protection against new IMI during the DP. The number of studies per comparison was low and the diversity between studies was high, which precluded proper comparison and hence conclusions on the protective effect of these interventions against new IMI during the DP.

CONCLUSIONS

DCT provided a significant protection from new quarter IMI caused by *Streptococcus* spp. during the DP up to 21 d post-calving. No protection was indicated against new coliform quarter IMI. After correction for publication bias, it became doubtful whether DCT is protective against new *Staphylococcus* spp. quarter IMI. Cloxacillin provided similar protection against all new quarter IMI and was similarly effective against new *Staphylococcus* spp. quarter IMI compared to other DCT. SDCT provided a significant protection against new quarter IMI when compared to no treatment. SDCT provided lower protection when compared to BDCT, with quarter SDCT the least effective. TS provided significant protection against new quarter IMI.

Because results were shown to change drastically through the effect of publication bias, publication bias should be included whenever attempts are made to review literature in a meta-analysis.

Acknowledgements

Authors acknowledge the help of Scott McDougall for providing some hard-to-find papers. This project was financially supported by the Dutch Udder Health Center (UGCN).

REFERENCES

Begg, C., and M. Mazumdar. 1994. Operating characteristics of a rank correlation test for publication bias. Biometrics 50:1088-1101.

Berry, E. A., and J. E. Hillerton. 2002a. The effect of selective dry cow treatment on new intramammary infections. J. Dairy Sci. 85:112-121.

Berry, E. A., and J. E. Hillerton. 2002b. The effect of an intramammary teat seal on new intramammary infections. J. Dairy Sci. 85:2512–2520.

Berry, E. A., and J. E. Hillerton. 2007. Effect of an Intramammary Teat Seal and Dry Cow Antibiotic in Relation to Dry Period Length on Postpartum Mastitis. J. Dairy Sci. 90:760–765.

Borenstein, M., L. Hedges, and H. Rothstein. 2007. Meta-Analysis: Fixed effect vs. random effects. Comprehensive meta-analysis, Biostat institute Inc., Englewood, New Jersey, USA.

Bradley, A. J., and M. Green. 2001. An investigation of the impact of intramammary antibiotic dry cow therapy on clinical coliform mastitis. J. Dairy Sci. 84:1632-1639.

Bradley, A. J., and M. Green. 2004. The importance of the lactating period in the epidemiology of intramammary infection and strategies for prevention. Vet. Clin. Food Anim. 20:547-568.

Bratlie, O. 1973. Dry cow therapy. Vet. Rec. 93:430–431.

Browning, J. W., G. A. Mein, P. Brightling, T. J. Nicholls, and M. Barton. 1994. Strategies for mastitis control: dry cow therapy and culling. Aust. Vet. J. 71:179–183.

Buddle, B. M., M. Herceg, M. J. Ralston, and H. D. Pulford. 1987. Reinfection of bovine mammary glands following dry cow antibiotic therapy. Vet. Microbiol. 15:191-199.

Comprehensive Meta Analysis. 2008. Meta-analysis manual. Biostat institute Inc., Englewood, New Jersey, USA.

Cummins, K. A., and T. A. McCaskey. 1987. Multiple infusions of cloxacillin for treatment of mastitis during the dry period. J. Dairy Sci. 70:2658–2665.

Davidson, T. J., I. R. Dohoo, and A. W. Donald. 1994. Comparing two dry cow treatments on the new infection and elimination rates of coagulase-negative staphylococci. Can. Vet. J. 35:775-776.

Dingwell, R. T., T. F. Duffield, K. E. Leslie, G. P. Keefe, L. DesCoteaux, D. F. Kelton, K. D. Lissemore, Y. H. Schukken, P. Dick, and R. Bagg. 2002. The efficacy of intramammary tilmicosin at drying-off, and other risk factors for the prevention of new intramammary infections during the dry period. J. Dairy Sci. 85:3250–3259.

Dohoo, I. R., S. W. Martin, and H. Stryhn. 2003. Veterinary Epidemiological Research, Atlantic Veterinary College Inc., Charlottetown, Prince Edward Island, Canada.

Duffield, T. F., A. R. Rabiee, and I. J. Lean. 2008. A meta-analysis of the impact of monensin in lactating dairy cattle. Part 2. production effects. J. Dairy Sci. 91:1334-1346.

Duval, S., and R. Tweedie. 2000. A non-parametric "trim and fill" method of accounting for publication bias in meta-analysis. J. Amer. Stat. Associ. 95:89-98.

Eberhart, R. J., and J. M. Buckalew. 1972. Evaluation of a hygiene and dry period therapy program for mastitis control. J. Dairy Sci. 55:1683–1691.

Egger, M., G. Davey Smith, M. Schneider, and C. Minder. 1997. Bias in meta-analysis detected by a simple graphical test. Br. Med. J. 315:629-634.

Ferguson, C. J. 2007. Evidence of publication bias in video game violence literature: A meta-analytic review, Agg. Viol. Beh. 12:470-482.

Funk, D. A., A. E. Freeman, and P. J. Berger. 1982. Environmental and physiological factors affecting mastitis at drying off and postcalving. J. Dairy Sci. 65:1258–1268.

Godden, S., P. Rapnicki, S. Stewart, J. Fetrow, A. Johnson, R. Bey, and R. Farnsworth. 2003. Effectiveness of an internal teat seal in the prevention of new intramammary infections during the dry and early-lactation periods in dairy cows when used with a dry cow intramammary antibiotic. J. Dairy Sci. 86:3899–3911.

Gregoire, G., F. Derderian, J. LeLorier. 1995. Selecting the language of the publications included in a meta-analysis: is there a tower of Babel bias? J. Clin. Epidemiol. 48:159-63.

Halasa, T., M. Nielen, T. Van Werven, H. Hogeveen, O. Østerås. Meta-Analysis of Dry Cow Management for Dairy Cattle. Part 2. Cure of Existing Intramammary Infections. J. Dairy Sci. Submitted in Sept. 2008.

Harmon, R. J., W. L. Crist, R. W. Hemken, and B. E. Langlois. 1986. Prevalence of minor udder pathogens after intramammary dry treatment. J. Dairy Sci. 69:843–849.

Hassan, Z., R. C. W. Daniel, D. O'Boyle, and A. J. Frost. 1999. Effects of dry cow intramammary therapy on quarter infections in the dry period. Vet. Rec. 27:635-639.

Heald, C. W., G. M. Jones, S. Nickerson, and T. L. Bibb. 1977. Mastitis control by penicillin and novobiocin at drying-off, Can. Vet. J. 18:171–180.

Hogan, J. S., K. L. Smith, D. A. Todhunter, and P. S. Schoenberger. 1994. Efficacy of dry cow therapy and a *Propionibacterum acnes* product in herds with low somatic cell count. J. Dairy Sci. 77: 3331–3337.

Hogan, J.S., R.N. Gonzales, R.J. Harmon, S.C. Nickerson, S.P. Oliver, J.W. Pankey, and K.L. Smith. 1999. Laboratory Handbook on Bovine Mastitis. National Mastitis Council, Madison WI.

Langley, O. H., J. Meaney, N. P. Cullen, and F. Cunningham. 1971. The control of mastitis, Vet. Rec. 89:315-316.

Meaney, W. J. 1976. Dry period teat seal, Vet. Rec. 99:30.

Meaney, W. J., and J. M. Mash. 1977. Comparison of two dry cow intramammary treatments, Vet. Rec. 100:492-493.

Morris, R. S., D. C. Blood, N. B. Williamson, C. M. Cannon, and R. M. Cannon. 1978. A healthy program for commercial dairy herds. 4. Changes in mastitis prevalence. Aust. Vet. J. 54:247-251.

Natzke, R. P., R. W. Everett, and D. R. Bray. 1975. Effect of Drying Off Practices on Mastitis Infection. J. Dairy Sci. 58:1828:1835.

Orwin, R. 1983. A fail-safe N for effect size in meta-analysis. J. Educ. Stat. 8:157-159.

Østerås, O., J. Aursjo, G. Gronningsaeter Gjul, and A. Jorstad. 1994. Effect of dry-cow therapy on subclinical mastitis - an evaluation of long acting and short acting intramammaria. J. Vet. Med. B 41:529-540.

Pankey, J. W., R. M. Barker, A. Twomey , and G.Duirs. 1982a. A note on effectiveness of dry cow therapy in New Zealand dairy herds, N. Z. Vet. J. 30:50–52.

Pankey, J. W., R. M. Barker, A. Twomey, and G. Duirs. 1982b. Comparative efficacy of dry-cow treatment regimens against *Staphylococcus aureus*. N. Z. Vet. J. 30:13–15.

Parkinson, T. J., J. J. Vermunt, and M. Merrall. 2000. Comparative efficacy of three dry cow antibiotic formulations in spring calving New Zealand dairy cows, New Zealand Vet. J. 48:129-135.

Pearson, J. K. L., and C.L. Wright. 1969. Dry cow therapy as a means of controlling bovine mastitis. Vet. Rec. 84:294–298.

Peters, J. L., A. J. Sutton, E. R. Jones, K. R. Abrams, and L. Rushton. 2006. Comparison of two methods to detect publication bias in meta-analysis. J. Am. Med. Ass. 295:676-680.

Postle, D. S., and R. P. Natzke. 1974. Efficacy of antibiotic treatment in bovine udder. Vet. Med. Small Anim. Clin. 1535-1940.

Rindsig, R. B., R. G. Rodewald, A. R. Smith, and S. L. Spahr. 1978. Complete versus selective dry cow therapy for mastitis control, J. Dairy Sci. 61:1483–1497.

Robert, A., H. Seegers., and N. Bareille. 2006. Incidence of intramammary infections during the dry period without or with antibiotic treatment in dairy cows – a quantitative analysis of published data. Vet. Res. 37:25–48.

Robinson, T. C., E. R. Jackson, and A. Marr. 1988. Mastitis incidence in quarters with different infection status at drying off and calving in two treatment groups. Br. Vet. J. 144:166– 173.

Rothstein, H., A. J. Sutton, and M. Borenstein. 2005. Publication Bias in Meta-Analysis: Prevention, assessment and adjustments, John Wiley & Sons, Ltd.

Rothstein, H. 2008. Publication bias as a threat to the validity of meta-analytic results. J. Exp. Criminol. 4:61-81.

Schukken, Y. H., J. van Vliet, D. van de Geer, and F. J. Grommer. 1993. A randomized blind trial on dry cow antibiotic infusion in a low somatic cell count herd. J. Dairy Sci. 76:2925– 2930.

Smith, A., D. R. Westgarth, M. R. Jones, F. K. Neave, F. H. Dodd, and G.C. Brander. 1967. Methods of reducing the incidence of udder infection in dry cows. Vet. Rec. 81:504–510.

Soback, S., G. Ziv, M. Winkler, and A. Saran. 1990. Systemic dry cow therapy – a preliminary report. J. Dairy Sci. 73:661–666.

Swanson, G. H. 1979. Posology and field efficacy study with novobiocin for intramammary infusion in nonlactating dairy cows. Can. J. Comp. Med. 43:440–447.

Tarabla, H., and V. Canavesio. 2003. Prevalence of intramammary infections by major pathogens at parturition in dairy cows after intramuscular antibiotic therapy at drying-off: a preliminary report. J. Dairy Res. 70:233–235.

Thornton, A., and P. Lee. 2000. Publication bias in Meta-analysis: its causes and consequences. J. Clin. Epidemiol. 53:207-216.

Williamson, J.H., M. W. Woolford, and A. M. Day. 1995. The prophylactic effect of a dry-cow antibiotic against *Streptococcus uberis*. N. Z. Vet. J. 43:228–234.

Ziv, C., M. Storper, and A. Saran. 1981. Comparative efficacy of three antibiotic products for the treatment and prevention of subclinical mastitis during the dry period. Vet. Q. 3:74-79.

APPENDIX

Appendix 1. Description of the inclusion criteria and the intramammary infection (IMI) pathogens[1] diagnosed per study.

Study	Inclusion criteria	Pathogens[1]								
		S	SA	CN	ST	Sag	SU	SD	C	O
Berry (2002a)	No or only *Coryn.*[2] IMI		X				X	X	X	X
Berry (2002b)	No clinical IMI		X			X	X	X	X	X
Berry (2007)	No major IMI pathogens			X			X	X	X	X
Browning (1994)	Medium SCC herds		X				X			X
Bratlie (1973)[3]	IMI status									
Cummins (1987)	Not available		X	X	X				X	X
Davidson (1994)	Herds with high IMI rate			X						
Dingwell (2002)	No pre-dry off treatment		X	X	X					X
Eberhart (1972)	Not available		X		X	X			X	
Funk (1982)	Not available	X			X	X				X
Godden (2003)	No Clinical IMI		X	X		X	X	X	X	
Harmon (1986)	Not available		X	X	X					X
Hassan (1999)	Not available		X			X	X	X	X	X
Heald (1977)	Not available		X		X	X			X	X
Hogan (1994)	3 functional quarters	X	X		X					X
Langley (1971)	Not available		X							
Meaney (1976)	No IMI		X					X		
Meaney and Mash (1977)	Not available		X			X				
Natzke (1975)	Not available		X		X	X			X	X
Østerås (1994)	Cows with IMI		X	X		X	X	X	X	X
Pankey (1982a)	Not available		X		X		X			
Pankey (1982b)	Had clinical IMI		X			X	X			
Pearson (1969)	Not available		X		X					X
Postle (1974)	Not available		X		X	X				X
Rindsig (1978)	Not available		X		X	X			X	
Robinson (1988)	Low IMI rate herds		X				X		X	X
Schukken (1993)	All dry off cows		X	X						X
Smith (1967)	Not available		X		X					
Soback (1990)	Cows with SA IMI		X							
Swanson (1979)	Major IMI pathogens		X		X	X				
Tarabla (2003)	No major IMI pathogen		X				X	X		
Williamson (1995)	Multiparous cows		X				X			X
Ziv (1981)	SCC level		X		X					

[1] Intramammary infection pathogens involved: *Staphylococcus* spp. (S), *Staphylococcus aureus* (SA), coagulase negative staphylococci (CNS), *Streptococcus* spp. (ST), *Streptococcus agalactiae* (Sag), *Streptococcus uberis* (SU), *Streptococcus dysgalactiae* (SD), coliforms (C), and other pathogens (O) which were mainly minor pathogens. [2] *Corynebacterium* spp. [3] No information was available about the isolated IMI pathogens

Appendix 2. The active ingredient per intervention[1] per study and the control group type included in the meta-analysis sorted by intervention and alphabetical order. When more than one study or protocol within a study applied the same intervention, they were grouped together.

Interventions[1] and active ingredients	Control[2]	Study
BDCT vs. no DCT		
CP (Clox, 500 mg)	NC	Cummins-I (1987), Postle (1974), Langley (1971), Hassan (1999)
CP (Clox, 1.5 g)	NC	Cummins-II (1987)
CP (Tilco, 1.5 g)	NC	Dingwell (2002)
CP (PP, 0.2^{m} IU) + (DHS, 100 mg)	NC	Eberhart (1972)
EP I-(PP, 1^{m} IU)+(DHS, 1 g) / II-(Clox, 500 mg) / III-(Nov, 50, 200, 400, 600 mg) / IV & V-(PP, 0.1, 0.2, 0.4, 1^{m} IU) + (Nov, 200, 400 mg)	NC	Funk[3] (1982)
CP I-(Nov, 400 mg) / II-(Cep, 300 mg) / III-(PP, 1^{m} IU) + (DHS, 1g)	NC	Harmon (1986)
EP I-(PP, 0.1, 0.2, 0.4^{m} IU) / II-(Nov, 400, 600 mg) / III-(PP, 0.1, 0.2, 0.4^{m} IU) + (Nov, 400 mg)	NC	Heald (1977)
CP I-(Cep, 300 mg)	NC	Hogan (1994)
CP (na, na)	NC	Natzke (1975)
CP I-(Nov, 550 mg) + (PP, 0.3^{m} IU) / II-(Nov, 400 mg) + (PP, 0.2^{m} IU)	NC	Pankey (1982a)
CP DCT I-(Neo, 500 mg) + (PP, 0.325^{m} IU) / CP II-(Clox, 500 mg)	NC	Pankey (1982b)
CP I-(PP, 0.35^{m} IU) / II-(Clox, 500 mg)	NC	Pearson (1969)
CP (PP, 0.3^{m} IU) + (DHS, 100 mg)	NC	Schukken (1994)
CP I-(Clox, 0.2 g) / EP-II-(Clox, 0.1 g)	NC	Smith (1967)
CP I-(Nor, 250 mg) / II-(Oxy, 10 mg/kg) / III-(Cep, 500 mg)	NC	Soback (1990)
EP I-(Nov, 50 mg) / II-(Nov, 200 mg) / III-(Nov, 400 mg) / IV-(Nov, 600 mg)	NC	Swanson (1979)
na (Spi, 2.5 g) + (Str, 5 g)	NC	Tarabla (2003)
CP (Cephl, 250 mg)	NC	Williamson (1995)
Cloxacillin vs. other DCT products		
CP (PP, 0.2mIU) + (Novo, 400 mg) or CP (Clox, 500 mg)	DCT	Davidson (1994)
CP I-(PP, 0.3mIU) + (Novo, 250 mg) / II-(BP, 3^{m} IU) + (DHS, 0.25^{m} IU) or CP (Clox, 500 mg)	DCT	Langley (1971)
CP (DHS, 500 mg) or CP (Clox, na)	DCT	Meaney (1977)
CP (PP, 300 mg) + (DHS, 100 mg) / CP (SN, 100 mg) or CP (Clox, 500 mg)	DCT	Ziv (1981)
SDCT vs. no SDCT or SDCT vs. BDCT		
CP I-(Cephl, 250 mg) / CP II-(Clox, 600 mg)	NC	Berry (2002a)
CP (Clox, 500 mg)	PC	Browning (1994)
CP (Clox, 500 mg)	NC/PC	Hassan (1999)
CP (PP, 1^{m} IU) + (DHS, 1 g)	PC	Rindsig (1978)
CP (Cephl, 250 mg)	NC/PC	Williamson(1995)
CP (PP, 1 g) + (DHS, 0.5 g) / CP (Clox, 500)	PC	Robinson (1988)
CP (PP, na) + (Neo, na) or (Neo, na) or (Clox, na)	NC	Bratlie (1973)
CP I-(BP, 0.1 mIU) + (PP, 0. 3^{m} IU) + (Neo, 100 mg) / II-(BP, 0. 3^{m} IU) + (DHS, 300 mg)	NC	Østerås (1994)
TS vs. no TS or TS + DCT vs. DCT		
CP-TS	NC	Berry (2002b), Meaney (1976)
CP-TS + CP-DCT (Cephl, na)	PC	Berry (2007)
CP-TS + CP-DCT (Clox, 500 mg)	PC	Godden (2003)

[1] Type of intervention and the active ingredient of each intervention applied; blanket dry cow therapy (BDCT), Cloxacillin (Clox) vs. other dry cow therapy (DCT) products, selective DCT (SDCT), and teat sealant (TS), preceded by the origin of the preparation; commercial (CP) or experimental preparation (EP); information not available (na) and the Roman numerals to represent protocols within a study. Preparations were: the dose is presented per million international units (^{m} IU); Procaine Penicillin (PP) or Benzyl Penicillin (BP); Dihydrostreptomycin (DHS), Novobiocin (Nov), Cephapirin (Cep), Norfloxacin (Nor), Neomycin (Neo), Spiramycin (Spi), Streptomycin (Str), Cephalonium (Cephl), Sodium nafcillin (SN), Tilcomicin sulfate (Tilco), Oxytetracycline (Oxy).
[2] Control group was either negative controls (NC: had no treatment or had a placebo) or positive controls (PC: had an application which was also applied to the treatment group).
[3] This study was also used in comparing the effect of Cloxacillin to the other preparations; Cloxacillin was used as treatment group and the other preparations as controls.

Appendix 3. Incidence[1] (%) of new quarter intramammary infections (IMI) in treated (T) and control (C) groups and (the number of healthy quarters at start in each group) for studies involved in the meta-analyses per intervention and pathogen group. Interventions were: Blanket dry cow therapy (BDCT), Cloxacillin vs. other dry cow therapy (DCT) products, selective dry cow therapy (SDCT), teat sealant (TS), external non-antibiotic formulation for the immune system (IS), and teat dipping (TD).

Study	Incidence[1] of new quarter intramammary infection									
	Overall		*Staphylococcus*		*Streptococcus*		Coliform		Other	
	T	C	T	C	T	C	T	C	T	C
BDCT vs. no DCT										
Cummins (1987)	22* (67)	42 (69)	1.5CP*, 0CN*	10CP, 15CN	0ST	10ST	0*	2.9	20*	4.3
Dingwell (2002)	14* (499)	19 (439)								
Eberhart-I[a] (1972)	27* (79)	37 (107)	1.2SA*	12SA	2.5Sag, 8.9ST*	3.7Sag, 15ST	13	11	2.5	0
Funk[b]^-I (1982)	9 (794)	12 (2093)	4.4SA	3.6SA	0.9Sag, 2.8ST	1.9Sag, 5.5ST	1.4	1		
Funk^-II (1982)	9 (710)	12 (2093)	4.4SA	3.6SA	0.1Sag, 4.4ST	1.9Sag, 5.5ST	0.6	1		
Funk^-III (1982)	5 (5610)	12 (2093)	3.2SA	3.6SA	0.5Sag, 1ST	1.9Sag, 5.5ST	1	1		
Funk^-IV (1982)	9 (2614)	12 (2093)	4.7SA	3.6SA	0Sag, 3.5ST	1.9Sag, 5.5ST	0.8	1		
Funk^-V (1982)	10 (938)	12 (2093)	2.1SA	3.6SA	5.3Sag, 7.5ST	1.9Sag, 5.5ST	0.6	1		
Harmon-I (1986)	15 (101)	23 (88)	0SA, 8.9CN	1.1SA, 10CN	4ST	6.8ST	2	1.1	0*	3.7
Harmon-II (1986)	7* (112)	23 (88)	0SA, 1.8CN*	1.1SA, 10CN	1.8ST	6.8ST	0	1.1	3.6	3.7
Harmon-III(1986)	22 (98)	23 (88)	0SA, 17CN	1.1SA, 10CN	1ST	6.8ST	1	1.1	3.1	3.7
Hassan-I (1999)	7* (153)	16 (173)	0SA	0SA	1Sag, 0SU, 2SD	0.6Sag, 0SU,0SD	2.6	0.8	1.3*	15
Heald-I^-I (1977)	12 (358)	19 (81)	3.1SA	2.5SA	0.6Sag, 1.1ST	2.5Sag, 16ST			7.8	0
Heald-I^-II (1977)	12 (186)	19 (81)	3.2SA	2.5SA	0.5Sag, 1.6ST	2.5Sag, 16ST			7	0
Heald-I^-III(1977)	22 (230)	19 (81)	4SA	2.5SA	0.6Sag, 2.1ST	2.5Sag, 16ST			15.6	0
Hogan-I (1994)	26* (274)	33 (291)	1.5SA, 16.4S	2.7SA, 17.2S	2.6ST*	4.8ST	0	0	7.3	11
Natzke (1975)	7* (397)	13 (402)								
Pankey (1982a)	10* (186)	15 (179)	4.8SA*	10SA	4.8SU	5.1SU				
Pankey (1982b)	17 (117)	22 (117)	10SA	12SA	0Sag, 6.8SU	3.4Sag, 6.8SU				
Pearson-I (1969)	14 (73)	16 (296)	11CP	11.8CP	1.4ST	3ST			1.4	1.4
Pearson-II (1969)	12 (108)	12 (106)	7.4CP	5.7CP	2.8ST	4.7ST			1.9	1.9
Schukken (1993)	37 (16)	50 (16)	0SA, 6.3CN	0SA, 6.3CN					31.2	44

Appendix 3 continued

Study	Incidence[1] of new quarter intramammary infection									
	Overall		*Staphylococcus*		*Streptococcus*		Coliform		Other	
	T	C	T	C	T	C	T	C	T	C
Postle (1974)	9 (1423)	16 (589)	1.7SA	2.2SA	0.8Sag, 4.9ST	3.7 Sag, 10.2ST	1.9	3.1		
Smith-I (1967)	2* (532)	16 (420)	0.8SA*	8.3SA	1.5ST*	7.6ST				
Smith-II (1967)	2* (544)	16 (420)	0.2SA*	8.3SA	1.8ST*	7.6ST				
Soback-I (1990)	(131)	(65)	18.3CP*	29.2 CP						
Soback-II (1990)	(62)	(65)	9.7CP*	29.2 CP						
Soback-III (1990)	(58)	(65)	51.7 CP*	29.2 CP						
Swanson-I (1979)	10 (1025)	10 (1017)	4.4SA*	2.6SA	1.7Sag, 5.1ST*	2.1Sag, 7.6ST				
Swanson-II(1979)	6* (1049)	10 (1017)	3.2SA	2.6SA	0.6Sag*, 2.3ST*	2.1Sag, 7.6ST				
Swanson-III (1979)	7* (1049)	10 (1017)	2.9SA	2.6SA	0.4Sag*, 3.7ST	2.1Sag, 7.6ST				
Swanson-IV (1979)	6* (1057)	10 (1017)	2.7SA	2.6SA	0.4Sag*, 2.9ST*	2.1Sag, 7.6ST				
Tarabla (2003)	2* (88)	11 (88)	0SA	2.3SA	2.3SD, 0SU*	1.1SD, 8SU				
Langley (1971)	(160)	(154)	2.5SA	4.6SA						
Williamson-I (1995)	4* (556)	10 (516)	0.7SA	0.8SA	0.4SU*	6.4SU			3.2	3.1
Cloxacillin vs. other DCT products										
Davidson (1994)			3.1CN (160)	5.5CN (164)						
Langley-I (1971)	(744)	(690)	1.7SA	1.7SA						
Langley-II (1971)	(744)	(703)	1.7*SA	3SA						
Meaney (1977)	1* (277)	3 (280)								
Ziv-I (1981)	45 (135)	40 (455)	27SA	20.7SA	18.2ST	19.6ST				
SDCT vs. no DCT or SDCT vs. BDCT										
Berry-I (2002a)	4* (318)	7 (341)	0SA	1.5SA	1.3SU*	4.1SU	2.5	1.5		
Berry-II (2002a)	0* (54)	17 (58)	0SA*	10.4SA	0SU*	6.9SU	0	0		
Bratlie (1973)	10 (313)	12 (143)								

Appendix 3 continued

Study	Incidence[1] of new quarter intramammary infection									
	Overall		*Staphylococcus*		*Streptococcus*		Coliform		Other	
	T	C	T	C	T	C	T	C	T	C
Browning-I (1994)	6 (1822)	2 (1805)								
Browning-II (1994)	4 (1837)	3 (1805)								
Hassan-II (1999)	2 (126)	18 (173)	0SA	0.8SA	0Sag, 0.8SU, 0.8SD	0Sag, 0SU, 3.2SD	0.8	0.8	0	13.4
Hassan-III (1999)	2 (126)	7 (153)	0SA	0.8SA	0Sag, 0.8SU, 0.8SD	1Sag, 0SU, 2SD	0.8	2.6	0	1.3
Østerås-I (1994)	16* (297)	24 (275)								
Østerås-II (1994)	19 (259)	24 (275)								
Rindsig-II (1978)	7 (216)	8 (181)	4.2SA	6SA	0.9Sag, 1.8ST	0.6Sag, 0ST	0	1.1	0.9	0
Robinson (1988)	15 (986)	7 (1424)								
Williamson-II (1995)	2 (120)	10 (516)								
Williamson-III (1995)	11 (141)	10 (516)								
TS vs. no TS and TS + DCT vs. DCT										
Berry (2002b)	2* (704)	7 (556)	1SA*	2.5SA	1SU*	3.4SU	0	0.8		
Berry (2007)	4* (574)	8 (492)	1.7CN	2.6CN	1.4SU, 0SD	3.5SU, 0.2SD	0.3	1	0.3	0.6
Godden (2003)	29* (575)	37 (552)	1.2SA, 9.2CN	1.8SA, 12CN	0Sag, 6.1SU, 0.3SD	0.4Sag, 3.3SU, 1.5SD	11.5	12.3	0.9	5.4
Meaney (1976)	4* (28)	32 (28)								

[a]The Roman numerals represent protocols within a study.

[b] This study was also used for the comparison of Cloxacillin vs. other DCT products, where the protocol of Cloxacillin treatment (Funk-II et al., 1982) was used as the treatment group and the other protocols as the positive control group.

[1] The number of new intramammary infections quarters during the dry period up to 21 d post-calving divided by the number of healthy quarters at dry off. The incidence is presented as percentage.

* Use of symbols: An asterisk (*) When significantly different than the comparative control group (P-value < 0.05),

A circumflex (^) when no statistical analysis was conducted,

No script when statistical significance was not found or not mentioned in the original study.

Pathogens are: all diagnosed *Staphylococcus* spp. (S), *Staphylococcus aureus* (SA), coagulase-positive *Staphylococcus* spp. (CP), *coagulase-negative Staphylococcus* spp. (CN), all diagnosed *Streptococcus* spp. (ST), *Streptococcus uberis* (SU), *Streptococcus dysgalactiae* (SD), and *Streptococcus agalactiae* (Sag).

Chapter 6

Meta-Analysis of Dry Cow Management for Dairy Cattle. Part 2. Cure of Existing Intramammary Infections

T. Halasa,‡[1] M. Nielen,‡ A. C. Whist,* and O. Østerås†

‡ Utrecht University, Department of Farm Animal Health and Reproduction, P.O. Box 80151, 3584 CN Utrecht, the Netherlands
***** TINE Norwegian Dairies, Department of Norwegian Cattle Health Services, PO Box 58, N-1431 Ås, Norway
† Norwegian School of Veterinary Science, Department of Production Animal Clinical Sciences, P.O. Box 8146 Dep., N-0033 Oslo, Norway

Accepted for publication in Journal of Dairy Science

ABSTRACT

A main goal of dry cow therapy (DCT) is to cure existing intramammary infections (IMI) at dry off. Although several studies have been published to estimate the cure rate of IMI after DCT, variation is large among studies, which makes it difficult to conduct a proper economic evaluation of DCT. The objective of the present meta-analysis of existing peer reviewed literature was to provide a summary quantification of quarter IMI cure based on DCT. A meta-analysis relative risk (RR) was calculated per intervention and pathogen group when at least 4 studies were available for analysis per comparison from the 22 selected studies, according to the selection criteria. Results of the meta-analyses were examined using publication bias tests. Blanket DCT with the 95% confidence interval (CI) provided a 1.78 (1.51-2.10) times higher calculated cure rate from quarter IMI, during the dry period (DP) up to 21 d post-calving, compared to no DCT. The RR of cure was similar when treatment was conducted for *Streptococcus* spp. IMI quarters compared to *Staphylococcus* spp. IMI quarters. The pooled RRs with the 95% CI were 1.83 (1.48-2.35) and 1.65 (1.38-1.96), respectively. There was no significant difference between cloxacillin and other DCT products in cure of quarter IMI during the DP up to 21 d post-calving. The pooled RR with the 95% CI was 1.00 (0.92-1.09). Similarly, there was no significant difference between cloxacillin and other DCT products in cure of quarter *Staphylococcus* spp. IMI. The pooled RR with the 95% CI was 1.00 (0.96-1.06). The pooled RR with the 95% CI of quarter IMI cure using selective DCT compared to no DCT were 1.76 (1.23-2.54).

Key words: Mastitis, Dairy Cattle, Management, Dry Period, Meta Analysis

INTRODUCTION

There are 2 main objectives of dry cow therapy (**DCT**): eliminating existing intramammary infections (**IMI**) present at drying off and preventing new IMI during the dry period (**DP**) and around calving. Pearson (1950, 1951) published some of the first papers on DCT in response to great concerns about summer mastitis in non-lactating or dry heifers and cows. However, since then scientists have sought more evidence of the efficacy of DCT. In 1962, Oliver et al. (1962) commenced an experimental DCT trial where they concluded that *Staphylococcus aureus* IMI during the DP is primarily due to pathogens that contaminate the teats after the last milking of lactation. They also concluded that infusion of antibiotics at the beginning of the DP can provide nearly full protection against infection with *Staphylococcus* spp. and *Streptococcus* spp. (Oliver et al., 1962).

During the 1960s and 1970s, the reports by Oliver et al. (1956a, b, c, d, e, f; 1962) were often cited, and blanket DCT (**BDCT**) was recommended as an important tool to reduce the level of IMI during the beginning of the DP (Kingwill et al., 1970). However, Oliver and Mitchell (1983) noted that these studies were performed under conditions markedly different from those in current dairy systems (that is 20 years after the studies in question). Since trial results from the 1960s and 1970s were limited and generally evaluated the efficacy of antibiotics intended for lactation treatment, additional investigations on DCT were needed (Oliver and Mitchell, 1983). A

major development was the formulation of DCT products that could achieve slow release over the DP and around calving (Pankey et al., 1982a, b). Rindsig et al. (1978) completed a trial comparing BDCT and selective DCT (**SDCT**) where the conclusion was that SDCT was as effective as BDCT in eliminating existing infections, but that BDCT should be preferred in situations where there was concern for new infections during the DP.

Frequently, studies investigated the efficacy of quarter IMI cure by comparing different DCT products (Pankey et al., 1982b; Davidson et al., 1994). Cloxacillin was frequently challenged owing to its common use as a DCT application (Ziv et al., 1981; Dingwell et al., 2003).

In the various efficacy trials, a large variation among studies was observed, which could be due to different study designs and the application of different DCT products (Erskine et al., 2003). Surprisingly, a systematic review of all estimates was not published, and this is essential for treatment decisions and economic evaluation of DCT applications. The objective of the present meta-analysis study was to provide a summary quantification of quarter IMI cure using different DCT management strategies based on existing peer reviewed literature.

MATERIALS AND METHODS

Selection of Papers

A search was conducted on published literature related to DP management published between 1930 and the beginning of 2008. The search was carried out using different key words in Pubmed (National Library of Medicine, Bethesda, USA) and the reference citation procedure in the ISI web of knowledge (The Thompson Cooperation, Philadelphia, USA).

Papers to be included in the meta-analysis had to: 1) be original research papers published in a peer reviewed journal; 2) report the number or the rate of IMI cure at quarter level in at least 2 groups (treatment and control group) and the total number of infected quarters in each group; 3) be published in or have a summary in English; 4) report the outcome of a new data set or protocol (when several studies were published based on the same data, the most detailed study was used.) Furthermore, only protocols that involved a therapeutic dose that was high enough (as recommended by the original studies) were included. Protocols with sub-therapeutic doses were not included, in order not to bias the weighted averages of cure rate. Further details per study were considered based on the interventions involved and are explained in the next section.

A total of 23 studies fitted the criteria, of which 22 were included in the meta-analysis. One study (Newbould, 1979) was excluded owing to serious design differences compared to the other studies. The studies report the outcome of one or of several protocols. When several protocols are reported, Roman numerals are added to distinguish between protocols. Two formats were observed in each type of study selected; treatments were applied either to the cow or to a single quarter. Table 1 and the descriptive results show data related to the study design and the level of analysis per study.

Table 1. Description of each study included in the meta-analysis ranked in alphabetical order.

Study	Year	Origin	Herd type[1]	Fund [2]	Study type[3]	ST[4]	PD[5]	Sample Herd/Cow	FPP[6]
Berry and Hillerton-I	2002	UK	I	I	FT	S	16	2/236	1
Berry and Hillerton-II	2002	UK	C	I	FT	S	49	2/54	1
Bratlie	1973	NO	C	Na[b]	FT	S	Na[b]	48/746	4
Cummins and McCaskey	1987	US	I	I	E	D	42	1/90	5
Davidson et al.	1994	CA	C	B	FT	S	12	7/84	7
Dingwell et al.	2003	CA	C	C	FT	S	30	77/288	30[c]
Harmon et al.	1986	US	I	I	E	S	29	1/156	7
Heald et al.	1977	US	C	C	FT	D	28	34/273	10
Hogan et al.	1994	US	C	I	FT	D	24	4/185	1
Langley[a] et al.	1971	IE	C	Na[b]	FT	S	Na[b]	1/91	1
Meaney[a] and Mash	1977	IE	C	I	FT	S	14	2/140	1
Natzke et al.	1974	US	C	I	FT	D	23	1/800	10
Østerås et al.	1994	NO	C	C	FT	S	38	288/684	1
Pankey et al.	1982a	NZ	C	C	FT	D	26	7/214	21
Pankey et al.	1982b	NZ	C	C	FT	D	40	6/330	21
Pearson and Wright[a]-I-II	1969	IE	B	I	FT	S	37	60/146	14
Pearson and Wright[a]-III	1969	IE	B	I	FT	S	50	1/49	4
Postle and Natzke	1974	US	C	I	FT	S	Na[b]	Na/678	14
Rindsig[a] et al.	1978	US	I	I	E	S	10	1/232	7
Schukken et al.	1993	NL	I	I	FT	D	88	1/68	1
Smith et al.	1967	ZA	I	I	FT	S	22	35/888	7
Soback et al.	1990	IL	C	I	FT	S	23	1/225	21
Swanson[a]	1979	US	C	I	FT	D	22	75/1318	10
Ziv et al.	1981	IL	C	C	FT	S	6	14/1253	14

[a] Within cow comparison was conducted, while other studies conducted between cow comparison. [b] not available. [c] Three consecutive samples were taken post-calving only to confirm cure during the DP. [1] Type of herds where the study was conducted: Institutional and research herds (I), commercial (C) or both herds (B). [2] Source of fund: Institutional and governmental (I), commercial (C) or both (B). [3] Study type: field trial (FT) or experiment (E). [4] Samples type used to confirm diagnosis: single (S), or double (D). [5] Prevalence (%) at dry off. [6] Follow up period post-calving in days.

Management Groups Involved in the Meta-analyses

Studies that reported the number or rate of quarter IMI cure during the DP up to 21 d post-calving using BDCT were included in the analysis. In these studies the treatment group was treated IMI quarters at dry off, while the control group was untreated IMI quarters at dry off.

A separate analysis was also conducted on studies reporting the cure rate during the DP using cloxacillin as the treatment group and the other DCT preparation as the control group.

Studies that measured the number or rate of quarter IMI cure using SDCT during the DP up to 21 d post-calving were included in the analysis. Selection of treatment unit was

either at quarter or cow level. However, the analysis could not be carried out separately per selection unit for treatment, because there was only one study (Østerås et al., 1994) for quarter as the selection unit for treatment. This would lead to potential malestimation of the summary estimate.

Definition of a Cure

The definition of cure considers 2 possibilities. Firstly, a quarter was considered cured when a pathogen was not isolated at calving or post-calving from a quarter that had had an isolate of a pathogen at drying off. This was the definition most frequently used. Secondly, when a pathogen was isolated in the post-calving sample from a quarter that had had a different isolate at dry off, the quarter was considered cured of the first pathogen and had acquired a new IMI. This was rarely encountered and the number of such quarters had not been mentioned in the original studies.

Re-calculating the Incidence of Cure

The definition of cure (see previous section) included quarters that had had the isolation of a pathogen at drying off that became bacteriologically negative or had had a different pathogen in the calving or post-calving sampling. Thus, the cumulative incidence of cure, which will be called 'cure rate' throughout this paper was re-calculated as the number of cured quarters divided by the total number of IMI quarters at dry off. The IMI quarters were assumed to be the total number of quarters at risk for cure during the DP up to 21 d post-calving.

Meta-analysis Procedure

Outcome parameters. The relative risk (**RR**) of cure (cure rate in the treatment group divided by the cure rate in the control group) was calculated per study, and then RRs per intervention were pooled in a meta-analysis using a commercial product (Comprehensive Meta analysis (CMA), 2008). The pooled RRs are presented per intervention as an overall effect (all pathogens together), and per pathogen group (*Staphylococcus* spp., and *Streptococcus* spp.) separately. In studies that included more than one protocol, a combined effect was calculated per study (CMA, 2008).

Meta-regression was not conducted because of small sample size per subgroups on variables presented in Table 1. The small number of studies per subgroup might indicate a lack of difference between the subgroups due to a lack of power and not due to a lack of difference. Therefore, a random effect model was used to calculate the pooled RR (CMA, 2008). The pooled RR with its 95% confidence interval (**CI**) and prediction interval (**PI**) are presented. The PI was calculated based on Chebyshev's inequality, which indicates that at least 95% of true effects would lie within 4.47 standard deviations of the mean effect (Higgins et al., 2009). The PI indicates the limits where the estimate of a future trial would exist (Higgins et al., 2009). The weighted average of cure was calculated by multiplying the estimated cure rate per study by the assigned weight to each study and summed over all studies then divided by the sum of the weights. The corresponding 95% CI was calculated based on the cure rates in the original studies.

Publication bias. The publication bias was assessed using Duval and Tweedie's fill and trim method (Duval and Tweedie, 2000), Begg and Mazumdar's rank correlation test (Begg and Mazumdar, 1994), and Egger's regression test (Egger et al., 1997). When significant publication bias and change in the estimated pooled RR was detected, the number of studies necessary to reverse the overall pooled effect was calculated using Orwin's fail-safe N method (Orwin, 1983). The influence of study was also examined using the one study removed method (Dohoo et al., 2003). When significant publication bias was deemed to exist, the pooled RR was presented based on the Duval and Tweedie's fill and trim method estimation after correcting for the bias.

RESULTS

Descriptive Results

Herds, Cows, and Funding. Fifteen studies were conducted in commercial herds, 5 in institutional or research herds and 2 in both research and commercial herds (Table 1). While the number of herds varied from 1 herd mainly in experimental studies to 288 herds in field trials, the number of cows varied from 68 cows to 1318 in field trials (Table 1). The funding of 13 papers was obtained from institutional and governmental funds, 6 from commercial funds, 1 from both institutional and commercial funds, and the source in 2 studies was unknown (Table 1).

Experimental Design. All studies involved were randomized field trials (19 studies) or 3 experimental studies (Table 1). Although the inclusion criteria varied among studies, frequently the infection status at dry off was used as the criterion to include cows. Most studies had a negative control group except studies comparing cloxacillin vs. other DCT products. In general most studies were based on between cows comparison (foot note in column 1 in Table 1).

Milk Samples for Bacterial Culturing. Milk samples were collected at dry off in all studies, and at calving or up to 21 d post-calving (Table 1). The dry off sample was used to confirm the existence of quarter IMI, while the sample taken at calving or post-calving was used to diagnose cure of quarter IMI.

In 14 studies single consecutive samples were collect to diagnose IMI, while a duplicate sample (at one point in time) was collected in the other studies (Table 1).

Bacteriological Culturing Procedure. The procedure of the National Mastitis Council was mainly the procedure followed in most studies (Hogan et al., 1999). Other studies used modified procedures based on the NMC procedures. Nevertheless, in all studies the handling of samples, from farm to processing, was similar, especially storage, freezing and processing. Milk samples were obtained by proper cleaning of the teat and after discarding the first 3 to 4 squirts of milk. In most studies, samples were stored at -20˚ C until processed.

Management Application

BDCT vs. no DCT (n= 14 studies). The treatment was based on intramammary injection of the antibiotics tested, in all quarters and all studies except in Soback et al. (1990) where systemic DCT was applied intramuscularly.

Cloxacillin vs. other DCT Product (n= 6 studies). In all studies, cloxacillin and the other product were applied by the intramammary route.

SDCT vs. no DCT (n= 4 studies). Treatment was carried out based on the unit of selection. At cow level, when one or more quarters had IMI, each quarter of the whole udder was injected with the antibiotics being tested (Bratlie, 1973; Rindsig et al., 1978; Berry and Hillerton, 2002). At quarter level, only quarters that had IMI were injected with the tested antibiotics (Østerås et al., 1994). Other quarters within the same udder were left untreated. In the control group, no DCT was given to IMI quarters.

Meta-analyses Results

BDCT vs. no DCT.

In general, cure rate after DCT was on average (with the 95% CI in parenthesis) 78% (71-85%), while the spontaneous cure was on average 46% (37-56%). IMI quarters that had had DCT had 1.78 (95% CI = 1.51-2.10) times higher cure rate during the DP and early lactation than untreated IMI quarters. The 95% PI was 1.15 to 2.42 (Table 2). The RR was not altered by removing any of the studies, and none of the publication bias tests indicated presence of significant bias.

On average (with 95% CI in parenthesis), cure rate from *Staphylococcus* spp. IMI after DCT was 77% (68-86%), while spontaneous cure was on average 44% (32-56%). DCT would lead to a 1.65 times higher calculated cure from *Staphylococcus* spp. IMI compared to no DCT (Table 2). All publication bias tests indicated an absence of significant bias (results not shown).

On average (with 95% CI in parenthesis), DCT led to 89% (83-95%) cure rate from *Streptococcus* spp. IMI, while spontaneous cure of *Streptococcus* spp. IMI was 47% (33-61%). The pooled RR was 1.86 and the 95% CI was 1.48 to 2.35. No significant publication bias was identified by any of the publication bias tests.

Cloxacillin vs. other DCT Products. There was no significant difference in the cure rate of IMI when cloxacillin was compared to other DCT products (Table 2). No bias was identified by any of the publication bias tests (results not shown).

When the cure from *Staphylococcus* spp. IMI using cloxacillin was compared to the cure using other DCT products, cloxacillin showed a similar cure rate to the other DCT products (Table 2). The effect was not altered by removing any of the studies (results not shown). Begg and Mazumdar's rank correlation test showed that there was no significant correlation between effect and study size (P-value = 0.14). This was also confirmed by Egger's regression test that showed no significant association between study size and effect (intercept = - 0.89 with 3 degrees of freedom; P-value = 0.22). Duval and Tweedie's trim and fill method suggests that there is a missing study on the right-hand side of the funnel plot to reach complete symmetry (Figure 1). Adding this study did not alter the effect significantly.

Table 2. Pooled Relative risk (RR[1]) of cure from quarter intramammary infection (IMI) during the dry period up to 21 d post-calving together with the 95% confidence interval (CI) and the predictive interval (PI) beneath the pooled RR, and the studies involved in each meta-analysis per intervention comparison for all pathogens together (overall), *Staphylococcus* spp., and *Streptococcus* spp. Interventions were: Blanket dry cow therapy (BDCT), cloxacillin vs. other dry cow therapy (DCT) products, and selective DCT (SDCT).

Interventions	Studies included in each meta-analysis	RR (95% CI) (95% PI)
BDCT vs. no DCT		
Overall	(Cummins and McCaskey, 1987; Harmon et al., 1986; Heald et al., 1977; Hogan et al., 1994; Langley et al., 1971; Natzke et al., 1974; Pankey et al., 1982a,b; Pearson and Wright, 1969; Postle and Natzke, 1974; Schukken et al., 1993; Smith et al., 1967; Soback et al., 1990; Swanson, 1979)	1.78 (1.51-2.10) (1.15-2.42)
Staphylococcus spp.	(Cummins and McCaskey, 1987; Harmon et al., 1986; Heald et al., 1977; Hogan et al., 1994; Langley et al., 1971; Natzke et al., 1974; Pankey et al., 1982a,b; Pearson and Wright, 1969; Postle and Natzke, 1974; Schukken et al., 1993; Smith et al., 1967; Soback et al., 1990; Swanson, 1979)	1.65 (1.38-1.96) (1.01-2.25)
Streptococcus spp.	(Cummins and McCaskey, 1987; Heald et al., 1977; Hogan et al., 1994; Natzke et al., 1974; Pankey et al., 1982b; Pearson and Wright, 1969; Postle and Natzke, 1974; Schukken et al., 1993; Smith et al., 1967; Swanson, 1979)	1.86 (1.48-2.35) (1.15-2.57)
Cloxacillin vs. other DCT		
Overall	(Davidson et al., 1994; Dingwell et al., 2003; Langley et al., 1971; Meaney and Nash, 1977; Pankey et al., 1982b; Ziv et al., 1981)	1.00 (0.92-1.09) (0.77-1.17)
Staphylococcus spp.	(Davidson et al., 1994; Dingwell et al., 2003; Langley et al., 1971; Pankey et al., 1982b; Ziv et al., 1981)	1.00 (0.96-1.06) (0.91-1.10)
SDCT vs. no DCT	(Berry and Hillerton, 2002; Bratlie, 1971; Østerås et al., 1994; Rindsig et al., 1978)	1.76 (1.23-2.54) (1.05-2.47)

[1] Cure rate of IMI using dry cow therapy (DCT) divided by the cure rate in the untreated IMI quarters. RR > 1; increased cure rate, RR = 1; no cure, and RR < 1; decreased cure rate.

SDCT vs. no DCT. On average (with 95% CI in parenthesis), the cure rate in the SDCT group was 83% (73-93%), and spontaneous cure was on average 52% (33-71%). SDCT provides a higher cure rate of IMI compared to no DCT (Table 2). Treated IMI quarters at dry off had a 1.76 (95% CI = 1.23-2.54) times higher cure rate during the DP and early lactation than untreated quarters (Table 2). The analysis was not carried out per pathogen group because the number of studies became fewer than 4. All publication bias tests suggested an absence of bias.

DISCUSSION

The RR of cure from *Staphylococcus* spp. IMI was not significantly different than that for the *Streptococcus* spp. IMI as the CIs overlap. This could be explained due to species

differences in cure within a pathogen group leading to a similar pooled cure rate between pathogen groups. For instance, Heald et al. (1977) found that the cure of IMI quarters was higher for *Streptococcus* spp. other than *S. agalactiae* compared to the cure rate from *S. aureus* IMI, but when the cure from *S. agalactiae* IMI was pooled with the other *Streptococci* spp. and compared to the cure rate from *S. aureus* IMI, no difference in the cure rate of IMI was observed between the *Streptococci* spp. and *S. aureus*. Another explanation could be the vast array of therapies and doses used in the different studies and the diversity among studies would limit the comparison between pathogen groups. This could have also been the reason for finding insignificant differences between cloxacillin and other DCT products (Table 2).

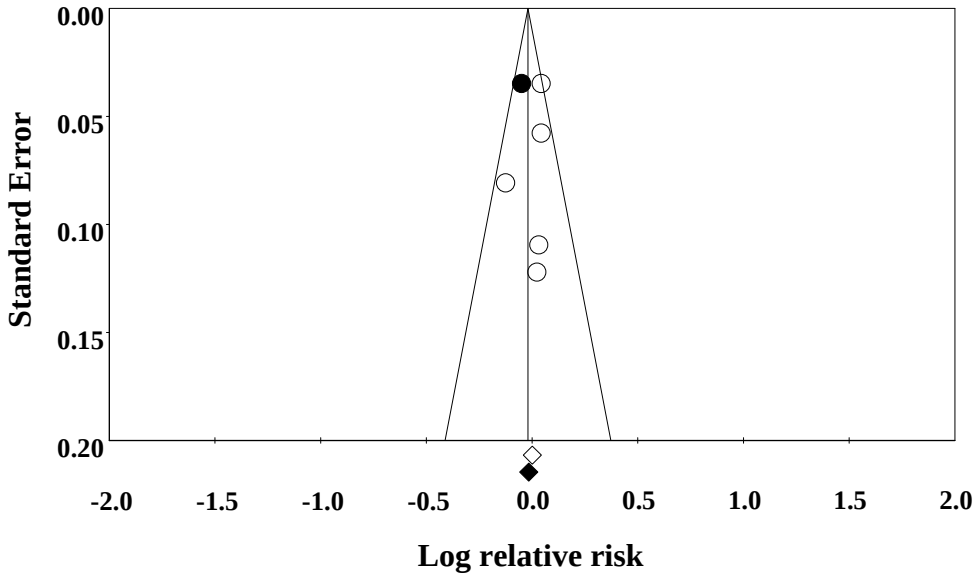


Figure 1. Funnel plot of studies involved in the estimation of the relative risk (RR) of cure from quarter *Staphylococcus* spp. intramammary infection (IMI) (RR = cure rate of quarter *Staphylococcus* spp. IMI using cloxacillin divided by the cure rate using other blanket dry cow therapy (BDCT) products) during the dry period together with the missing study (black spot) as identified using Duval and Tweedie's (2000) fill and trim method for the 5 studies included. The empty and the dark diamonds indicate the RR and its 95% confidence interval before and after including the effect of the missed study; respectively.

SDCT showed a similar RR of cure compared to BDCT (Table 2). In a recent field trial, cows were selected for SDCT based on their SCC in the month prior to drying off (Whist et al., 2007). The cure rate at the population level increased when high SCC cows (> 400,000 cells/mL) were not included for SDCT analysis (Whist et al., 2007). This indicates that the prognosis of treatment could be highly dependent on cow factors (Østerås et al, 1999).

The current study provided the PI around the estimated pooled RR. Traditionally, the CI indicates the uncertainty around the mean effect estimation, where this uncertainty is reduced by additional observations, leading to narrow 95% CI. For economic analysis, the true population variation is important because economic analysis should be comprehensive and represent extreme situations (Halasa et al., 2007). Thus, empirically wider, the PI may be

better than the CI to represent the range of situations when distributions are selected or sensitivity analysis is conducted in economic models.

The way the PI was calculated in the current paper was based on a basic, but valid approach (Higgins et al., 2009). Another approach was suggested using Bayesian analysis, which can predict a RR value for a new study with its PI (Higgins et al., 2009). However this approach is more complicated and our meta-analysis software did not support this approach.

Overall discussion of management to prevent and cure IMI during the DP up to 21 days post-calving, related to part 1 and 2

Because DCT aims to cure existing IMI and prevent new IMI during the DP and around calving, the current study and the companion paper (Halasa et al., In Press) summarized these two important aspects based on the peer reviewed research published in English. The current study found that DCT provided high cure of *Staphylococcus* spp. and *Streptococcus* spp. IMI at dry off. Significant publication bias was not detected. The companion study (Halasa et al., In Press) shows that DCT provided significant protection against new *Streptococcus* spp. quarter IMI. However, protection was doubtful against new *Staphylococcus* spp. quarter IMI after correcting for publication bias. Using DCT the cure rate against *Staphylococcus* spp. IMI was high in most studies, which made it easy to achieve publication for them because they reached significant levels even in small studies (Dohoo et al., 2003). However, again using DCT, because the rate of prevention of new *Staphylococcus* spp. IMI is not as great as the cure rate, we expected studies that measured new *Staphylococcus* spp. IMI, not to show significant effects and consequently not to succeed in being published. These studies appeared to be missing in the meta-analysis; see the discussion section of the companion paper (Halasa et al., In Press). This could explain the absence of certain studies for *Staphylococcus* spp in the companion paper (Halasa et al., In Press), but not in the current one.

On the basis of our two meta-analytical studies it can be concluded that DCT is effective in curing and protecting against new quarter IMI during the DP up to 21 d post-calving (Halasa et al., In Press), although this depends on the pathogen causing the IMI. However, internal teat sealant showed a good potential to protect against new IMI. Therefore the combination of DCT and internal teat sealant might be a good control procedure in some farms, as DCT would cure existing IMI at dry off and teat sealant would prevent new IMI during the DP; see the companion paper for detailed information about the effect of teat sealant to prevent new IMI during the DP (Halasa et al., In Press). However, such a recommendation should be supported by field data and thereafter proper calculations of their economic importance.

The two meta-analytical studies provided an important observation in relation to SDCT. When the cow was the selection unit for treatment, SDCT provided protection against new IMI as well as BDCT did, and it also showed similar efficacy of cure of existing IMI. This might make SDCT based at cow level a good choice to optimize production and limit the use of antibiotics. However, the data were limited and more studies on SDCT at cow and quarter level might be helpful to confirm or reject this important observation. Moreover, such

recommendation should be supported by economic analysis that takes into account field situations.

CONCLUSIONS

BDCT provided 1.78 times higher calculated cure rate from quarter IMI compared to no DCT, whereas for SDCT this was similar at 1.76. The RR of cure for BDCT was not significantly different when treatment was conducted for *Streptococcus* spp. IMI quarters compared to *Staphylococcus* spp. IMI quarters.

There was no significant difference between cloxacillin and other DCT products in curing quarter IMI from all pathogens and specifically from quarter *Staphylococcus* spp. IMI during the DP up to 21 d post-calving. The estimated cure rates can be used in further economic analysis.

Acknowledgements

Authors acknowledge the Norwegian School of Veterinary Science for financial support.

REFERENCES

Begg, C., and M. Mazumdar. 1994. Operating characteristics of a rank correlation test for publication bias. Biometrics 50:1088-1101.

Berry, E. A., and J. E. Hillerton. 2002. The effect of selective dry cow treatment on new intramammary infections. J. Dairy Sci. 85:112-121.

Bratlie, O. 1973. Dry cow therapy. Vet. Rec. 93:430–431.

Comprehensive Meta Analysis. 2008. Meta-analysis Manual. Biostat Institute Inc., Englewood, New Jersey, USA.

Cummins, K. A., and T. A. McCaskey. 1987. Multiple infusions of cloxacillin for treatment of mastitis during the dry period. J. Dairy Sci. 70:2658–2665.

Davidson, T. J., I.R. Dohoo, and A. W. Donald. 1994. Comparing two dry cow treatments on the new infection and elimination rates of coagulase-negative staphylococci. Can. Vet. J. 35:775-776.

Dingwell, R. T., K. E. Leslie, T. F. Duffield, Y. H. Schukken, L. Des Coteaux, G. P. Keefe, D. F. Kelton, K. D. Lissemore, W. Shwefelt, P. Dick, and R. Bagg. 2003. Efficacy of Intramammary Tilmicosin and Risk Factors for Cure of *Staphylococcus aureus* Infection in the Dry Period. J. Dairy Sci. 86:159–168.

Dohoo, I. R., S. W. Martin, and H. Stryhn. 2003. Veterinary Epidemiological Research, Atlantic Veterinary College Inc., Charlottetown, Prince Edward Island, Canada.

Duval, S., and R. Tweedie. 2000. A non-parametric “trim and fill” method of accounting for publication bias in meta-analysis. J. Amer. Stat. Associ. 95:89-98.

Egger, M., G. Davey Smith, M. Schneider, and C. Minder. 1997. Bias in meta-analysis detected by a simple graphical test. Br. Med. J. 315:629-634.

Erskine, R., S. Wagner, and F. De Graves. 2003. Mastitis therapy and pharmacology. Vet. Clin. Food Anim. 19:109–138.

Halasa, T., K. Huijps, O. Østerås, and H. Hogeveen. 2007. Economic effects of bovine mastitis and mastitis management: a review. Vet. Q. 29:18–31.

Halasa, T., M. Nielen, T. Van Werven, H. Hogeveen, O. Østerås. Meta-Analysis of Dry Cow Management for Dairy Cattle. Part 1. Protection against New Intramammary Infections. J. Dairy Sci. Submitted in Sept. 2008.

Harmon, R. J., W. L. Crist, R. W. Hemken, and B. E. Langlois. 1986. Prevalence of minor udder pathogens after intramammary dry treatment. J. Dairy Sci. 69:843–849.

Heald, C. W., G. M. Jones, S. Nickerson, and T. L. Bibb. 1977. Mastitis control by penicillin and novobiocin at drying-off. Can. Vet. J. 18:171–180.

Higgins, J. P. T., S. G. Thompson, D. J. Spiegelhalter. 2009. A re-evaluation of random-effects meta-analysis. J. R. Statist. Soc. A. 172:137-159.

Hogan, J. S., K. L. Smith, D.A. Todhunter, and P. S. Schoenberger. 1994. Efficacy of dry cow therapy and a *Propionibacterum acnes* product in herds with low somatic cell count. J. Dairy Sci. 77: 3331–3337.

Hogan, J.S., R.N. Gonzales, R.J. Harmon, S.C. Nickerson, S.P. Oliver, J.W. Pankey, and K.L. Smith. 1999. Laboratory Handbook on Bovine Mastitis. National Mastitis Council, Madison WI.

Kingwill, R. G., F. K. Neave, F. H. Dodd, T. K. Griffin, and D. R. Westgarth. 1970. The effect of a mastitis control system on levels of subclinical and clinical mastitis in two years. Vet. Rec. 87: 94-100

Langley, O. H., J. Meaney, N. P. Cullen, and F. Cunningham. 1971. The control of mastitis. Vet. Rec. 89:315-316.

Meaney, W. J., and J. M. Mash. 1977. Comparison of two dry cow intramammary treatments. Vet. Rec. 100:492-493.

Natzke, R. P., R. W. Everett, and D. R. Bray. 1975. Effect of Drying Off Practices on Mastitis Infection. J. Dairy Sci. 58:1828:1835.

Oliver, J., F. H. Dodd, and F. K. Neave. 1956a. Infection and mastitis in a dairy herd 1945-53. J. Dairy Res. 23: 169-180

Oliver, J., F. H. Dodd, F. K. Neave, and G. L. Bailiy. 1956b. Variations in the incidence of udder infection and mastitis with stage in lactation, age and season of the year. J. Dairy Res. 23: 181-193

Oliver, J., F. H. Dodd, F. K. Neave, and J. M. Lee. 1956c. Udder infection in the "dry period" II. The effect of withdrawing secretion from the dry udder on the incidence of infection. J. Dairy Res. 23: 194-196

Oliver, J., F. H. Dodd, and F. K. Neave. 1956d. Udder infection in the "dry period". III. The method of drying-off cows at the end of lactation. J. Dairy Res. 23: 197-203

Oliver, J., F. H. Dodd, and F. K. Neave. 1956e. Udder infection in the "dry period" IV. The relationship between the new infection rate in the early dry period and the daily milk yield at drying-off when lactation was ended by either intermittent or abrupt cessation of milking. J. Dairy Res. 23: 204-211

Oliver, J., F. H. Dodd, and F. K. Neave. 1956f. Udder infection in the "dry period. V. The effect of teat disinfection at drying-off on the incidence of infections in the early dry period. J. Dairy Res. 23: 212-216

Oliver, S. P., and B. A. Mitchell. 1983. Susceptibility of bovine mammary gland to infections during the dry period. J. Dairy Sci. 66: 1162-1166

Oliver, J., F. Neave, and E. Sharpe. 1962. The prevention of infection of the dry udder. J Dairy Res. 29: 95-100

Orwin, R.1983. A fail-safe N for effect size in meta-analysis. J. Educ. Stat. 8:157-159.

Østerås, O., J. Aursjo, G. Gronningsaeter Gjul, and A. Jorstad. 1994. Effect of dry-cow therapy on subclinical mastitis - an evaluation of long acting and short acting intramammaria. J. Vet. Med. B 41:529-540.

Østerås, O., V. L. Edge, and S. W. Martin. 1999. Determinants of success or failure in the elimination of major mastitis pathogens in selective dry cow therapy. J. Dairy Sci. 82:1221–1231.

Pankey, J. W., R. M. Barker, A. Twomey, and G. Duirs. 1982a. Comparative efficacy of dry-cow treatment regimens against *Staphylococcus aureus*. N. Z. Vet. J. 30:13–15.

Pankey, J. W., R. M. Barker, A. Twomey, and G. Duirs. 1982b. A note on effectiveness of dry cow therapy in New Zealand dairy herds. N. Z. Vet. J. 30:50–52.

Pearson, J. K. L. 1950. The use of penicillin in the prevention of *C pyogenes* infection of the non-lactating udder. Vet. Rec 62: 166-168.

Pearson, J. K. L. 1951. Further experiments in the use of penicillin in the prevention of *C pyogenes* infection of the non-lactating udder. Vet. Rec 63: 215-220

Pearson, J. K. L, and C. L. Wright. 1969. Dry cow therapy as a means of controlling bovine mastitis. Vet. Rec. 84:294–298.

Postle, D. S., and R. P. Natzke. 1974. Efficacy of antibiotic treatment in bovine udder. Vet. Med. Small Anim. Clin. 1535-1940.

Rindsig, R. B., R. G. Rodewald, A. R. Smith, and S. L. Spahr. 1978. Complete versus selective dry cow therapy for mastitis control. J. Dairy Sci. 61:1483–1497.

Schukken, Y. H., J. Van Vliet, D. Van de Geer, and F. J. Grommer. 1993. A randomized blind trial on dry cow antibiotic infusion in a low somatic cell count herd. J. Dairy Sci. 76:2925– 2930.

Smith, A., D. R. Westgarth, M. R. Jones, F. K. Neave, F. H. Dodd,. and G.C. Brander. 1967. Methods of reducing the incidence of udder infection in dry cows. Vet. Rec. 81:504–510.

Soback, S., G. Ziv, M. Winkler, and A. Saran. 1990. Systemic dry cow therapy – a preliminary report. J. Dairy Sci. 73:661–666.

Swanson, G. H. 1979. Posology and field efficacy study with novobiocin for intramammary infusion in nonlactating dairy cows. Can. J. Comp. Med. 43:440–447.

Whist, A. C., O. Østerås, and L. Sølverød. 2007. Staphylococcus aureus and Streptococcus dysgalactiae in Norwegian herds after introduction of selective dry cow therapy and teat dipping. J. Dairy Res. 74:1-8.

Ziv, C., M. Storper, and A. Saran. 1981. Comparative efficacy of three antibiotic products for the treatment and prevention of subclinical mastitis during the dry period. Vet. Q. 3:74-79.

Chapter 7

Stochastic Simulation Model to Calculate Costs and Benefits of Dry Period Interventions in Dairy Cattle

T. Halasa, ‡† M. Nielen, ‡ T. van Werven, ‡ H. Hogeveen‡†

‡ Department of Farm Animal Health and Reproduction, Utrecht University, P.O. Box 80151, 3584 CN Utrecht, the Netherlands
†Business Economics Group, Wageningen University, P.O. Box 8130, 6706 KN Wageningen, the Netherlands

Submitted for publication

ABSTRACT

A developed stochastic bio-economic model of intramammary infection (IMI) caused by *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Escherichia coli* was extended to model the dynamics of IMI during the dry period. The extended model was used to calculate the cost-effectiveness of different dry period interventions in relation to the annual costs of IMI in a herd of 100 dairy cows. The dynamics of IMI during the dry period were modeled based on a Greenwood model. The interventions were blanket dry cow therapy (BDCT) as the default scenario, BDCT combined with teat sealant (TS), selective dry cow therapy (SDCT) or TS, and SDCT combined with TS. Input parameters of the dynamics of IMI during the dry period and the economic parameters were based on literature. The costs of clinical and subclinical IMI during lactation, clinical IMI during the dry period, and the costs of intervention were used to calculate the combined total annual net costs of IMI per herd. The combined total annual net costs of IMI using the other intervention scenarios was compared to the default scenario (BDCT) to estimate the cost-effectiveness of the other intervention scenarios. Sensitivity analysis was conducted on the involved parameters. The results showed that a considerable number of cows acquire new IMI during the dry period and start the new lactation with IMI. Furthermore, the combined total annual net costs of IMI per herd using BDCT was € 8,336 distributed as € 4,313 due to clinical IMI, and € 2,871 due to subclinical IMI during lactation, € 84 due to clinical IMI during the dry period, and € 1,068 due to antibiotic therapy and labor costs at dry off. Application of BDCT combined with TS resulted in € 967 higher combined total annual net costs of IMI compared to the BDCT scenario. Similarly, the SDCT scenarios resulted in higher combined total annual net costs of IMI compared to the BDCT scenario. The SDCT or TS was € 586 higher and the SDCT combined with TS was € 596 higher. Sensitivity analysis results showed that the rate of new IMI during the dry period was the most influential parameter on the combined total annual net costs of IMI. Although the differences between the 4 simulated scenarios were minor, BDCT was the most cost-effective scenario in the default situation.

Key words: Dry Cow Therapy, Teat Sealant, Mastitis, Stochastic Economic Model

INTRODUCTION

The dry period (**DP**) is considered to be one of the most important periods affecting the health of the bovine udder (Green et al., 2005). DP management was already recommended in the "5 points intramammary infection (**IMI**) control plan" (Dodd et al., 1969). In recent meta-analyses (Halasa et al., 2009a,b) the efficacy of DP interventions was quantified. These interventions focused on dry cow therapy (**DCT**), which was indeed found to both cure existing and prevent new IMI during DP and the first few weeks of lactation. This effect was not similar for all pathogens. Emerging awareness about antibiotic bacterial resistance combined with economic incentives led to the introduction of selective DCT (**SDCT**) based on cow characteristics such as SCC at dry off or clinical IMI history as indicated in the meta-analyses. A more recent intervention, teat sealant (**TS**) was also found to prevent new IMI (Halasa et al., 2009a).

Several studies indicated that DCT can be economically beneficial (McNab and Meek, 1991; Oliver et al., 2003). One study indicated that DCT is economically beneficial and TS could be economically beneficial, depending on the incidence of IMI (Berry et al., 2004). Another study indicated that blanket DCT (**BDCT**) is economically beneficial, but SDCT could be better in certain situations, depending on the selection procedure for treatment (Huijps et al., 2007). Nevertheless, variation in the calculated costs and benefits between the different studies was observed (Halasa et al., 2007). Furthermore, the studies focused only on DCT and the effect of TS separately, without economic assessment of combined use of DCT and TS in a blanket and/or selective manner, as advised sometimes (Berry and Hillerton, 2007). Additionally, the transmission dynamics of IMI during the DP were not considered in previously published studies. Prevention and cure of IMI during the DP might indirectly prevent transmission of IMI during lactation. The transmission dynamics of IMI during lactation were recently incorporated in a dynamic and stochastic bio-economic model simulating IMI in bovine dairies (Halasa et al., 2009c). This model included the transmission of pathogen-specific IMI for four major IMI pathogens, *S. aureus*, *S. uberis*, *S. dysgalactiae*, and *E. coli* during lactation, in order to assess the economic impact of IMI.

The objective of this research was to estimate the costs and benefits of IMI interventions during the DP. The analysis was based on the total annual net costs of IMI in a herd of 100 dairy cows in Dutch circumstances, with incorporation of the dynamics of pathogen-specific IMI during the dry period in a previously developed bio-economic model.

MATERIALS AND METHODS

A previously developed dynamic and stochastic bio-economic model (Halasa et al., 2009c) was updated by incorporating the dynamics of pathogen-specific IMI during the DP. Briefly, the previous version of the model simulated the dynamics of *S. aureus*, *S. uberis*, *S. dysgalactiae*, and *E. coli* IMI in a herd of 100 dairy cows in a situation where milk quota is applied. Dynamics of IMI were modeled per 2-weeks time steps. The probability to obtain an IMI was determined at the beginning of each time period based on the number of pathogen-specific IMI cows, the number of susceptible cows, and the transmission rate of each of the involved IMI pathogens at the previous time period, for the contagious IMI pathogens. For *E. coli* IMI, a constant probability of infection was used, based on the cumulative incidence of *E. coli* IMI per 14 cow-days at risk. All cows that entered the DP were not included in the equations anymore for 4 time periods. Cows re-entered the milking herd with a probability of IMI relative to the pathogen-specific IMI prevalence in the milking herd in the previous time period. This previous model was updated to include mechanistically the dynamics of pathogen-specific IMI during the 4 time periods of the DP as explained in the next section.

Modeling IMI during the DP

In the Netherlands, dry cows are usually separated from the lactating cows (Schukken et al., 1993). Therefore, the dry cows were modeled separately from the lactating herd. Due to the absence of transmission parameter estimates during the DP and because the environment is believed to be the source of infection to dry cows (Eberhart, 1986), the rate of new IMI was

based on the risk of new IMI per pathogen from our meta-analysis study (Halasa et al., 2009a). Cure rates for existing IMI were also based on a meta-analysis study (Halasa et al., 2009b). A Greenwood model (Becker et al., 1989) was used to represent the dynamics of infection during the DP for each of the simulated pathogens. Per time period, a dry cow could become a clinical or subclinical IMI, based on the rate of new IMI per 14 cow-days at risk. Because the risk of clinical IMI is rare and usually encountered in early or late DP (Dingwell et al., 2003; Bradley and Green, 2004), clinical IMI was possible only in the first and last 2 weeks of the DP. Clinical IMI cows were treated with antibiotics (Woolford et al., 2001) and consequently they bacteriologically recovered, based on the recovery probabilities in Table 1, or persisted as subclinical IMI.

Table 1. Input parameters (% per time period) with default and limits for sensitivity analysis of the dynamics of intramammary infection (IMI) during the dry period (DP). The rate of new IMI (NIMI) per 2 weeks using dry cow therapy (DCT) and teat sealant (TS), the probability of recovery of IMI during the first period of the DP (R_{IMI}) using DCT, and the probability of spontaneous recovery of subclinical IMI per 2-weeks (SR), the flare up rate (FR) of subclinical IMI to become clinical IMI during the first and last periods of the DP, and the proportion of new infections to become subclinical IMI (IMI_{SC}) per pathogen during the DP.

Rates per intervention	*S. aureus*	*S. uberis*	*S. dysgalactiae*	*E. coli*
NIMI[1]				
DCT	0.7 (0.6-0.85)	0.6 (0.5-0.7)	0.6 (0.5-0.7)	0.5 (0.4-0.65)
TS	0.4 (0.1-0.7)	1.4 (0.6-2.2)	1.4 (0.6-2.2)	0.5 (0.4-0.65)
R_{IMI} [2]				
DCT[a]	77 (60-94)	89 (80-98)	89 (80-98)	90 (83-100)
SR	11	12	12	18
FR [3, 4]	9	6	6	6
IMI_{SC} [4]	90	90	90	90

[a] If an IMI cow did not bacteriologically recover after DCT based on these values, the cow persisted as subclinical IMI during the next time period, in which the subclinical IMI cow was subjected to the spontaneous recovery (SR) probabilities per time period with subclinical IMI.

[1] Halasa et al. (2009a, original data) [2] Halasa et al. (2009b, original data), [3] Green et al. (2005), [4] Bradley and Green (2004)

Clinical and subclinical IMI cows could recover and become susceptible based on cure rates presented in Table 1. A subclinical IMI cow could flare up to a clinical IMI cow, based on a flare up probability; only in early and late DP. The Greenwood model for the DP for *S. aureus*, S. *uberis*, and *S. dysgalactiae* was activated only when IMI cows were present in the herd during the previous time period. Specifically, if no IMI existed in the herd during the previous time period, no IMI caused by that pathogen occurred during the DP in the next time period, except for *E. coli*, which was considered environmental and always present. It has been indicated that the dry off and late stage of the DP comprise a higher risk of new IMI than the rest of the DP (Ostergaard et al., 2005). Thus, the risk of new IMI was scaled 3 times higher during the first and last time periods of the DP than the 2 middle time periods (Bradley and Green, 2004; Ostergaard et al., 2005).

The lactational pathogen-specific transmission rate parameter was calculated in the original studies from farms that applied BDCT at dry off to all cows (Zadoks et al., 2001; Zadoks et al., 2002). Thus, in the default situation BDCT was applied to insure consistent dynamics of IMI in the DP in relation to the lactation.

Modeling management scenarios during the DP

Quantitative knowledge related to DP management is quite rare (Green et al., 2007) also in Dutch circumstances (Huijps and Hogeveen, 2007). Therefore, interventions that are recommended by the NMC (Verona, USA) and the Dutch Udder Health Center (Deventer, the Netherlands) were simulated. The interventions were BDCT (default scenario), SDCT, and TS. The effect of these interventions was modeled in the Greenwood model using the incidence of IMI with or without that specific intervention (Table 1) in several scenarios as presented in Table 2. In the SDCT protocol, cows that had at least one test-day in the milk production record with a SCC > 200,000 cells/mL from the last 3 test-days before dry off, or had a history of clinical IMI during the lactation were treated in the 4 quarters. The milk production records were assumed to be conducted once every 4 weeks (NRS, 2005).

Table 2. The simulated scenarios of dry period (DP) interventions are shown. The default scenario (1) in which blanket dry cow therapy (BDCT) was applied to all cows, scenario 2 in which BDCT and teat sealant (TS) were applied to all cows, scenario 3 in which selected DCT (SDCT) was applied and unselected cows had only TS, and scenario 4 in which SDCT was applied and all cows had TS. Application of the intervention was always at dry off.

Scenario	BDCT	SDCT	TS
1	X		
2	X		X
3		X	X[1]
4		X	X

[1] Only cows without SDCT

In the scenarios where DCT and TS were applied, the rate of new IMI was based on the lowest value of new IMI rate using DCT and TS from Table 1. Generally, Halasa et al. (2009a) found no difference in prevention of new quarter IMI between quarters that had DCT and TS versus quarters that had only TS. Thus no cumulative effect of using DCT and TS on the risk of new IMI was modeled.

Economic analysis

The cost and benefit factors of the simulated interventions were included as recommended by Halasa et al. (2007).

DCT was carried out by the farmer. The costs of intervention included costs of antibiotics and the labor time to treat the cow (Table 3). For TS, all 4 quarters had TS applied. The costs included the teat sealer material and the labor time to apply the TS (Table 3). The intervention costs of applying both DCT and TS included the costs of 4 DCT injectors, the costs of TS material and the labour time to apply both applications (Table 3). Costs of

selection for SDCT such as the costs of milk production records and clinical IMI recording were set to be 0, because these were assumed standard management practices.

Table 3. Input values for economic parameters to apply dry cow therapy (DCT) and/or teat sealant (TS) together with the reference. Between brackets the values used for sensitivity analysis.

Parameter	Value	Reference
Price antibiotics, €/4 injectors	9.5 (5-15)	Huijps and Hogeveen, (2007)
Price of TS, €/4 injectors or dips	9.5 (9-12)	Commercial products[1] and expert opinion
Hourly wage, €/hour	18 (10-30)	Huijps and Hogeveen, (2007)
Time to apply DCT, min/cow	8 (6-10)	Huijps and Hogeveen, (2007)
Time to inject/dip TS, min/cow	8[2] (6-10)	Experts' opinion
Time to apply DCT and TS, min/cow	12[2] (10-15)	Experts' opinion

[1] Averaged based on different commercial products.
[2] Averaged based on the opinion of 5 Experts.

The costs of a clinical case during the early or late DP consisted of the costs of antibiotics for 3 days, and the labor time to apply the antibiotics. The costs of clinical and subclinical IMI during lactation were calculated as explained by Halasa et al. (2009c). A combined annual net costs of IMI caused by the 4 simulated pathogens was calculated for clinical and subclinical IMI separately. The combined annual net costs of clinical IMI was calculated as the sum of the costs of milk yield loss due to clinical IMI, costs of culling, costs of veterinary service, and costs of labour caused by the 4 simulated pathogens. The combined annual net costs of subclinical IMI was calculated as the sum of the costs of milk yield loss due to subclinical IMI, costs of culling, and bulk tank somatic cell count penalty caused by the 4 simulated pathogens.

A combined total annual net costs of IMI caused by the 4 simulated pathogens was calculated as the sum of the combined annual net costs of clinical IMI and subclinical IMI and the intervention costs. All costs were presented as mean, 5^{th} and 95^{th} percentiles to represent the variability.

The total annual net effects of scenario 2, 3, and 4 were compared to the combined total annual net costs of IMI using the default scenario (BDCT).

Stabilization and implementation of scenarios

Each scenario was run for 2 quota years, starting from the same herd situation to insure a stable infection process over time. The rate of pathogen-specific new IMI at the end of the second year was used to start the model run per scenario to be used for economic assessment of each scenario. After stabilization, the model was run again for 2 quota years. Only the output from the second quota-year was used to calculate the combined annual net costs of IMI per scenario. This would insure that the model simulated the effect of the applied management in a herd, not the initial herd states (Østergaard et al., 2005) and insure that the majority of the cows would have been subjected to the simulated intervention scenario. The model was replicated until the change in the combined total annual net costs of IMI was $\leq 1\%$ when extra iterations were added, which was the case using 5,000 iterations.

Sensitivity analysis

Important input parameters of the DP were included in the sensitivity analysis to quantify their impact on the combined total annual net costs of IMI. These parameters were the rate of new pathogen-specific IMI, the cure rate of pathogen-specific IMI, price of DCT and TS, and labor wage. The values of the parameters were changed for the sensitivity analysis using the limits in Table 1 and 3, which represent a realistic change, based on the corresponding references. The sensitivity analysis was carried out on the above mentioned parameters for all scenarios separately and in separate model runs per parameter. When one parameter was changed for the sensitivity analysis, it was changed for the 4 simulated pathogens simultaneously.

Sensitivity analysis was also carried out on the threshold of SCC for selection to DCT in scenarios 3 and 4, by changing the threshold from 200,000 cells/mL to 100,000 cells/mL or 300,000 cells/mL.

RESULTS

Descriptive results

The number of cows that dried off within a year differed, depending on the simulated scenario. In the default scenario, the average number of cows that dried off was 90, varying from 79 to 101 cows per year. The average number of cows dried off with the 5^{th} and 95^{th} percentiles in parentheses, for scenarios 2, 3 and 4 were respectively 94 (83-106), 98 (86-111), 96 (85-109).

When selection for DCT was carried out in scenarios 3 and 4, on average 27 (9-47) cows per year were selected for treatment.

Dynamics of IMI during the DP

The median annual number of new IMI during the DP, when BDCT was applied, was 5 cases per year, with the 5^{th} and 95^{th} percentiles 2 and 8 cases per year (Figure 1a). BDCT combined with TS resulted in the lowest number of new IMI cases during the DP (Figure 1a). When the scenario of SDCT or TS was applied, the median annual number of new IMI during the DP was 5 (Figure 1a). The scenario of SDCT combined with TS resulted in a 4 new IMI cases during the DP (Figure 1a). The SDCT scenarios showed the largest variability in the number of new IMI cases during the DP.

The frequency and variation of IMI at calving is presented in Figure 1b per intervention scenario. Using the default scenario (BDCT), the median annual number of IMI at calving was 4 and the 5^{th} and 95^{th} percentiles were 1 and 8 cases, respectively. In scenario 2, in which BDCT and TS were applied, the median annual number of IMI at calving was 3 cases (Figure 1b). This indicates the positive effect of combining DCT and TS to control IMI cases during the DP. When scenario 3 (SDCT or TS) was applied, the median annual number of IMI at calving was 5 (Figure 1b). SDCT combined with TS scenario resulted in a median annual number of IMI at calving of 5 cases (Figure 1b). The SDCT scenarios showed the largest variability in the number of IMI cases at calving.

Cost-benefit of DP interventions

The average costs and benefits of the simulated scenarios of DP interventions are presented in Table 4 for clinical and subclinical IMI during the lactation, clinical IMI during the DP, and intervention costs. The combined total annual net cost of IMI using BDCT was on average € 8,336, and varied largely with the 5th percentile € 2,031 and the 95th percentile € 17,304. Fifty two percent of the combined total annual net cost in the BDCT scenario was due to clinical IMI during the lactation. The costs of BDCT intervention was on average € 1,068, and the 5th and 95th percentiles were € 940 and € 1,202 per year, respectively. The combined total annual net costs of IMI when BDCT was combined with TS was € 9,303 (Table 4). Applying this scenario would lead to € 18, € 66, and € 7 lower costs of clinical IMI during lactation, subclinical IMI during lactation, and clinical IMI during the DP per year, respectively. These lower costs were mainly due to less culling costs (Table 4). Nevertheless, these lower costs did not pay off the higher costs of BDCT and TS intervention, which was on average € 2,126, leading to a total annual net loss of € 967 for the combined application of BDCT and TS compared to the BDCT scenario (Table 4).

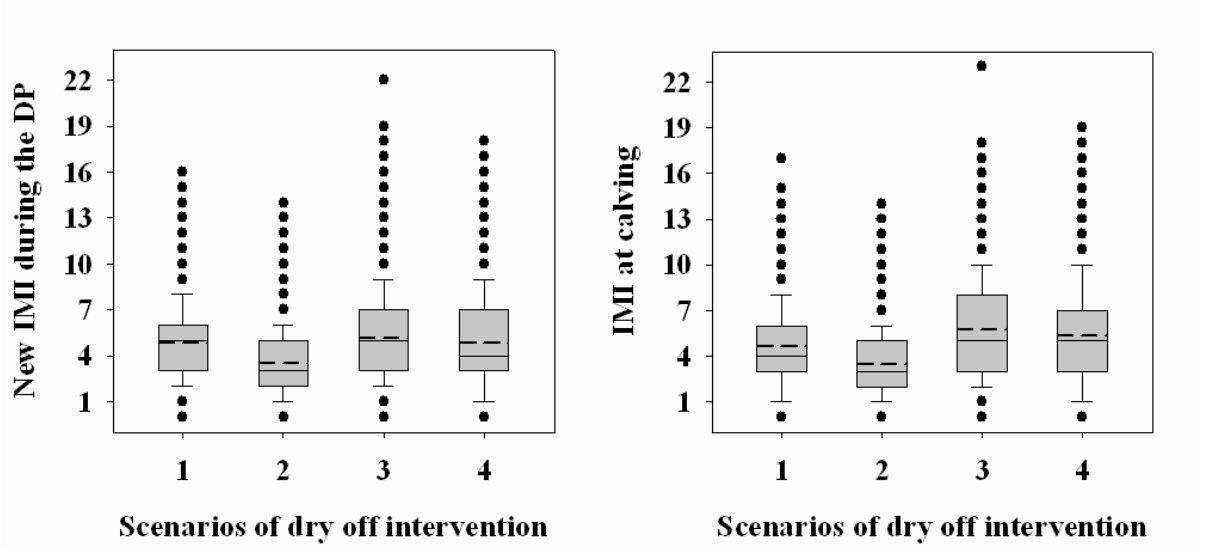


Figure 1a Figure 1b

Figure 1: Box plots of: a) the number of new IMI cases during the dry period; b) the total number of IMI cases at calving for scenario 1 that included blanket dry cow therapy, scenario 2 that included blanket dry cow therapy combined with teat sealant, scenario 3 that included selective dry cow therapy or teat sealant, and scenario 4 that included selective dry cow therapy combined with teat sealant. The interrupted line represents the average value, the box represents the 25th and 75th percentiles, and the whiskers represent the 5th and 95th percentiles.

Scenario 3, in which SDCT or TS was applied showed a higher combined total annual net costs of IMI compared to the default scenario (Table 4). The combined total annual net costs of IMI when SDCT or TS was applied were on average € 8,922 (Table 4). Although the costs of intervention of this scenario were close to that of the default scenario, applying SDCT or TS would lead to a total annual net loss of € 586. These losses were mainly due to higher costs of clinical and subclinical IMI during the lactation (Table 4). When the scenario of SDCT combined with TS was applied, the combined annual net costs of IMI were € 8,932 (Table 4). The costs of intervention using this scenario was € 366 higher than the default

scenario, and the associated total annual net loss compared to the default scenario was € 596 (Table 4).

Sensitivity analysis

The changes in the combined total annual net costs of IMI, due to the changes in the input values included in the sensitivity analysis, are presented in Table 5. The combined total annual net costs of IMI was most sensitive to changing the rate of new IMI during the DP, but depending on the simulated scenario (Table 5). For instance, BDCT was always the most cost-effective scenario, except when the rate of new IMI was lowest, where the SDCT became more cost-effective. This indicates that under low rate of infection during the DP, SDCT scenarios become the economically feasible scenarios to apply. The cure rate of IMI at dry off corresponding to antibiotic treatment did also influence the combined total annual net costs of IMI noticeably, and the ranking of the intervention scenario changed accordingly (Table 5). For instance, under a high cure rate, the SDCT or TS scenario was economically most effective (Table 5).

The price of DCT and TS was most influential on the combined total annual net costs of IMI in case of scenario 2 in which BDCT and TS were applied, because the intervention costs was highest in this scenario. The SCC threshold for DCT influenced the combined total annual net costs of IMI slightly (Table 5).

DISCUSSION

In scenario 1, the model predicted 5 new IMI that developed during the DP, and 4 cases to exist at calving. This shows that a considerable number of cows start the lactation with IMI originating from the DP. Bradley and Green (2004) found, using DNA analysis, that most of the IMIs during early lactation were persistent cases from the DP. The number of new IMI cases during the DP and the number of IMI cases at calving differed between the different scenarios and were lowest when BDCT combined with TS was applied (Figures 1). This was because recovery after therapy was high, leading to fewer persistent IMI cases than when the BDCT was not applied. In addition, the combination of BDCT and TS protected well against new IMI with *Streptococci* spp. and *S. aureus* IMI, which resulted in a lower new IMI rate during the DP, compared to the other scenarios (Figure 1). When the SDCT scenarios were applied, they showed close median numbers of IMI cases during the DP to the default scenario (BDCT), but with larger variability, and a tendency to show worse situations (Figure 1). This was because cows that had not had antibiotic therapy at dry off were not protected against new IMI with the *Streptococci* spp. during the DP, leading to a higher chance of potential lactational transmission of IMI of these pathogens.

Table 4. Average cost (5th and 95th percentiles) and benefit of dry period (DP) interventions based on the combined total annual net costs of intramammary infection (IMI) that consisted of the costs of clinical IMI during lactation (CIMI), costs of subclinical IMI during lactation (SCIMI), costs of clinical IMI during the DP (DPCIMI), and the costs of DP intervention per scenario, which were blanket dry cow therapy (BDCT) as the default scenario, BDCT combined with teat sealant (TS), selective DCT (SDCT) or TS, and SDCT combined with TS.

Cost-benefit factors	DP intervention Scenario						
	BDCT (Default)	BDCT + TS		SDCT or TS		SDCT + TS	
	Costs	Costs	Benefits	Costs	Benefits	Costs	Benefits
Net cost-benefits	**8,336 (2,031-17,304)**	**9,303 (3,203-18,757)**	**-967**	**8,922 (2,133-18,389)**	**-586**	**8,932 (2,216-18,649)**	**-596**
Costs of CIMI	**4,313 (760-9,345)**	**4,295 (762-9,510)**	**18**	**4,543 (677-9,847)**	**-230**	**4,384 (677-9,764)**	**-71**
Milk loss	901 (167-1,971)	924 (176-2,069)	-23	948 (159-2,087)	-47	927 (144-2,075)	-26
Medicines	1,257 (280-2,680)	1,242 (280-2,640)	15	1,305 (240-2,760)	-48	1,267 (240-2,720)	-10
Vet. service	236 (53-503)	233 (53-495)	3	245 (45-518)	-9	238 (45-510)	-2
Labour	1,131 (252-2,412)	1,118 (252-2,376)	13	1,174 (216-2,484)	-43	1,140 (216-2,448)	-9
Culling	1,657 (0-4,018)	1,668 (0-4,017)	-11	1,785 (0-4,520)	-128	1,706 (0-4,520)	-49
Saved costs	869 (161-1,900)	890 (170-1,994)	-21	914 (154-2,012)	-45	894 (139-2,001)	-25
Costs of SCIMI	**2,871 (0-7,546)**	**2,805 (0-8,043)**	**66**	**3,149 (0-8,048)**	**-278**	**3,054 (0-8,052)**	**-183**
Milk loss	157 (4-395)	148 (5-390)	9	172 (5-414)	-15	164 (4-405)	-7
Culling	2,866 (0-7,534)	2,800 (0-8,036)	66	3,143 (0-8,035)	-277	3,048 (0-8,036)	-182
Saved costs	152 (4-381)	143 (5-376)	9	166 (5-399)	-14	158 (4-391)	-6
Penalty	0	0	0	0	0	0	0
Costs of DPCIMI	**84 (0-228)**	**77 (0-228)**	**7**	**64 (0-228)**	**20**	**60 (0-228)**	**24**
Costs DP intervention	**1,068 (940-1,202)**	**2,126 (1,876-2,396)**	**-1,058**	**1,166 (1023-1,321)**	**-98**	**1,434 (1,199-1,700)**	**-366**
Materials	853 (751-960)	1,787 (1,577-2,014)	-934	931 (817-1055)	-78	1,170 (969-1,397)	-317
Labor	215 (190-242)	339 (299-382)	-124	235 (206-266)	-20	264 (228-302)	-49

The model included a risk of new IMI after TS during the whole DP. This could contradict expectations that TS, as a physical barrier, should prevent bacterial access to the mammary gland and thus prevent new IMI. Parker et al. (2008) found that on average 8.4 % of the heifers that had a TS application and had no pathogen isolated at the time of the TS application, developed IMI that was detected during the 1st week in lactation. A possible biological reason to explain occurrence of IMI during the DP while TS was applied could be that some IMI species might have resided within the mammary gland in late lactation, and were not isolated due to shedding patterns and imperfect test sensitivity, the bacteria would be sealed inside the mammary gland causing IMIs that were detected at calving or early lactation.

Table 5. Sensitivity analysis on the combined total annual net costs of intramammary infection (IMI) using different dry period (DP) interventions, which were blanket dry cow therapy (BDCT), BDCT combined with teat sealant (TS), selective dry cow therapy (SDCT) or TS, and SDCT combined with TS, when the lower limit and the upper limit of each parameter was examined. Parameters of the DP were: the rate of new IMI during the DP, the cure rate after antibiotic therapy at dry off, the price of antibiotic therapy and TS, the labor wage, and the threshold of SCC for selection for dry cow therapy (DCT) at dry off.

Parameters in the sensitivity analysis	Scenario of dry period intervention							
	BDCT		BDCT + TS		SDCT or TS		SDCT + TS	
	Low limit	Upper limit	Low limit	Upper limit	Low limit	Upper limit	Low limit	Upper limit
IMI during the DP[1]								
Rate of new IMI	8,226	9,039	8,493	9,742	7,890	10,187	8,053	10,473
Cure rate	9,294	8,259	10,137	8,664	9,158	8,400	9,401	8,836
Economic inputs[2]								
Price of DCT/TS	7,931	8,331	8,833	10,055	8,761	9,251	8,762	9,321
Labor wage	8,242	8,480	9,156	9,529	8,820	9,079	8,817	9,107
Threshold of SCC for SDCT[3]	-	-	-	-	8,423	9,114	8,725	9,208

[1] Values for the lower and upper limits are presented in Table 1
[2] Values for the lower and upper limits are presented in Table 3
[3] The low limit of the threshold of SCC for SDCT was 100,000 cells/mL and the upper limit was 300,000 cells/mL.

For the default assumptions, on average, BDCT was the most cost-effective scenario of DP intervention scenarios (Table 4). Although it did not result in the lowest costs of clinical and subclinical IMI during the lactation, and clinical IMI during the DP, it resulted in the lowest costs of intervention, which was a determinant cost factor (Table 4). Obviously, there is no drastic difference in the combined total annual net costs of IMI between the different scenarios, especially between the BDCT and the 2 SDCT scenarios (Table 4). This can also be seen from the sensitivity analysis results (Table 5), in which the ranking of the scenarios changed corresponding to some input parameters changes. This indicates that a scenario could be chosen depending on the herd IMI situation and the farmer's preference. Most important, it indicates that under the simulated DP intervention scenarios and the current prices, further attention should be given to control IMI during the lactation as the major source of loss. When

a basic scenario in which no DP intervention was assumed, the average combined annual net costs of clinical IMI during lactation, subclinical IMI during lactation, and clinical IMI during the DP were € 6,315, € 4,558, and € 168, respectively, leading to an average combined total annual net costs of IMI € 11,041 with the 5^{th} and 95^{th} percentiles € 1,933 and € 20,810, respectively. On average, this shows that applying any of the 4 intervention scenarios is necessary to minimize losses due to IMI.

The sensitivity analysis showed that a high rate of new IMI during the DP would lead to a great loss under the 4 simulated IMI interventions (Table 5), which indicates the importance of proper control of new IMI during the DP. The lowest combined annual net costs of IMI was reached using the low limits of new IMI rate during the DP when the SDCT scenarios were applied. This indicates that costs of IMI could be minimized when the rate of new IMI was lowest using SDCT and TS. Huijps and Hogeveen (2007) indicated that the costs of IMI around the DP were lowest when a good selection strategy for SDCT would be carried out. Cure of IMI at dry off was also an influential parameter (Table 5). A lower cure rate would lead to a higher chance of persistent cows over the DP that start the lactation as IMI cases. Combined with transmission of pathogens during the lactation, this resulted in a high combined annual net costs of IMI. A higher cure rate influenced the costs slightly, because the cure rates were already high, and because the main influence on the costs was the rate of new IMI during the DP. Based on the sensitivity analysis results, the use of SDCT could be economically beneficial when a good selection strategy is available, and when the rate of new IMI during the DP is low, possibly due to good hygiene and feeding regimes. If these 2 necessities are not available, then BDCT may be economically a better choice to minimize costs. Good hygiene and feeding during the DP have been shown to reduce the risk of clinical and subclinical mastitis in early lactation (Bradley and Green, 2004; Green et al., 2007). Nevertheless, the efficacy of hygiene and feeding during the DP in reducing the rate of new IMI during the DP are not available, making it hard to assess their economic efficiency.

In experiments and field trials, selection for treatment was mostly based on SCC and bacteriological culture samples to diagnose IMI (Østerås et al., 1994; Whist and Østerås, 2007). In Dutch practice, farmers generally tend to select cows based on the SCC values of the last 3 test-days and/or the clinical IMI history of the cow. In the current model, we simulated a field situation, in which a SCC threshold value of 200,000 cells/mL was used. The threshold combined with the clinical IMI history resulted in an average 29 % selection of cows for DCT, which is close to the 35 % finding of Huijps and Hogeveen (2007). Nevertheless, a farmer might miss a subclinical IMI case, which could persist over the dry period until calving, or the farmer could select an uninfected cow for DCT and TS, which would cause unnecessary costs. With the current model settings, infected cows would rarely be missed and uninfected cows would not be treated, which would lead to high positive and negative predictive values of selection, which might be too optimistic compared to the field. The consequence could be an overestimation of the profit of using SDCT by underestimating the costs of intervention and over-estimating the cure effect. Adding bacteriological culture costs to the SDCT scenarios to mimic reality would only confirm that BDCT was the most cost-effective scenario. A low SCC threshold for selection to DCT resulted in lower costs

(Table 5). Using a low threshold, IMI cows will definitely not be missed, and hence prevent persistent IMI cases to the next lactation. In addition, because of the high positive predictive value, uninfected cows will not be unnecessarily selected for DCT, which will lead to lower costs. Oppositely, a high threshold would lead to neglect IMI cows at dry off, leading to persistent IMI cases to the next lactation and hence a higher combined total annual net costs of IMI (Table 5).

CONCLUSIONS

A substantial number of cows acquire new IMI during the DP and start the lactation with IMI affecting the dynamics of IMI during the lactation and consequently the costs of IMI. The combined total annual net costs of IMI following the application of DP interventions were around € 9,000. Although the difference between the 4 simulated scenarios was minor, on average, BDCT was the most cost-effective scenario. Applying any of the DP interventions was essential to minimize economic losses due to IMI.

Acknowledgement

We would like to acknowledge Martin Green and other experts who provided realistic input parameter values.

References

Becker, N.G.1989. Analysis of infectious disease data. Chapman and Hall, London, UK.

Berry, E. A., H. Hogeveen, and J. E. Hillerton. 2004. Decision tree analysis to evaluate dry cow strategies under UK conditions. J. Dairy Res. 71:409-418.

Berry, E. A., and J. E. Hillerton. 2007. Effect of an Intramammary Teat Seal and Dry Cow Antibiotic in Relation to Dry Period Length on Postpartum Mastitis. J. Dairy Sci. 90:760–765.

Bradley, A. J., and M. J. Green. 2004. The importance of the non-lactating period in the epidemiology on intramammary infection and strategies to prevention. Vet. Clin. Food Anim. 20:547–568.

Dingwell, R. T., K. E. Leslie, T. F. Duffield, Y. H. Schukken, L. Des Coteaux, G. P. Keefe, D. F. Kelton, K. D. Lissemore, W. Shwefelt, P. Dick, and R. Bagg. 2003. Efficacy of Intramammary Tilmicosin and Risk Factors for Cure of *Staphylococcus aureus* Infection in the Dry Period. J. Dairy Sci. 86:159–168.

Dodd, F. H., D. R. Westgarth, F. K. Neave, and R. G. Kingwell. 1969. Mastitis - The strategy of control, J. Dairy Sci. 52:689–695.

Eberhart, R. J. 1986. Management of dry cows to reduce mastitis. J. Dairy Sci. 69:1721-1732.

Green, M. J., L. E. Green, A. J. Bradley, P. R. Burton, Y. H. Schukken, and G. F. Medley. 2005. Prevalence and associations between bacterial isolates from dry mammary glands of dairy cows. Vet Rec. 156:71-77.

Green, M. J., A. J. Bradley, G. F. Medley, and W. J. Browne. 2007. Cow, farm, and management factors during the dry period that determine the rate of clinical mastitis after calving. J. Dairy Sci. 90:3764–3776.

Halasa, T., K. Huijps, O. Østerås, and H. Hogeveen. 2007. Economic effects of bovine mastitis and mastitis management: A review. Vet. Q. 29:18–31.

Halasa, T., O. Østerås, T. van Werven, H. Hogeveen, and M. Nielen. Meta-analysis on dry cow management for dairy cattle. Part 1: Protection against new intramammary infections. J. Dairy Sci. Submitted.

Halasa, T., M. Nielen, A. C. Whist, and O. Østerås. Meta-analysis on dry cow management for dairy cattle. Part 2: Cure of existing intramammary infections. J. Dairy Sci. Submitted.

Halasa, T., M. Nielen, R. B. M. Huirne, and H. Hogeveen. Stochastic Bio-economic model of bovine intramammary infection. Livestock Sci. Submitted.

Huijps, K., and H. Hogeveen. 2007. Economic evaluation of dry cow therapy. J. Dairy Sci. 90:1225-1234.

McNab, B. W., and A. H. Meek. 1991. A benefit cost analysis of dry-cow mastitis therapy in dairy cattle in Ontario. Can. Vet. J. 32:347-353.

NRS, 2005. Annual statistics (2005). CR-Delta, Arnhem, the Netherlands.

Oliver, S. P., M. J. Lewis, B. E. Gillespie, H. H. Dowlen, E. C. Jaenicke, and R. K. Roberts. 2003. Prepartum antibiotic treatment of heifers: Milk production, milk quality and economic benefit. J. Dairy Sci. 86:1187-1193.

Østerås, O., J. Aursjo, G. Gronningsaeter Gjul, and A. Jorstad. 1994. Effect of dry-cow therapy on subclinical mastitis - an evaluation of long acting and short acting intramammaria. J. Vet. Med. B 41:529-540.

Østergaard, S., Chagunda, M.G.G., Friggens, N.C., Bennedsgaard, T.W., Klaas, I.C., 2005. A stochastic model simulating pathogen-specific mastitis control in a dairy herd. J. Dairy Sci. 88, 4243–4257.

Parker, K. I., C. Compton, F. M. Anniss, C. Heuer, and S. McDougall. 2008. Quarter-Level Analysis of Subclinical and Clinical Mastitis in Primiparous Heifers Following the Use of a Teat Sealant or an Injectable Antibiotic, or Both, Precalving. J. Dairy Sci. 91:169–181.

Schukken, Y. H., J. van Vliet, D. van de Geer, and F. J. Grommer. 1993. A randomized blind trial on dry cow antibiotic infusion in a low somatic cell count herd. J. Dairy Sci. 76:2925– 2930.

Whist, A. C., and O. Østerås. 2007. Associations between somatic cell counts at calving or prior to drying-off and clinical mastitis in the remaining or subsequent lactation. J. Dairy Res. 74:66-73.

Woolford M. W., J. H. Williamson, T. M. Day, S. J. Lacy-Hulbert, and H. V. Handerson. 2001. Effect of localised antibiotic infusions applied to the teat-canal and teat sinus at drying-off on mastitis in the dry-period and at calving. J. Dairy Res. 68:551-558.

Zadoks, R.N., Allore, H.G., Barkema, H.W., Sampimon, O.C., Grohn, Y.T., Schukken, Y.H., 2001. Analysis of an outbreak of *Streptococcus uberis* mastitis. J. Dairy Sci. 84, 590–599.

Zadoks, R.N., H.G. Allore, T. J. Hagenaars, H. W. Barkema, and Y. H. Schukken. 2002. A mathematical model of *Staphylococcus aureus* control in dairy herds. Epidemiol. Infect. 129:397-416.

Chapter 8

General Discussion

This thesis was conducted with the main objective to develop a pathogen-specific intramammary infection (**IMI**) bio-economic model, to be used as a tool to support decision making in relation to udder health management in bovine dairy herds.

Some important topics were not covered in the previous chapters, but will be discussed in the next sections.

What can be a good definition of new subclinical mastitis on cow level?

It has been shown earlier, that SCC is a good indicator of subclinical mastitis (Dohoo and Leslie, 1991). However, no general consensus was reached to define a cut-off value to discriminate between healthy quarters or udders and new subclinical IMI cases. So, what would be an appropriate definition of new subclinical mastitis based on SCC on cow level? Definitely the answer of this question is not absolute, but it is interesting and important to be discussed here.

From an economic point of view, definition of new subclinical mastitis based on SCC has been used for 2 main objectives:

1) To define cut off values to assign attention cows to monitor the bulk tank SCC (Borne et al., 2008). These cows required more attention than herd mates, and could be eventually treated with antibiotics when the cow SCC continued to be higher than the assigned limits (Borne et al., 2008) or the milk of these cows would not be delivered to prevent penalties on high bulk tank SCC (Chapter 2).

2) To determine economic values, for instance production loss corresponding to the SCC levels. In our study (Chapter 3) and according to literature, a healthy udder was defined when cow SCC < 50,000 cells/ml. So, is it worth to decrease cow SCC levels to values < 50,000 cells/ml for all cows? This demands more strict management and most likely more antibiotics, which is quite costly. Van Asseldonk et al. (2009) found that some farmers would react to decrease bulk tank SCC after receiving advices supplemented with economic evaluation of production loss due to new subclinical mastitis cases. However, economic assessment of antibiotic treatment of subclinical mastitis cows was not carried out. Thus the economic impact of decreasing cow SCC < 50,000 cells/ml should be properly assessed. Thereafter, the definition of subclinical mastitis based on cow SCC can be set for production loss evaluation.

A main reason for the dispute in literature to define a healthy quarter or udder and a subclinical mastitis case based on SCC is perhaps due to the absence of a gold standard test to be compared to SCC data. Bacterial culture of quarter milk samples is believed to represent IMI (Reksen et al., 2008). However, sometimes the bacteria colonize the teat canal without major involvement of the udder parenchyma (Persson et al., 1995; Green et al., 2004). Bacterial isolation of these cases would indicate IMI and contradict low SCC of that quarter (Dürr et al., 2008). Infection with major pathogens is accompanied with considerable milk production loss (Grohn et al, 2004). On the other hand, no evidence was found for milk production loss after infection with minor pathogens such as the coagulase-negative

Staphylococcus spp. (Grohn et al., 2004), in which mammary gland damage might not occur. Thus, presence of pathogens in the milk might not be the best indication of inflammation and consequently mastitis, where economic losses are expected to occur. Therefore, absence of production loss could be an indicator worth to consider when defining subclinical mastitis.

Physiologically a quarter can not have a zero SCC even when no known pathogen resides in the udder. Polymorphonuclear cells and phagocytes, besides other cells, are permanent residents of the udder (Leitner et al., 2003). When an infectious agent successfully passes the teat canal, the innate immunity represented by the resident cells of the mammary gland would react to clear this infectious agent (Van Werven et al., 1999). The adaptive immune response could start within 4 to 7 days to help fighting the infectious agents (Van Werven et al., 1999). The degree of these reactions might differ, depending on the stimulating antigen in the mammary gland and influence the severity of infection (Van Werven et al., 1998). The mammary gland may be under constant exposure to infectious agents from the surrounding environment, but because of rapid clearance carried out by the resident cells of the mammary gland, successful inflammations are not initiated. The body immune defense reactions as a response to infectious agents from the environment and the occurrence of subclinical mastitis are not fully understood, making it difficult to define a true gold standard to indicate subclinical mastitis. Because infectious agents could have an access to the udder without major involvement of the glandular tissue, theoretically, there could be equilibrium between the body immune defenses, the mammary gland (as the milk producing unit) and the micro flora of the environment. At some point in time, the equilibrium might shift towards one side, for instance an increased number of the resident cells could prevent an inflammation, because of possible rapid clearance of infectious pathogens, which could also prevent production loss. On the other hand, the equilibrium might shift towards co-existence with the infectious pathogen leading to an inflammation and consequently a high reactionary SCC and unavoidable production losses. Thus, a single perfect gold standard of mastitis might not exist, but should be based on equilibrium between the body immune defense, the mammary gland and the micro flora of the environment. If so, definition of subclinical mastitis based on SCC and/or bacterial isolation from the milk would not be ultimately successful, but including milk production loss when defining new subclinical mastitis for economic analysis might help to better indicate new subclinical mastitis than only using SCC and/or bacterial isolation.

Bio-economic model

Applied methodology:

In the past, several modeling approaches were used to model IMI. Recently, discrete-event simulation was frequently used to model IMI (Allore et al., 1998; Seegers et al., 2000; Ostergaard et al., 2005). This method has several advantages over the other common approaches such as Markov processes and differential equations. The main advantage over the Markov processes is less memory necessary to run a complex process like IMI. This permits mechanistic modeling of IMI, with less concerns of infinite matrix enlargement that could

result in computer memory failure (Allore and Erb, 1999). When the process of interest is simple enough and can be represented with a set of mathematical equations, differential equations are usually used (Allore and Erb, 1999). However, the use of differential equations is often limited to deterministic modeling and frequently on population level (Allore and Erb, 1999). Differential equation models are therefore currently not suited to model the complex process of IMI on cow level and over time, which is necessary for cow level economic evaluation.

Advantages and limitations:

The developed model (Chapter 4 and 7) simulated the IMI process based on a suggested model structure of Allore et al. (1999). In case of cow-to-cow infection, this was the first bio-economic model to include the IMI process based on the transmission rate of pathogen-specific IMI. This was a major improvement over previously published bio-economic models, leading to better mechanistic modeling of the spread of pathogen-specific IMI over time within a herd. The stochastic nature of the model made it possible to include uncertainty to estimate the cost of pathogen-specific IMI. Recent knowledge in relation to production losses due to clinical mastitis (Grohn et al., 2004), and to new subclinical mastitis (Chapter 3) were incorporated. All the above mentioned reasons made it possible to estimate pathogen-specific IMI costs based on a realistic model (as adapted in Chapter 7), which is an important requirement to further assessment of IMI management in dairy cattle (Seegers et al., 2003).

In previous bio-economic models, IMI was treated as a single disease that has a specific prevalence and can be caused by different pathogens over time. In the current pathogen-specific IMI model, the dynamics of pathogen-specific IMI were treated separately, but taking into account the existence of the other IMI pathogens in the herd. This major improvement of IMI modeling allows simulation of pathogen-specific IMI control strategies, taking into account the existence of the different IMI types. This could for instance allow studying the biological and economical effects of pathogen-specific IMI antibiotic treatment to optimize herd-level treatment efficacy.

Despite the innovative approach to model IMI, the described bio-economic model has limitations. For instance, the model does not solve for optimum solutions, which is a restriction due to the selected methodology. The model demands a long running time and adaptation of the model to include extra processes would also consume time, due to the complexity of the modeled processes. The model included the 4 major IMI pathogens in the Netherlands, and therefore, it is not comprehensive in the representation of all possible IMI in Dutch dairies. Therefore, the model is a good research tool, for general recommendations, but not for farm-specific decision support.

Model structure:

In the original studies (Zadoks et al., 2001; Zadoks et al., 2002), the pathogen-specific transmission rates were estimated on quarter level, while in the current model, these parameters were assumed to reflect the transmission on cow level. The validity and the impact

of this assumption on the economic outcomes are hard to predict, due to the complexity of the process of transmission of infection and the absence of field data. It was not possible to re-calculate the transmission parameters from the field trial on cow level due to the absence of essential epidemiologic knowledge on transmission of infection between quarters within the same cow or to quarters of different cows.

The choice to model on cow level was made mainly because the economic outcome was the major interest, which is in fact on herd level, but many decisions of dairy herds are taken on cow level (Houben et al., 1994; Zadoks et al., 2002). Moreover, most estimates of IMI effects such as production loss and culling are only available on cow level making cow level modeling for economic analysis a valid choice.

Perhaps the best approach to model pathogen-specific IMI would have been to model IMI on quarter level and then cluster economic analysis on cow and herd level. Although this sounds as the perfect approach, it would make the model much more complicated, and still one more essential problem would arise: missing knowledge. To our knowledge, it is unknown whether an infected quarter (s) would have an equal chance to infect other healthy quarters within the same cow or to infect healthy quarters of other cows. This means that the transmission between quarters within the same cow could differ from that between quarters of different cows. If so, it would be necessary to have two parameters to reflect transmission between quarters within and between cows. Additionally, an udder could be infected by different pathogens at the same time. As the effects on production and culling are unknown on quarter level IMI, it would be hard to incorporate these effects for economic calculations on cow level.

We believe that our cow-level model represents current knowledge as good as it is available, despite the possible weak points that are mainly due to the lack of essential epidemiological knowledge, as well as caused by the complexity of the underlying pathogen-specific IMI processes.

Validity and credibility:

Several methods were used to validate the developed bio-economic model (Chapter 4). Nonetheless, the methods were all internal validation methods (Sørensen, et al., 1990). They were conducted in all stages of the model development to ensure correct validation. An important validation method is to compare model outcome to field data, which is referred to as external validity (Sørensen, et al., 1990). Although this was partly discussed in chapter 4, comparison of the model output to the original studies that provided the transmission rate parameters was not presented.

We compared the model prediction (Chapter 7) to the original study where the transmission rate parameter of *S. aureus* was obtained (Zadoks et al., 2002). However, because that field study was conducted on quarter level, we assumed different scenarios of the number of IMI quarters per cow to make it comparable to our model outcomes. Thereafter the incidence of IMI on cow level was re-calculated from the original field study, assuming a cow to have IMI only in one quarter, in 1.5 quarters as the average number of IMI quarters per IMI

cow (Barkema et al., 1997), or in 4 quarters per cow. The outcome of the comparison is presented in Table 1, corrected for similar herd size.

The model prediction of the annual median incidence of clinical *S. aureus* IMI was higher than the incidence of clinical *S. aureus* IMI from the field trial assuming 1, 1.5, or 4 IMI quarter per clinical IMI cow (Table 1). The predicted annual median incidence of subclinical *S. aureus* IMI was close to the incidence from the field trial when 1.5 IMI quarters per subclinical IMI cow was assumed (Table 1). The difference in the incidence of clinical and subclinical IMI between our model and the original study could be attributed to the other parameters in the model and the consideration of new IMI. More important, the study of Zadoks et al. (2002) could have not represented a median or an average farm in the Netherlands. We actually believe that such a farm does not exist, due to the large variability in management systems over time. Nevertheless, the recalculated number of IMI quarters per IMI cow from the field study (Table 1) did actually exist within the limits of the model predictions.

Table 1. The incidence with the 5th and 95th percentiles in parentheses of new *Staphylococcus aureus* intramammary infection (IMI) per 100 cows per year as predicted by the simulation model presented in chapter 7 and as re-calculated from the data of Zadoks et al. (2002) assuming an IMI cow would have IMI in 1, 1.5, or 4 quarters

IMI form	Model prediction of new *S. aureus* IMI	Recalculated from field data , with 1, 1.5, or 4 *S. aureus* IMI quarters per IMI cow		
		1	1.5	4
Clinical IMI	11 (0-51)	5	3	1
Subclinical IMI	17 (0-65)	35	23	9

It was not possible to compare our model prediction of the incidence of *S. uberis* IMI to the original study, from which the transmission rate parameter was obtained (Zadoks et al., 2001), because the incidence of *S. uberis* IMI during the steady state, which we modeled, was not mentioned in the field study.

In conclusion, external validation was somewhat possible for *S. aureus*, and did not disqualify the model results. However, it was deemed too weak methodology to be presented in the original papers as formal external validation.

Future applications of the model

Control and prevention of IMI during lactation:

Control and prevention of IMI during lactation can be achieved by limiting the transmission of infection between cows, which can be implemented in 3 main areas. First, eliminate existing IMIs. Second, reduce the transmission of infection. Third, reduce susceptibility and increase resistance to IMI.

Existing IMI could be well controlled by implementation of strict antibiotic treatment plans by, for instance, longer duration of antibiotic treatment of clinical IMI cases, which was found to provide better bacteriological recovery of clinical IMI (Wilson et al., 1997;

Schukken et al., 2008). The economic benefits of applying long duration antibiotic treatment can be estimated in the current model (Chapter 7) taking into account the benefits of shorter duration of IMI, and hence lower the chance of IMI spread between cows. Similarly, the economic benefits of subclinical IMI treatment, which has been advised in certain situations (Swinkels et al., 2005, Steeneveld et al., 2007), can be estimated in the current bio-economic model. Economic benefits of other prevention measures during lactation, such as pathogen-specific culling can also be evaluated by a slight modification of the culling decisions in the model (Chapter 7).

Transmission of infection between cows is the major source of contagious IMI such as *S. aureus, S. uberis,* and *S. dysgalactiae*. It was shown in chapter 4 (Figure 4) that doubling the transmission rate of any of the 3 contagious IMI pathogens greatly influenced the calculated loss. The milking parlour is believed to be the main source of new infections with contagious pathogens (Allore et al., 1999). Recent knowledge showed that transmission was actually strain specific, and hence the solution to better control transmission of IMI may be strain specific (Schukken et al., 2008). Currently, it might not be economically beneficial to identify the IMI cases at strain level, because of the high costs. However, the use of molecular diagnostic techniques might increase in the next years, due to the progress in this field, which might lead to development of rapid and cheap molecular diagnostic techniques (Schukken et al., 2008). The current bio-economic model (Chapter 7) can be adapted to include strain-specific IMI by changing the input parameters to represent a specific strain. Subsequently, economic analysis of strain-specific IMI can be conducted.

Reducing the susceptibility and increasing resistance against IMI can be achieved by using vaccines (Hogan et al., 1995) and by genetic improvements of cows (Heringstad et al., 2000). It has been shown that vaccination decreased the duration and the severity of clinical mastitis (Hogan et al., 1995). This can be simulated in our bio-economic model to estimate the cost-effectiveness of vaccination. Genetic improvement was not included in the current bio-economic model, due to 2 two main reasons. First, the transmission rate parameter represents partly the susceptibility of cows to IMI, which is what genetic improvement target. The effect of genetic improvement on the transmission rate parameters is completely unknown and hard to predict. Thus the change in pathogen-specific IMI transmission as a result of genetic improvement is very difficult to predict and was hence impossible to include in the model. Secondly, because genetic improvements are usually observed after several generations or rather decades, in which management and other parameters could also change over time, genetic effects were not included.

Further Research

Several recommendations for further research were given in the previous chapters. We have seen in chapter 4 the high impact of transmission rate parameters on the pathogen-specific net cost of IMI. Because the currently available transmission rates are uncertain, and not available on cow level and are not classified on cow factors, pathogen-specific transmission rate parameters should be estimated more precisely from field data on cow

level and classified on cow factors. Thereafter the model can be adapted to include cow factors for future economic analysis in relation to IMI management.

We have observed as well in chapter 4 the high impact of a higher rate of bacteriologic recovery of subclinical IMI on the net cost of IMI. Thus the current model could be adapted to estimate the economic impact of treating subclinical IMI cases during lactation rather than at dry off.

Conclusions

The results of this research have contributed to our epidemiologic and economic understanding of the dynamics of IMI in dairy cattle. The main conclusions are:

1- Literature estimates of the cost and/or cost-benefit of mastitis control strategies varied largely, which was partly due to different consideration of the cost factors of mastitis. Thus, the proposed guideline in chapter 2 provides a credible framework to be followed when attempts are made to conduct economic calculations on mastitis control and prevention, which will also ease comparing literature outcomes.

2- New subclinical mastitis cases can be defined based on individual consecutive SCC values. These new cases contribute to a substantial production loss in primiparous and multiparous dairy cows.

3- The proposed bio-economic model is a valid and credible tool to estimate the cost of pathogen-specific IMI, and to support decision making in relation to IMI control and prevention. The net economic effect of IMI varied largely and was most sensitive to the pathogen-specific IMI transmission rate.

4- Prevention of new IMI during the dry period up to 21 days post-calving can be achieved using dry cow therapy and/or teat sealant. Moreover, peer-reviewed literature estimates might have been biased due to possible publication bias.

5- Cure of existing IMI at dry off can be successfully achieved using dry cow therapy. No indication of publication bias existed in the estimates of the peer-reviewed literature.

6- A substantial number of cows acquire new IMI during the dry period and start the lactation with IMI affecting the dynamics of IMI during the lactation and consequently the cost of IMI. Applying dry period intervention was essential to minimize economic losses due to IMI. Despite relatively small differences, blanket dry cow therapy was the most cost-effective dry period intervention in a quota situation, followed by selective dry cow therapy or teat sealant, selective dry cow therapy combined with teat sealant, and blanket dry cow therapy combined with teat sealant.

7- It might be necessary to include milk production loss besides increased SCC and bacterial isolation to define new subclinical mastitis.

8- The bio-economic model represented the variability in the cost of IMI caused by the variability due to the dynamics of pathogen-specific IMI and it can be used in

further research to assess the economic impact of various IMI control strategies during lactation.

References:

Allore H.G., Erb, H.N., 1998. Partial budget of the discounted annual benefits of mastitis control strategies. J. Dairy Sci. 81:2280-2292.

Allore, H.G., Erb H.N. 1999. Approaches to modeling intramammary gland infections in cattle. Prev. Vet. Med. 39:279-293.

Barkema, H.W., Schukken Y.H., Lam T.J.G.M., Galligan D.T., Beiboer M.L., Brand A. 1997. Estimation of Interdependence Among Quarters of the Bovine Udder with Subclinical Mastitis and Implications for Analysis. J. Dairy Sci. 80:1592-1599.

Borne, B. van der, van Schaik G., Lam T.J.G.M., Nielen M. 2008. A comparison of the occurrence of mastitis in Dutch primi- and multiparous cows. In proc. Mastitis control; from science to practice, 30th September to 3rd October, The Hague, the Netherlands, pp 55.

Coldebella, A., Machado P.F., Demetrio C.G.B., Junior P.J.R., Meyer P.M., Corassin C.H., Cassoli L.D. 2004. Somatic cells count and milk yield in confined Holstein cows. Braz. J. Animal Sci. 33:623–634.

Dohoo, I. R., Leslie K.R. 1991. Evaluation of changes in somatic cell counts as indicators of new intramammary infections. Prev. Vet. Med. 10:225–237.

Green, M.J., Green L.E., Schukken Y.H., Bradley A.J., Peeler E.J., Barkema H.W., De Haas Y., Collis V.J., Medley G.F. 2004. Somatic cell count distribution during lactation predict clinical mastitis. J Dairy Sci. 87:1256-1264.

Grohn, Y.T., Wilson D.J., Gonzalez R.N., Hertl J., Schulte H., Bennett G., Schukken Y.H., 2004. Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. J. Dairy Sci. 87:3358–3374.

Heringstad, B., Klemetsdal G., Ruane J. 2000. Selection for mastitis resistance in dairy cattle: A review with focus in the situation in the Nordic countries. Livestock Prod. Sci. 64:95-106.

Hogan, J.S., Weiss, A.P., Smith K.L., Todhunter D.A., Schoenberger P.S., and Sordillo L.M. 1995. Effects of an Escherichia coli J5 Vaccine on mild clinical coliform mastitis. J. Dairy Sci. 78:285-290.

Houben, E.H.P., Huirne R.B.M., Dijkhuizen A.A., Kristensen A.R. 1994. Optimal replacement of mastitis cows determined by a hierarchic Markov process. J. Dairy Sci. 77:2975 – 2993.

Leitner, G., Eligulashvily R., Krifucks O., Perl S., Saran A. 2003. Immune cell differentiation in mammary gland tissues and milk of cows chronically infected with Staphylococcus aureus. J. Vet. Med. B. 50:45-52.

Østergaard S., Chagunda M.G.G., Friggens N.C., Bennedsgaard T.W., Klaas I.C. 2005. A stochastic model simulating pathogen-specific mastitis control in a dairy herd. J. Dairy Sci. 88:4243–4257.

Persson, K., Amolina B., Jonsson P. 1995. Inflammation in the bovine teat cistern induced by *Staphylococcus aureus*. Zentralbl. Veterinarmed. B 42:435–442.

Reksen, O., Sølverød L., Østerås O. 2008. Relationships Between Milk Culture Results and Composite Milk Somatic Cell Counts in Norwegian Dairy Cattle. J. Dairy Sci. 91:3102–3113.

Schrick, FN., Hockett M.E., Saxton A.M., Lewis M.J., Dowlen H.H., Oliver S.P. 2001. Influence of subclinical mastitis during early lactation on reproduction parameters. J. Dairy Sci. 84:1407-1412.

Schukken, Y.H., Barkema, H.W., Lam T.J.G.M., Zadoks R.N. 2008. Improving udder health on well managed farms: mitigating the perfect storm. In Proc of international conference on Mastitis control: from Science to practice, 30 September to 2nd October 2008, The Hague, The Netherlands, pp. 21-35.

Seegers, H., Fourichon C., Sørensen J.T., Hortet P., Billon D. 2000. Modelling the cost-benefits of prevention and control of mastitis in dairy herds. Pages 55–61 in Proc. Symp. Economic Modelling of Animal Health and Farm Management. Wageningen University, Wageningen, The Netherlands.

Seegers, H., Fourichon C., Beaudeau F. 2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet. Res. 34:475–491.

Sørensen, J.T. 1990. Validation of livestock herd simulation models: A review. Livest. Prod. Sci. 26:79–90.

Steeneveld, W., Swinkels, J., Hogeveen, H., 2007. Stochastic modelling to assess economic effects of treatment of chronic subclinical mastitis caused by *Streptococcus uberis*. J. Dairy Res. 74, 459-467.

Swinkels, J.M., Hogeveen, H., Zadoks, R.N., 2005. A partial budget model to estimate economic benefits of lactational treatment of subclinical *Staphylococcus aureus* mastitis, J. Dairy Sci. 88, 4273-4287.

Van Asseldonk, M.A.P.M., Reint Jan Renes, Lam T.J.G.M., Hogeveen H. 2009. Awareness and perceived value of economic information in controlling somatic cell count. Vet. Rec. Accepted.

Van Werven, T., Piens K., van den Broek J., Heyneman R., Noordhuizen-Stassen E. N., Schukken Y. H., Brand A., 1998. Flow cytometric measurement of neutrophil alkaline phosphatase before and during initiation of an induced *Escherichia coli* mastitis in cattle. Vet. Immunol. Immunopathol. 62:235-244.

Van Werven, T. 1999. the role of leukocytes in bovine *Escherichia coli* mastitis. PhD dissertation, Department of Farm Animal Health and Reproduction, Utrecht University, the Netherlands.

White, L.J., Lam T.J.G.M., Schukken Y.H., Green L.E., Medley G.F., Chappell, M.J. 2006. The transmission and control of mastitis in dairy cows: A theoretical approach. Prev. Vet. Med. 74:67-83.

Zadoks, R.N., Allore H.G., Hagenaars T.J., Barkema H.W., Schukken Y.H., 2002. A mathematical model of *Staphylococcus aureus* control in dairy herds. Epidemiol. Infect. 129, 397-416.

Zadoks, R.N., Schukken Y.H., 2006. use of molecular epidemiology in veterinary practice. Vet. Clin. North Am. Food Anim. Pract. 22:229-261.

Summary

Mastitis is considered the most frequent and costly disease in bovine dairy herds in developed countries. To estimate the efficacy of mastitis control strategies, a proper assessment of the economic consequences of mastitis should be available. This economic assessment should take into account all cost factors involved in mastitis. Furthermore, specific market circumstances, such as the existence of a milk quota system, should be considered. Because production losses are one cost factor of mastitis, proper assessment of production losses are necessary to obtain reliable economic assessment of mastitis. More important, mastitis can be caused by different pathogens that differ in the dynamics of infection and consequently give a different economic impact. Thus, it is important to consider the dynamics of pathogen-specific intramammary infection (**IMI**), so that advices can be given for farm specific situations, in which the mastitis problem can be caused by different pathogens. The dynamics of IMI should be considered during both the lactation and the dry period (**DP**). The DP is responsible for a considerable number of cows starting the lactation with an IMI. By taking into account the dynamics of different mastitis pathogens for both the lactation and the DP, realistic economic assessments of mastitis and possible control strategies may be made. These assessments can support decision making on mastitis.

Several studies have been published since 1990 on the economics of mastitis and mastitis control strategies. However, hardly any of these studies has discussed the consistency of results with other studies. In **chapter 2**, the economic factors associated with mastitis are explained, providing a framework for economic analysis. As a second step, calculations of the costs of mastitis and the costs in relation to the benefits of mastitis control strategies published since 1990 in peer-reviewed journals are extensively reviewed and analysed. The result shows a large variation in the calculated costs and benefits of mastitis and mastitis control strategies between the different studies. Moreover, it is clear that important factors were ignored in some of the studies. The framework provided in **chapter 2** can provide future studies a basis for economics assessment of mastitis and mastitis control strategies.

Milk, fat, and protein loss due to a new subclinical mastitis case may be economically important. Hardly any study estimated the production loss due to new subclinical mastitis cases. The aim of **chapter 3** was to estimate this loss. The loss was estimated based on test-day (**TD**) cow records collected over a 1-year period from 400 randomly selected Dutch dairy herds. After exclusion of records from cows with clinical mastitis, the dataset comprised 251,647 TD records from 43,462 lactations of 39,512 cows. The analysis was carried out using the random regression TD modeling approach that predicts the production of a cow at each TD based on the actual production at all previous TDs. The definition of new subclinical mastitis was based on literature and assumed a new subclinical case if SCC > 100,000 cells/mL after a TD with SCC < 50,000 cells/mL. A second dataset was created by applying an adjustment to correct low SCC for the dilution effect when determining if the previous TD SCC was < 50,000 cells/mL. Thereafter the loss was estimated for records with SCC >

100,000 cells/mL. The production (milk, fat, or protein) losses were modeled as the difference between the actual and predicted production (milk, fat or protein) of a cow at the TD of new subclinical mastitis, for 4,382 cow records before, and 2,545 cow records after dilution correction. Primiparous cows were predicted to lose 0.31 (0.25-0.37) and 0.28 (0.20-0.35) kg/d milk at a SCC of 200,000 cells/mL, for unadjusted and adjusted low SCC, respectively. For the same SCC increase, multiparous cows were predicted to lose 0.58 (0.54-0.62) and 0.50 (0.44-0.56) kg/d milk, respectively. Moreover, it was found that with an increasing SCC over 100,000 cells/mL, the production losses also increased. Because of the used methodology, the estimated production losses were more precise than previously reported estimates.

Although the dynamics of transmission play an important role in the occurrence of IMI, they have not been considered in previous simulation models used to estimate the cost of IMI. The bio-economic model described in **chapter 4** includes within-herd dynamics of pathogen-specific IMI. The model simulated *Staphylococcus aureus, Streptococcus uberis, Streptococcus dysgalactiae,* and *Escherichia coli* IMI stochastically and estimated the cost of these IMI in bovine dairy herds in a situation with a milk quota. A Reed-Frost model was used for *Staph. aureus, Strep. uberis,* and *Strep. dysgalactiae* IMI and a Greenwood model for *E. coli* IMI. Economic analysis was conducted per pathogen for both clinical and subclinical IMI. The parameters used in the model were based on scientific literature and were deemed credible and valid. Median annual incidence of clinical and subclinical IMI for all pathogens varied considerably. This variation was greater for *Staph. aureus* IMI than for the other pathogens. The annual incidence of IMI in a herd with 100 dairy cows caused by *Staph. aureus* varied between 0 and 88 cases, with a median of 5 cases and the 5^{th} and 95^{th} percentiles of 0 to 36 cases for clinical IMI, and a median of 7 cases with the 5^{th} and 95^{th} percentiles of 0 to 52 cases for subclinical IMI. In consequence, the average total annual net costs also varied widely for *Staph. aureus* IMI. Clinical IMI costs were € 1,375 per herd, with the 5^{th} and 95^{th} percentiles of 0 to 4,716 and subclinical IMI costs were € 1,219 per herd, with the 5^{th} and 95^{th} percentiles of 0 to 4,030. The average annual net cost due to the 4 simulated pathogens combined was € 4,896 and varied from € 915 to € 11,287 per herd of 100 dairy cows. The bio-economic model developed in chapter 4 was utilized (in chapter 7) as a tool to investigate the economic impact of pathogen-specific IMI management during the DP.

The objective of **chapter 5** was to estimate the preventive effect of various dry cow control strategies against new quarter IMI during the DP up to 21 days post-calving using meta-analysis. Moreover, the potential publication bias was assessed in the studies selected for this analysis. The intervention measures were blanket dry cow therapy (**BDCT**), selective DCT (**SDCT**), Cloxacillin compared to other DCT products, and teat sealant (**TS**). A meta relative risk (**RR**) was calculated per intervention and pathogen group when enough studies were available from the 45 selected studies. BDCT showed significant protection against new IMI caused by *Streptococcus* spp., the pooled RR was 0.39 (0.30-0.51), but no protection was observed against coliform new IMI; the pooled RR was 0.95 (0.81-1.10). After correction for publication bias, protection against new *Staphylococcus* spp. IMI was doubtful. SDCT

showed higher protection against new IMI compared to no DCT; pooled RR was 0.51 (0.30-0.86). However, BDCT showed more protection when compared to SDCT; pooled RR was 0.55 (0.37-0.80), depending on the unit of treatment (quarter or cow). Cloxacillin showed similar protection against new quarter IMI compared to other DCT products with a pooled RR of 1.09 (0.94-1.25). Internal TS showed high protection against new IMI during the DP with a pooled RR of 0.39 (0.18-0.82).

A main goal of DCT is to cure existing IMI at dry off. Although several studies have been published to estimate the cure rate of IMI after DCT, variation is large between studies, which makes it difficult to conduct a proper economic evaluation of DCT. The objective of the meta analysis of existing peer reviewed literature in **chapter 6** was to provide a summary quantification of quarter IMI cure using DCT. Meta-analysis RR was calculated per intervention and pathogen group when enough studies were available from the 22 studies selected. Results of the meta-analyses were examined using publication bias tests. BDCT provided a 1.78 (1.51-2.10) times higher calculated cure rate from quarter IMI, during the DP up to 21 days post-calving, compared to no DCT. The RR of cure was similar when treatment was conducted for *Streptococcus* spp. IMI quarters compared to *Staphylococcus* spp. IMI quarters. The pooled RRs were 1.83 (1.48-2.35) and 1.65 (1.38-1.96), respectively. There was no significant difference between cloxacillin and other DCT products in cure of quarter IMI during the DP up to 21 d post-calving. The pooled RR with the 95% CI was 1.00 (0.92-1.09). Similarly, there was no significant difference between cloxacillin and other DCT products in cure of quarter *Staphylococcus* spp. IMI. The pooled RR with the 95% CI was 1.00 (0.96-1.06). The pooled RR with the 95% CI of quarter IMI cure using SDCT compared to no DCT were 1.76 (1.23-2.54).

Despite the high importance of the DP in affecting the udder health of dairy cattle, less attention has been given to model the dynamics of IMI during the DP and to estimate the subsequent cost-effectiveness of DP interventions. The developed stochastic bio-economic model of intramammary infection (IMI) caused by *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Escherichia coli* (chapter 2) was extended in **chapter 7** to model the dynamics of IMI during the dry period. The extended model was used to calculate the costs and benefits of different DP interventions in relation to the annual cost of IMI in a herd of 100 dairy cows. The dynamics of IMI during the DP were modeled based on a Greenwood model. The interventions were BDCT as the default scenario, BDCT combined with TS, SDCT or TS, and SDCT combined with TS. Parameters of the dynamics of IMI during the DP and the economic parameters were based on scientific literature. The cost of clinical and subclinical IMI during lactation, clinical IMI during the dry period, and the investment cost of intervention were used to calculate the combined total annual net cost of IMI per herd. The combined total annual net cost of IMI using the other intervention scenarios was compared to the default scenario (BDCT) to estimate the costs and benefits of the other intervention scenarios. The results showed that a considerable number of cows acquire new IMI during the dry period and start the lactation with IMI. Furthermore, the combined total annual net cost of IMI per herd using BDCT was € 8,336 distributed as € 4,313 due to clinical

IMI, and € 2,871 due to subclinical IMI during lactation, € 84 due to clinical IMI during the dry period, and € 1,068 due to antibiotic therapy and labor costs at dry off. Application of BDCT combined with TS resulted in € 967 higher combined total annual net cost of IMI compared to the BDCT scenario. Similarly, the SDCT scenarios resulted in higher combined total annual net cost of IMI compared to the BDCT scenario. The SDCT or TS was € 586 higher and the SDCT combined with TS was € 596 higher. Although the differences between the 4 simulated scenarios were not drastic, BDCT was the most cost-effective scenario in the default situation.

In **chapter 8** the definition of subclinical mastitis based on SCC, and the validity and the applications of the developed bio-economic model are evaluated and discussed. From an economic point of view, the use of only SCC and bacterial isolation might not be sufficient to indicate new subclinical mastitis, in which milk production loss is expected to occur. Therefore, it might be more relevant to include milk production loss when definition of new subclinical mastitis is carried out for economic analysis. It might be hard to validate the developed bio-economic model, because of the unavailability of relevant epidemiologic data and the complexity of the modeled processes. However, the bio-economic model seems to represent the dynamics of IMI in bovine dairy herds properly, including the large variability between herds.

In conclusion, the developed bio-economic model in chapter 4 and as updated in chapter 7 is a valid and credible tool to estimate the cost of pathogen-specific IMI in bovine dairy herds. Furthermore, it is a useful tool to evaluate the efficacy of several IMI control strategies that can lead to decrease the transmission rate of pathogen-specific IMI and the number of infectious cows in the herd during the DP and the lactation.

Samenvatting (Dutch summary)

In ontwikkelde landen wordt mastitis beschouwd als de meest voorkomende en kostbare ziekte bij melkvee. Om de kosten-effectiviteit van preventieve maatregelen tegen mastitis te beoordelen, is het noodzakelijk een goede schatting van de kosten van mastitis te hebben. Deze schatting moet alle kosten factoren die betrekking hebbenop mastitis meenemen. Bovendien moeten specifieke marktomstandigheden, zoals het melkquotum, worden meegenomen in de berekeningen. Omdat melkproductieverliezen een belangrijke kosten factor voor mastitis vormen, is het voor een betrouwbare economische berekening, belangrijk een goede inschatting te hebben van de productieverliezen ten gevolge van mastitis. Ook is het belangrijk, onderscheid te maken tussen de verschillende pathogenen die mastitis kunnen veroorzaken. Deze pathogenen hebben een verschillende dynamiek en hebben, dientengevolge, verschillende economische gevolgen. Ook verschilt de effectiviteit van preventieve maatregelen tussen pathogenen. De dynamiek van IMI is niet alleen belangrijk gedurende de lactatie van een koe, ook de droogstand speelt een belangrijke rol. De dynamiek van IMI gedurende de droogstaand is verantwoordelijk voor een aanzienlijk aantal koeien welke de lactatie beginnen met een IMI. Het is daarom belangrijk dat bij economische berekeningen de dynamiek van intramammaire infecties (IMI), veroorzaakt door verschillende pathogenen, gedurende de lactatie en de droogstand meegenomen wordt. Dergelijke berekeningen kunnen gebruikt worden om de kosten-effectiviteit van mogelijke mogelijke preventieve maatregelen te kunnen schatten, wat te gebruiken is bij de besluitvorming rondom mastitis op een melkveebedrijf.

Sinds 1990, zijn diverse studies gepubliceerd met betrekking tot de economische aspecten van mastitis en mastitis preventie. In geen van deze studies zijn echter de resultaten vergeleken met resultaten van andere studies. Ook werden in de diverse studies verschillende kosten factoren meegenomen. In hoofdstuk 2 worden de economische factoren die verband houden met mastitis nader uitwerkt. Deze uitwerking vormt een kader voor economische analyse van mastitis. Daarnaast worden de berekeningen van de kosten van mastitis en effecten van mastitis preventie, die sinds 1990 gepubliceerd zijn peer-reviewed tijdschriften, uitvoerig geëvalueerd en geanalyseerd. Het resultaat toont een grote variatie in de berekende kosten van mastitis en effecten van mastitis preventie tussen de verschillende studies. Bovendien wordt duidelijk dat belangrijke factoren werden genegeerd in sommige van de gepubliceerde berekeningen. Het economisch kader dat in hoofdstuk 2 uitgewerkt is, kan voor toekomstige studies de basis vormen voor economische berekeningen rondom mastitis en mastitis preventie.

De verlaging van de melk-, vet-, en eiwitproductie is een belangrijke kostenfactor van mastitis. Niet alleen klinische mastitis leidt tot een verlaging van de productie, ook subklinische mastitis geeft een verlaging van de productie van een koe. In hoofdstuk 3 wordt een schatting gemaakt van de melkproductie verliezen (kg melk, kg vet en kg eiwit) ten gevolge van subklinische mastitis. De berekeningen zijn uitgevoerd op testdag (TD) gegevens

van alle koeien op 400 willekeurig geselecteerde Nederlandse melkveebedrijven. Na uitsluiting van de gegevens van koeien met klinische mastitis, bestond de dataset uit 251.647 TD records van 43.462 lactaties van 39.512 koeien. Met behulp van het random regressie testdag model, werd de productie van iedere koe op iedere TD voorspeld, gebaseerd op de werkelijke productie in alle voorgaande TDen. De definitie van een nieuw geval van subklinische mastitis was gebaseerd op beschikbare kennis uit de wetenschappelijke literatuur en. Een koe had een nieuw geval van subklinische wanneer het celgetal (SCC) op een testdag groter was dan 100.000 cellen/ml, terwijl de voorgaande TD een SCC had lager dan 50.000 cellen/ml. Op deze wijze, werden 4.382 koeien gevonden die een nieuw geval van subklinische mastitis hadden. Een zelfde procedure werd uitgevoerd in een tweede dataset, waar het SCC gecorrigeerd was voor het verdunningseffect. In deze tweede dataset, waren 2.545 koeien met een nieuw geval van subklinische mastitis. In beide datasets werden de productieverliezen (melk, vet en eiwit) van koeien met een SCC > 100.000 cellen/ml geschat door het verschil te nemen van de voorspelde productie en de werkelijke productie. Voor koeien in de eerste lactatie met subklinische mastitis en een SCC van 200,000 cellen/ml, bedroegen de berekende verliezen respectievelijk 0,31 (0.25-0.37) kg/koe/dag en 0,28 (0,20-0,35) kg/koe/dag voor de niet gecorrigeerde en gecorrigeerde SCC gegevens. Voor koeien met een hogere lactatie bedroegen deze verliezen respectievelijk 0,58 (0,54-0,62) en 0,50 (0,44-0,56) kg/koe/dag voor de niet gecorrigeerde en gecorrigeerde SCC gegevens. Bovendien werd vastgesteld dat met een toenemende SCC, de productieverliezen verder toenamen. Door de gebruikte methodologie, konden productieverliezen van nieuwe gevallen van subklinische mastitis nauwkeuriger worden geschat dan in eerdere berekeningen.

Hoewel de dynamiek van de transmissie van verschillende mastitis pathogenen een belangrijke rol spelen bij IMI, zijn deze factoren niet meegenomen in eerdere simulatie modellen voor de schatting van de kosten van IMI. In hoofdstuk 4 wordt een bio-economisch model beschreven. Dit stochastische model simuleert de dynamiek van IMI, veroorzaakt door *Staphylococcus aureus, Streptococcus uberis, Streptococcus dysgalactiae* en *Escherichia coli* op een melkveebedrijf van 100 koeien. De kosten van de gesimuleerde IMI worden berekend voor een situatie met melkquotum. Een Reed-Frost model werd gebruikt voor de dynamiek van *Staph aureus, Strep uberis* en *Strep dysgalactiae* IMI. Een Greenwood model werd gebruikt om de *E. coli* IMI te modelleren. Economische berekeningen werden uitgevoerd voor het hele bedrijf, per pathogeen en voor klinische en subklinische IMI. De gebruikte parameters in het model zijn gebaseerd op wetenschappelijke. De mediaan van de incidentie (gevallen per 100 koeien per jaar) van klinische en subklinische IMI voor varieerde voor alle pathogenen aanzienlijk. Deze variatie is groter voor *Staph aureus* IMI dan voor de andere pathogenen. De incidentie (gevallen per 100 koeien per jaar) van klinische IMI veroorzaakt door *Staph aureus,* varieerde tussen de 0 en 88 gevallen, met een mediaan van 5 gevallen en 5 en 95 % percentielen van 0 en 36 gevallen. De mediaan en 5 en 95 % percentielen van subklinische IMI veroorzaakt door *S aureus* waren respectievelijk 7, 0 en 52 gevallen. Als gevolg hiervan bedroegen de totale jaarlijkse netto kosten ten gevolge van klinische *S aureus* IMI gemiddeld € 1.375, met 5 en 95 % percentielen van € 0 en € 4716. De totale jaarlijkse netto kosten ten gevolge van subklinische IMI veroorzaakt door *Staph aureus* kosten

bedroegen gemiddeld € 1.219 met 5 en 95 % percentielen van € 0 en € 4030. Voor alle IMI gezamenlijk (ten gevolg van *Staph aureus, Strep uberis, Strep dysgalactiae* en *E coli,* klinisch, zowel als subklinisch) bedroegen de gemiddelde jaarlijkse netto kosten € 4896, variërend van € 915 tot € 11.287 voor een veestapel van 100 melkkoeien. Het bio-economisch simulatiemodel, beschreven in hoofdstuk 4 is gebruikt (in hoofdstuk 7) om te onderzoeken wat de economische gevolgen van pathogeen-specifieke maatregelen tijdens de droogstand waren.

Het doel van hoofdstuk 5 was een schatting te maken van de preventieve werking van maatregelen om IMI te genezen of te voorkomen gedurende de droogstand. Hiervoor is een meta-analyse van bestaande, wetenschappelijke, literatuur gemaakt (45 artikelen). In deze meta-analyse is ook gekeken naar de publicatie bias van de artikelen die voor dit onderzoek geselecteerd zijn. De onderzochte maatregelen waren: volledige droogzet therapie (VDT; alle kwartieren van alle koeien worden op het moment van droogzetten met antibiotica behandeld), selectieve droogzet therapie (SDT; alle kwartieren van geselecteerde koeien worden op het moment van droogzetten met antibiotica behandeld), gebruik van Cloxacilline in vergelijking met andere droogzet antibiotica en het gebruik van teat sealant (TS; een kunstmatige afdichting van de speen op het moment van droogzetten). Een gepoolde relatief risico (RR) werd berekend per maatregel en pathogeen groep. VDT liet een aanzienlijke bescherming zien tegen nieuwe IMI veroorzaakt door Streptococcus spp. De gepoolde RR was 0,39 (0,30-0,51). VDT liet geen bescherming zien tegen nieuwe IMI veroorzaakt door *E. coli.* De gepoolde RR was 0,95 (0.81-1.10; de getallen tussen haakjes geven het 95% betrouwbaarheidsinterval weer). Na correctie voor publicatie bias, leek bescherming van VDT tegen nieuwe IMI veroorzaakt door Staphylococcus spp twijfelachtig. SDT gaf een hogere bescherming tegen nieuwe IMI in vergelijking met geen DCT. De gepoolde RR was 0,51 (0,30-0,86). VDT gaf echter meer dan SDT. De gepoolde RR was 0,55 (0.37-0.80). Cloxacilline toonde vergelijkbare bescherming tegen nieuwe IMI met andere droogzet antibiotica. De gepoolde RR was 1,09 (0,94-1,25). TS liet een hoge bescherming zien tegen nieuwe IMI tijdens de droogstand. De gepoolde RR was 0,39 (0,18-0,82).

Naast het voorkomen van nieuwe IMI is een ander doel van droogzet therapie het genezen van bestaande IMI. Hoewel verschillende studies zijn gepubliceerd met een schatting van het genezingspercentage van IMI na droogzet therapie, is de tussen de verschillende studies groot. Dit maakt het moeilijk om een goede economische evaluatie van droogzet therapie uit te voeren. Het doel van de meta-analyse van bestaande wetenschappelijke literatuur (22 artikelen) in hoofdstuk 6, was een samenvatting te maken van de genezing van IMI (op kwartierniveau) door bij gebruik van droogzet therapie. Een gepoolde RR werd berekend per maatregel en pathogeen groep. Ook werden testen uitgevoerd om de publicatie bias te kunnen onderzoeken. Op basis van de gepoolde artikelen gaf VDT een 1,78 (1.51-2.10) keer hoger percentage genezen IMI tijdens de droogstand in vergelijking met het niet gebruiken van droogzet therapie. De gepoolde RR van genezen was vergelijkbaar voor IMI veroorzaakt door Streptococcus spp en IMI veroorzaakt door Staphylococcus spp. De gepoolde RR waren respectievelijk 1,83 (1.48-2.35) en 1,65 (1,38-1,96). Er was geen

significant verschil tussen het gebruik van Cloxacilline en andere antibiotica in de genezing van IMI tijdens de droogstand. De gepoolde RR was 1,00 (0,92-1,09). Ook was er geen significant verschil tussen het gebruik van Cloxacilline en andere antibiotica in de genezing van Staphylococcus spp. De gepoolde RR was 1,00 (0,96-1,06). De gepoolde RR van SDT in vergelijking met geen droogzet therapie was 1,76 (1.23-2.54).

Ondanks het grote belang van de droogstand bij de uiergezondheid van melkvee, wordt er bij simulatiemodellen voor de dynamiek van IMI relatief weinig aandacht aan gegeven. Dat betekent dat deze modellen ook niet goed geschikt zijn om de kosten-efficiëntie van preventieve maatregelen tegen IMI in de droogstand te bepalen. Het ontwikkelde bio-economische model van IMI verooraakt door *Staph aureus, Strep uberis, Strep dysgalactiae* en *E coli* (hoofdstuk 2) werd in hoofdstuk 7 uitgebreid met de dynamiek van IMI in de droogstand. Dit model is gebruikt om de kosten en baten van verschillende preventieve maatregelen rondom de droogstand te schatten in een veestapel van 100 melkkoeien. De dynamiek van IMI tijdens de droogstand is gemodelleerd op basis van een greenwood model. Als standaard scenario werd VDT gebruikt. Alternatieve maatregelen waren: VDT gecombineerd met TS, SDT, TS en SDT gecombineerd met TS. Parameters die de dynamiek van IMI tijdens de droogstand bepalen en economische parameters zijn geschat op basis van wetenschappelijke literatuur. De kosten van klinische en subklinische IMI tijdens de lactatie, de kosten van IMI tijdens de droogstand, en uitgaven voor de preventieve maatregelen zijn gebruikt om de totale jaarlijkse netto kosten van IMI voor de verschillende preventieve maatregelen te berekenen. De berekende kosten van IMI bij de verschillende preventieve maatregelen werden vergeleken met het standaard scenario (VDT). Het verschil tussen de uitkomsten bepaalde het netto economisch effect van een preventieve maatregelen ten opzichte van het standaard scenario. Uitkomsten van het model lieten zien dat een aanzienlijk aantal koeien een nieuw IMI tijdens de droogstand kregen en de lactatie met een IMI begonnen. Bij VDT waren de totale jaarlijkse netto kosten van IMI € 8.336 voor een veestapel van 100 koeien. Hiervan was € 4.313 een gevolg van klinische IMI tijdens de lactatie, € 2.871 een gevolg van subklinische IMI tijdens de lactatie, € 84 een gevolg van klinische IMI tijdens de droogstand en € 1.068 een gevolg van antibiotica en arbeidskosten om koeien droog te zetten. Toepassing van VDT gecombineerd met TS resulteerde in € 967 hogere totale jaarlijkse netto kosten van IMI in vergelijking met het VDT scenario. Ook de twee SDT scenario's leidden tot hogere totale jaarlijkse netto kosten van IMI in vergelijking met het VDT scenario. De kosten voor SDT waren € 586 hoger dan het standaard scenario en de kosten voor SDCT gecombineerd met TS waren € 596 hoger. Hoewel de verschillen tussen de 4 gesimuleerde scenario's niet groot waren, kon geconcludeerd worden dat voor een standaard bedrijf, VDT de meest kosten efficiënte preventieve maatregel in de droogstand is.

In hoofdstuk 8 wordt de definitie van subklinische mastitis, nu vaak op basis van het SCC bediscussieerd. Ook de validiteit en mogelijke toepassingen van het ontwikkelde bio-economisch model worden geëvalueerd en bediscussieerd. Vanuit economisch perspectief, is alleen het gebruik van SCC en isolatie van pathogenen wellicht niet voldoende om subklinische mastitis te definiëren. In dat kader is meer relevant om ook de verminderde

melkproductie bij de definitie van nieuwe subklinische mastitis mee te nemen. Hoewel het bio-economische model zeer moeilijk te valideren is, vanwege het ontbreken van relevante epidemiologische gegevens en de complexiteit van de gemodelleerde processen, lijkt dit model de dynamiek van IMI op melkveebedrijven goed te modelleren. Ook de gevonden variatie, binnen bedrijf, lijkt de werkelijkheid goed te benaderen. .

Samengevat, het ontwikkelde bio-economisch model in hoofdstuk 4 en uitgebreid in hoofdstuk 7, is een geldig en betrouwbaar instrument om de kosten te schatten van pathogeen-specifieke IMI bij melkveebedrijven. Bovendien is het een nuttig instrument voor het evalueren van de kosten efficiëntie van verschillende preventieve maatregelen om de transmissie van IMI en het aantal infectieuze koeien op melkveebedrijven te verminderen.

Acknowledgments

The past three and a half years have been in some ways difficult, stressful and long, but in other ways, they were interesting, full of fun, and passed quickly. Although they were sufficient to finish my PhD, they were not enough to do all what I wanted to do. During this period, I had constant support from several people that I would like to thank. First of all, my family was the continuous source of support and encouragement to me. Father and Mother, without your support, this work would have never existed. Thank you very much. My love (Birgitte), meeting you during my PhD was the happiest moment of all. You were very supportive and never complained from my extra work. Your words have always comforted me. Thank you so much. My sisters, brothers, and brothers in law, thank you for the constant encouragement and help.

Second of all, my supervisors - working with you was brilliant. I was privileged to do my PhD under your supervision. Henk, you have given me great guidance and have always made my life easier. Mirjam, you have never preserved an advice, even during your overwhelming periods of work. Both of you were very tolerant, supportive, and have always given me brilliant ideas. Without you, I could not have made it to the end. Thank you very much for believing in me and for your patience and constant support. Tine, thank you for the interesting discussions and the advices you gave me.

Colleagues from Utrecht and Wageningen Universities and from the Dutch Udder Health Center (UGCN), thank you for the encouragement, advices, help, and the nice time during my PhD. Friends in Jordan and in Europe, you have filled my life with great fun and joy. I always looked forward to meet with you and to enjoy your company, which kept me out of my work world.

Olav Østerås and researchers from the Epicenter in Oslo school of veterinary sciences, thank you for the great discussions we had and the nice time during my stay in Norway.

Curriculum Vitae

Tariq Halasa was born on 15th April 1979 in Jordan. He followed the primary and secondary school in Karak city, located in the middle part of Jordan. In 1997, he joined the Faculty of Veterinary Sciences in Jordan University for Science and Technology and graduated in June 2002 as a Bachelor in Veterinary Medicine and Surgery. On 1st July 2002, he started working as a veterinary practitioner in Karak city, where his duties were to fight animal diseases and to assist farmers in herd health management. In September 2003, he followed an M.Sc. program in Veterinary Epidemiology and Economics at Utrecht University in the Netherlands. He graduated with an M.Sc. degree in February 2005. Thereafter, he started writing his PhD proposal, and in October 2005 he started his PhD at Utrecht University with strong collaboration with Wageningen University. During his PhD he collaborated with the National Recording System (NRS) and the Dutch Udder Health Center (UGCN). He was invited for a short-term stay at Oslo School of Veterinary Sciences to work with Professor Olav Østerås on part of his PhD project.