Epidemiological and partial budget analysis for treatment of subclinical *Staphylococcus aureus* intramammary infections considering microbiological and cytological scenarios

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Highlights

- Udder health models using current data to predict future outcomes were developed.
- Treating subclinical *S. aureus*infections lead to a decline in treatments numbers.
- Substantial reductions in new and persistant intramammary infections was indicated.
- Models combinedfinancial and epidemiological reductions of *S. aureus* infections.
- Assist in optimising decisions balancing cost/benefit with end point IMI dynamics.

SIGNIFICANCE OF THE WORK

Antimicrobial resistance is currently an actual global topic. This study is a fresh approach towards this challenge. The aims for developing the models were: firstly to provide a consultant, veterinarian or dairy producer with a tool to base decisions on current farm information to accurately predict future events so that measurements could be taken to prevent disease in advance; secondly we wanted to predict cost effectiveness of management changes in combination with an indication of epidemiological outcome in the *Staphylococcus aureus* population in herds. Current availability of advances on farm technology and data capabilities based on scientific approach predictions is making this approach become a reality.

Predictions were based on the combination of financial cost benefit but included also predictions of probable epidemiological outcomes such as number of clinical mastitis cases, new intramammary infections, persistant cases likely to occur and number of subclinical *Staphylococcus aureus cases* that would require treatment for the period chosen. The study further compare these outcomes for different initial herd prevalence of *Staphylococcus* intramammary infections, different management levels and varying duration of intramammary treatments. When results of economic and epidemiological models were compared, the best financial option differed in some scenarios. Such models may assist producers, consultants and veterinarians in optimising decisions balancing cost/benefit with end point IMI dynamics. Where initial effective treatment was applied, a lower number of treatments were needed for the 255 days.

We believe that the models can make a positive contribution to enhance current herd udder health decisions by providing more accurate predictions of outcome of inventions such as the treatment of subclinical *Staphylococcus aureus*.

ABSTRACT

An innovative method was investigated to aid in the elimination of *Staphylococcus aureus* (*S. aureus*) intramammary infections (IMI) from dairy herds. A stochastic model explore the economic benefit of three-day or eight-day treatment of subclinical IMI in all *S. aureus* infected cows or in only those with a somatic cell count (SCC) exceeding 200,000 cells/ml. An epidemiological model was developed to run parallel to the economic model that would predict the *S. aureus* IMI likely to persist, develop new infections and clinical mastitis.

In the economic model a first algorithm was used to consider the low prevalence (LP) scenario and made use of *S. aureus* prevalence information provided by retrospective analysis of microbiological and cytological results in South Africa (2008-2012). The data used considered *Staphylococcus aureus* prevalence from $[1.495; 1.595]_{95\%}$ to $[6.72; 6.95]_{95\%}$ for SCC≤200,000 and SCC>200,000 cells/ml respectively. A second algorithm considered the

high prevalence (HP) scenario to evaluate a simulated situation with a 5 to 25% prevalence. Scenarios of low or high transmission ratio (TR) were included in the model according to the hygiene management on the farm. Probabilities and costs were calculated over 255 days. The economic models predicted average cost indices for low *S. aureus* IMI and low TR to vary from -3,179 ZAR (South African Rands) when subclinical cases with SCC higher than 200,000 cell/ml were treated for eight days, to -3,663 ZAR when all subclinical *S. aureus* IMI were treated for three days. With a HP and high TR of *S. aureus* the average cost indices changed from -18,042 ZAR when none to -5,433 ZAR per 255 days when all *S. aureus* IMI were treated for eight days.

The epidemiological model in this study predicted substantial benefit of treatment mainly in high TR scenarios. New IMI decreased up to77% in the three-day and up to 91% in the eight-day treatment scenarios. In the HP scenarios, persistent IMI were reduced by 94%. The number of clinical cases predicted with no treatment for subclinical infections was higher than the total number of clinical and subclinical cases in scenarios where cows were treated three or eight days.

Initial prudent treatment of subclinical IMI resulted in less overall treatments and less new, persistent and clinical cases. Combined results of economic and epidemiological models indicated that the option that cost the least did not always have the best epidemiological outcome. Models may assist in optimising and balancing decisions relating to financial and IMI.

Key words: mastitis treatment, subclinical *Staphylococcus aureus*, stochastic economic model

1. INTRODUCTION

Bovine mastitis is the single most important disease of dairy cows and imposes economic burdens worldwide on dairy farms in first world countries (Schepers and Dijkhuizen, 1991, Halasa et al. 2007). Despite decades of intensive research and management strategies, bovine mastitis still remains an immense challenge. While the occurrence of clinical mastitis has been reported to have decreased there has been almost no reduction in the prevalence of subclinical mastitis (Pyörälä 2002, Guimarães et al. 2017). Economic pressure drives modern dairy farmers to exert continuous efforts to optimize profitability. Many however appear to be motivated by losses from clinical mastitis and failure to obtain milk price premiums and are often unaware of the presence of subclinical mastitis and of the vast losses caused by it (Huijps et al. 2007). Decisions are often based on their limited perception of the economic losses and not on the actual loses (Vaarst et al. 2002, Huijps et al. 2007). Only once farmers can be convinced of the actual losses caused by subclinical mastitis can they be motivated to invest in pro-active udder health management (Valeeva et al. 2007).

The epidemiology of *S. aureus* IMI depends on specific bacterial characteristics as well as on the susceptibility of the cow to this organism (Piccinini et al. 1999). Irregular shedding patterns of many *S. aureus* strains complicate the diagnosis (Sears et al. 1990), control and management of these infections, while *S. aureus* strains differ in their ability to resist phagocytosis (Piccinini et al. 1999). This bacteria is known to lead often to chronic udder infection, extensive parenchyma damage and lower lifetime expectancy.

Modern dairy farms in South Africa have been characterized by amalgamation of smaller herds into herds of 800 or more lactating cows (Lactodata 2013). As the risk of new infections increase due to movement of cows between herds, the demand for effective and pro-active udder health management grows. This increased demand for pro-active management requires ways to detect and eliminate mastitis at an early stage when animals in the herd are infected. Accurate information such as prevalence of intra-mammary infection (IMI) and identification of individual cows that are infected with contagious udder pathogens are valuable in the quest to eliminate in particular *Staphylococcus aureus* (*S. aureus*) and *Streptococcus agalactiae* (*Str. agalactiae*) IMI from dairy herds (Petzer et al. 2016). Nevertheless, the identification of subclinical IMI in individual cows or quarters can be costly especially in large herds, and many farmers often have to rely on somatic cell count (SCC) as the only test to indicate possible IMI. Somatic cell count has been used for many decades as an important tool to indicate udder health status (NMC Guidelines 2001, 2015) and is closely correlated with economic losses (Raubertas and Shook 1982, Halasa et al. 2007, Guimarães et al. 2017). Cows with high somatic cell counts have been estimated to produce less milk (8% for primiparous cows) and up to 8% less milk solids per lactation for every increase of 250 000 cells/ml milk in the range between 100 000 and 600 000 cells/ml (Raubertas and Shook 1982, Halasa et al. 2007). Losses from cows with subclinical mastitis and high SCC have been estimated at 1.8 kg/day for primiparous cows, and at 2.5 kg/day in older cows (Bar et al. 2007). Guimarães et al. (2017) found a reduction of 32.3% due to subclinical and 18.2% due to clinical mastitis.

Reviews of past calculations of the economic losses resulting from mastitis have been shown to differ meaningfully (Schepers and Dijkhuizen, 1991; Halasa et al. 2007) but there is agreement that mastitis is responsible for major economic losses. Different costs, losses and benefits have been taken into account in these calculations and economic losses have been shown to differ between farms, farming systems, regions and countries. Losses are higher under intensive farming conditions compared to extensive farming (Schepers and Dijkhuizen, 1991, Halasa et al. 2007). Mastitis losses and costs can be direct, indirect and invisible. Invisible losses are those not noticed by the farmer. Direct costs may include treatment costs (drugs used and time of the labourer or veterinarian), losses due to discarded milk (during treatment and the milk withholding period), cow fatalities (market value of the cow and the lost milk production due to an incomplete lactation) and recurring clinical mastitis in a lactation (Petrovski et al. 2006, Bar et al. 2007) as well as lower reproductive performance (Lavon et al. 2011). Indirect or invisible mastitis losses may

include decreased milk yield (current and subsequent lactations), inferior milk quality (composition and hygienic quality, increasing zoonotic risks, lower end-product yields, poorer quality and shorter shelf-life of the product), increased risk of culling (and loss of future income) and loss of milk quality premiums (Hortet and Seegers 1998). Other costs might include the need for continuing farmer education, pre- and post-milking disinfectants, teat sealants, dry-cow treatment, mastitis vaccines and laboratory costs (Petrovski et al. 2006).

This study used stochastic modelling in an innovative way to evaluate the cost of no treatment, compared to three-day or eight-day treatment of subclinical *S. aureus* IMI over a 255 day period when either no cows, all cows or only those cows with SCC exceeding 200 000cells/ml were treated. A duration of 255 days were chosen in this study for this time scale fitted the management plan of farmers that were willing to participate in future research that involve testing the epidemiologic models in practise. Decisions that optimize financial cost and cow health are never easy to make as the causes of mastitis are multifactorial and the benefit of interventions should outweigh the cost and predicted losses. Benefits should however not only be measured in economic terms but should also consider the epidemiological outcome in the herd namely the *S.aureus* status at the end of a selected period.

Staphylococcus aureus is a contagious udder pathogen (Fox and Gay 1993) that is transmitted from cow to cow mainly during milking and it remains a challenge in South African herds. It is known for its chronic destructive nature and for shortening the productive lives of cows that need to be culled prematurely. The epidemiology of IMI caused by *S. aureus* depends on specific bacterial characteristics (Sutra and Poutrel, 1994), the susceptibility and immune status of the cow to the specific organism (Piccinini et al. 1999), cow management and the environmental conditions surrounding the cow. Middleton et al. (2002) asserted that there was not much difference in the severity of parenchyma damage caused by the various *S. aureus* strains. Irregular shedding patterns of *S. aureus* can complicate the diagnosis (Sears et al. 1990), control and management of these infections (Piccinini et al. 1999). The epidemiological elements of interest in this study were to compare the number of new IMI, persistent infections, cure rate and the number of treatments required within the scenarios of variable treatment duration, treatment groups and transmission risks. Further challenges confirming effective diagnosis may include sample type (udder quarter or composite samples), the small volume of milk used for bacterial culturing, and sub-detectable levels of bacteria in samples (Nelson 1991). These uncertainties were not taken into consideration when developing the current models for they are experienced on an ongoing basis in routine milk sampling and microbiological evaluation for all scenario. Conventional intramammary antibiotic therapy used in the treatment of clinical mastitis has been found to be not very successful in eliminating *S. aureus* (Barkema et al. 2006). A meta-analysis by Sol et al. (1997) found that the bacterial cure rate of subclinical *S. aureus* IMI varied greatly. Sol et al. (1997) developed a formula to

calculate the probability of cure based on the following criteria: the age or parity of the infected cow; days in milk (DIM); number of quarters per udder infected with *S. aureus,* the SCC level of infected quarters and quarter position (hind or front). Petzer et al. (2016) included the level of udder parenchyma damage found on udder palpation by an experienced veterinarian into these criteria. Adequate treatment duration was indicated as a prime factor for successful treatment (Sol et al. 1997, Deluyker et al. 2005). Barkema *et al.* (2006) found cure rates to vary from 3 to 74% depending on the product used, duration of treatment and whether treatment was administered during lactation or the dry period. According to Sol et al. (1997) a three-day treatment period for subclinical *S. aureus* IMI cured 35% while Sol et al. (2000) and Deluyker et al., (2005) found a 60% cure rate for *S. aureus* IMI when treatment duration was increased to eight days. The outcome of treatment of subclinical *S.aureus* should be used in combination with improved hygiene and parlour management (Allore et al. 1998).

The concept of treatment of subclinical IMI on a herd basis in order to eliminate *S. aureus* from a herd is not an established practice as it has been in the case of a *Str. agalactiae* outbreaks when "blitz therapy" has often been advised. Prudent treatment of subclinical mastitis however may have both direct and indirect benefits. Direct benefits for the cow involve lower SCC, higher potential milk yield and a reduced probability of contracting clinical mastitis, while healthy animals in the herd will benefit indirectly by a lower risk probability for new IMI (Ott 1999; Ruegg 2000). Swinkels et al. (2005) used a deterministic partial budget model to evaluate the cost/benefit of antibiotic treatment of subclinical *S. aureus* IMI using epidemiological parameters and the estimated losses in milk production. However no indication of the likely *S. aureus* IMI status namely new infections, chronic infections and infections cured during the period or the end point of these treatments has been provided.

The epidemiological model included the expected number of persistent cases and the number of clinical cases assumed to have occurred. This study included both the economic and epidemiological outcomes that would aid in the decision making by veterinarians and farmers. The goal of a dairy producer for his/her herd to eradicate *S. aureus* will have an influence on the choice of a scenario. To our knowledge, so far no cost/benefit predictions have been developed and used in parallel with IMI dynamic outcomes in an attempt to aid decision making.

The objectives of this study were to provide a science-based modeling system to determine cost/benefit when treating or not treating subclinical *S. aureus* IMI in herds; to compare epidemiological outcomes for herds with different initial *S. aureus* prevalence and to simulate the cost/benefit and epidemiology after improved management and intramammary treatment durations.

2. MATERIAL AND METHODS

2.1. Stage 1 - Basis for both the economic- and epidemiological models

Estimations used in the models were reliable data that were observed at the time based on various previous research results as indicated in the text to follow. The economic model used a stochastic approach to incorporate mainly individual variability (production, production losses and prevalence) adapted to the South African context while the epidemiological model used a deterministic approach based on data obtained from the literature.

2.1.1. *Staphylococcus aureus* dynamic

Persistent cases were those *S. aureus* IMI that remained infected for longer than 30 days (Swinkels et al. 2005). According to Lam (1996) and Zadoks et al. (2002) an existing subclinical *S. aureus* IMI (taken as an old case) could either persist in 78% (76% to 80%) of IMI, develop into clinical mastitis in 19% (17% to 21%) of cases or according to Deluyker et al. (2005) or cure spontaneously in 3% (3% to 6%) of cases. Zadoks et al. (2002) and Lam et al. (1996) found that less new infections compared to old existing infections of *S. aureus* IMI developed into clinical mastitis 17% (11.5% to 22.8%). They observed that 21% (18.1% to 23.9%) of the new cases were perceived to have cured spontaneously (Zadoks et al. 2002) and an estimated 62% (59.1% to 64.9%) of the new *S. aureus* IMI persisted (Swinkels et al. 2005) (Figure 1).

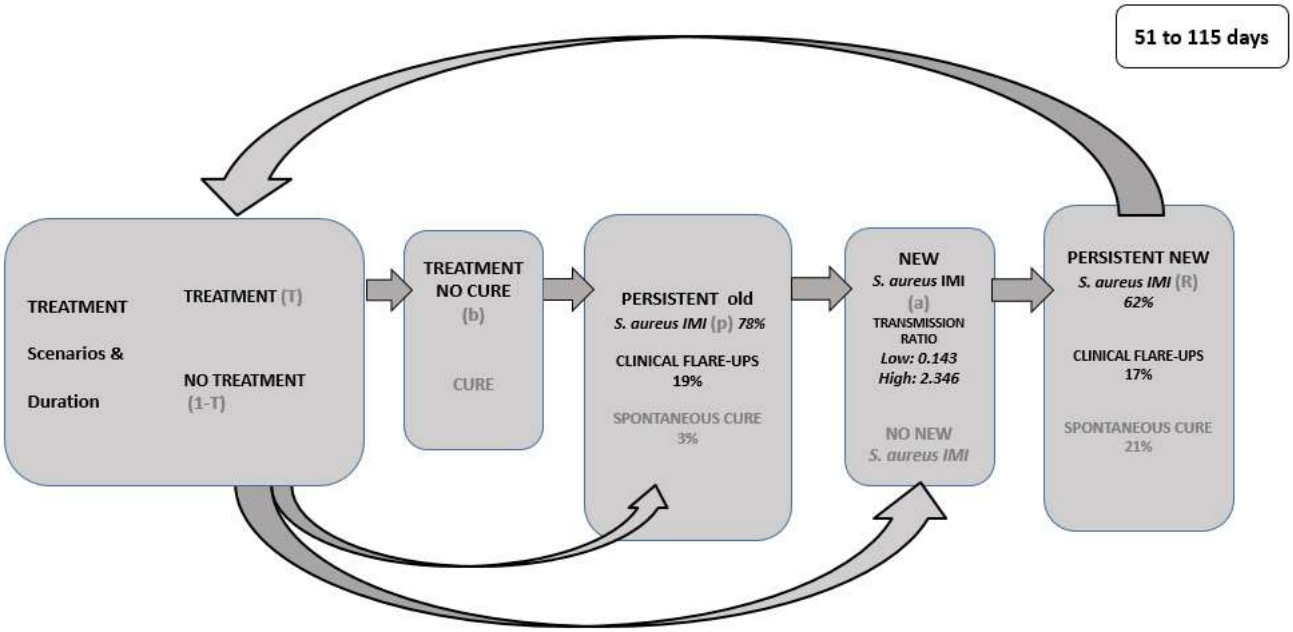

Figure 1. Flow diagram used in the modulation of the models.

2.1.2. Transmission parameter for new *S. aureus* IMI

Cows with *S. aureus* IMI may expose other cows in their herds to *S. aureus.* The magnitude of the risk of new infections for fellow herd mates could be expressed as a transmission parameter indicating the average number of new infections that were anticipated to occur from one infected cow (Lam 1996; Zadoks et al. 2002). The transmission parameters used to calculate new *S.aureus* IMI in this study for a herd with a low risk scenario was 0.0028 per day and it was 0.0460 per day in those where management was deficient (Lam et al. 1996, Zadoks et al. 2002, Swinkels et al. 2005).

According to Zadoks et al. (2002) and Swinkels et al. (2005) in herds under field conditions with strict measurements to identify *S. aureus* and where treatment of IMI was undertaken, the duration of persistent *S. aureus* cases was on average 51 days. Herds with low risk (well managed) used post teat dip, backwash of clusters and hand disinfection of milkers as standard operating procedures whereas herds with high transmission risk did not perform these procedures. Models based their calculations on 51 day persistent infection of *S. aureus* in low risk (well managed) herds and 115 days in high risk (poorly managed) (Zadoks et al. 2002, Swinkels et al. 2005) (Figure 1). The transmission ratio was calculated for the treatment scenario by multiplying the daily transmission parameter with the estimated duration of a persistent *S. aureus* IMI. In case of the low and high risk scenarios transmission ratio of 0.1428 (0.0028 x 51 days) and 5.29 (0.046 x 115 days) were used respectively (Figure 2). As we considered a duration of 255 days we needed to consider 5 time steps in the low risk scenario and 2.2 in the high risk scenario.

2.1.3. Herd prevalence of SCC

According to South African historical data on quarter milk (2008-2011) (Petzer et al. 2017a) a SCC distribution of mean 45.1986% and standard deviation of 0.0765% was present.

2.1.4. Herd prevalence of *S. aureus* IMI

The retrospective analysis of microbiological and cytological results from South African dairy herds (Petzer et al. 2017b) tested in quarter milk from 2008-2012 were used in the current study models. The prevalence of *S. aureus* IMI in samples with SCC ≤ 200,000 and >200,000 cells/ml were separated and used as normal distribution to integrate the variability of the herds. This somatic cell count level was chosen based on the National Mastitis Council Guidelines (NMC 2001) whom define that subclinical mastitis is an infected quarter with a SCC equal to or above 200,000 cells/ml in the absence of clinical changes. *S. aureus* prevalences of $[1.495\%; 1.595\%]_{90\%}$ and $[6.72\%; 6.95\%]_{90\%}$ respectively were used in the models as low prevalences and the simulated situation of a high prevalence of $[4.50\%; 5.50\%]_{90\%}$ to $[24\%; 26\%]_{90\%}$ respectively (Figure 2) were explored.

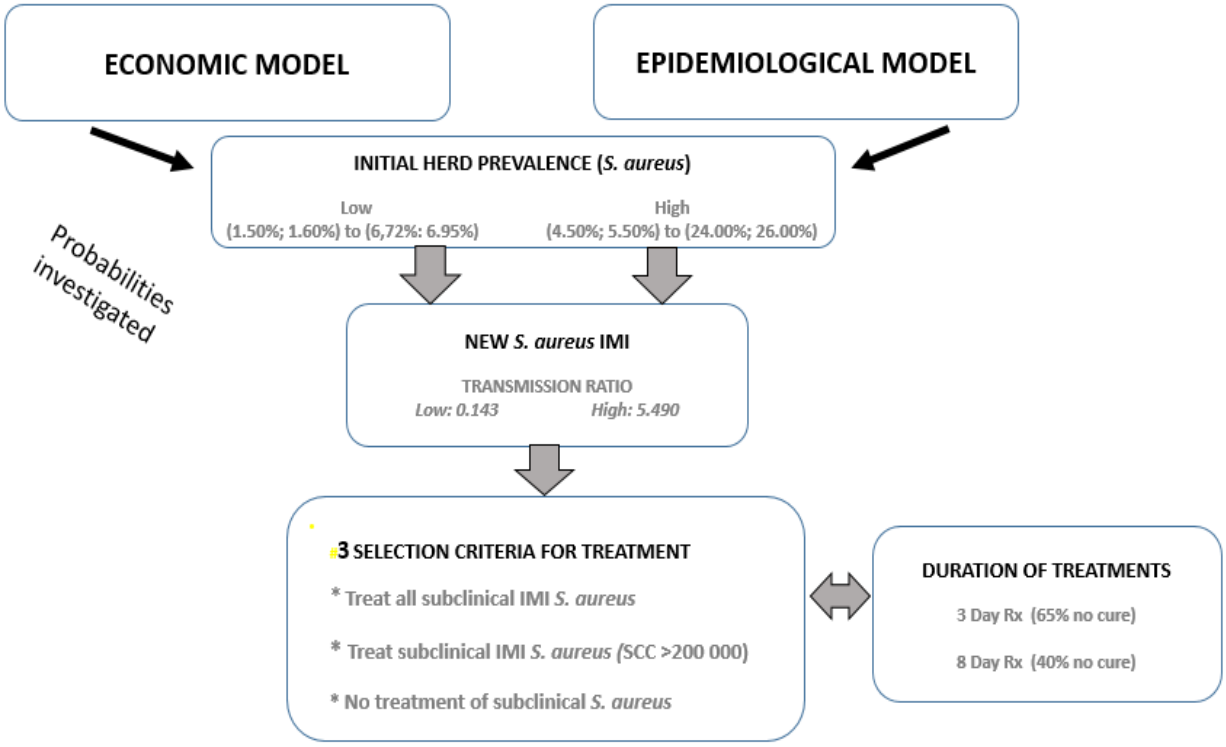

Figure 2. Flow chart of treatment scenarios of subclinical IMI S. aureus that were used in both the economic and epidemiological models.

2.1.5. Different treatment scenarios used in models

The models explored three treatment regimens: a) no subclinical *S. aureus* IMI were treated; b) all subclinical *S. aureus* IMI were treated; c) only subclinical *S. aureus* IMI with a SCC in excess of 200,000 cells/ml in udder quarters were treated.

The final number of clinical flare-ups, new IMI and number of subclinical *S. aureus* IMI that required treatment during the study period were compared between scenarios with different risk and prevalence of *S. aureus* IMI. In order to obtain accurate total number of treatments required per scenario, the number of clinical cases anticipated and the number of subclinical treatments that were projected were added.

2.2. Stage 2: Model development

2.2.1. Epidemiological models (Determining *S. aureus* infection status during the study period and at the end point of measurement)

The model used the *S. aureus* dynamics indicated in Figure 1. Epidemiological models were developed to calculate the persistent *S. aureus* IMI, number of clinical flare-ups, number of subclinical *S. aureus* IMI treated during the three scenarios and number of new *S. aureus* IMI

derived from those subclinical cases based on a herd of 100 lactating cows. Developing formulations are advantages for the variables, such as the test duration, could be change under field conditions and new predictions would be immediately available. Clinical mastitis cases were assumed to be treated or culled including those in scenario A where no subclinical mastitis cases were treated. Models in this study did not consider the remission of clinical *S. aureus* cases to subclinical cases, new infections or persistent infections in order to avoid too much complexity in a first attempt.

2.2.2. Epidemiological model to predict persistent *S. aureus* IMI

The *S. aureus* IMI cases that still persisted at the end point of the simulation in the model were calculated as arithmetic progression using the following model. The calculation of the number of persistent cases of *S. aureus* IMI after a 255 days period was developed as an arithmetic progression incorporating the initial number of *S. aureus* IMI and recalculating the new number of persistent cases at each step based on the model of the *S. aureus* dynamics indicated in Figure 1.

$$U_n = U_0 (1-T)\, paR\, (aR\, (1-T+Tbp))^{n-1} + U_0 TbpaR\, [aR\, (1-T+Tbp)]^{n-1} \qquad \text{formula (a)}$$

Where:

U_n = number of persistent cases of *S. aureus* IMI at end of the period

U_0 = Initial number of *S. aureus* IMI prevalence at the start of the simulation

T = percentage of the *S. aureus* IMI in the herd that was treated

p = percentage of *S. aureus* IMI that was persistent form old cases

R = percentage of persistent *S. aureus* IMI form new IMI

a = percentage of new IMI with *S. aureus* (RR)

b = percentage of *S. aureus* IMI that was not cured

n = number of repetitions of the 51 day or 115 day cycle

(See supplementary material for more detail)

2.2.3. Epidemiological model indicating probable clinical flare-ups

The number of clinical flare-ups anticipated from subclinical cases of *S. aureus* (Sn), compared in the three treatment scenarios, were also calculated using arithmetic progression as follow:

$$Sn = \sum_{i=1}^{n} Cl_i$$

Cl_1 = Clinical Flare-up + New clinical cases after the first cycle

$= U_o(1\text{-}T)\,C_0 + U_0\,Tb\,C_0 + U_0(1\text{-}T)\,pa\,C_n + U_0\,TbpaC_n$

$S_n = A + \frac{ßY\left(1-Y^{n-1}\right)}{1-Y}$

with

$A = Cl_1$

$ß = U_0\,paR\,(1\text{-}T+Tb)\,(TbC_0 + aC_n - aC_nT)$

$Y = aR\,(1\text{-}T+Tbp)$

Where

U_0 = Initial number of *S. aureus* IMI prevalence at the start of the simulation

T = percentage of *S. aureus* IMI in the herd that was treated

p = percentage of *S. aureus* IMI that persisted from old IMI

R = percentage of persistent *S. aureus* IMI from new IMI

a = percentage of new IMI with *S. aureus*

b = percentage of *S. aureus* IMI that was not cured

n = number of repetitions of the 51 day cycle

C_n= % of clinical onset

Cl_1 = number of clinical cases after 1 cycle

Cl_i = number of clinical cases after i cycles

C_o = % of clinical flare-up

(See Supplementary Information 3 for detailed calculations)

2.2.4. Epidemiological model indicating new intramammary infections and numbers of treatment of subclinical cases

In a similar fashion that the number of clinical cases were determined the number of anticipated new *S. aureus* IMI ($\sum_{i=1}^{n} N_i$) and the numbers of subclinical IMI that were treated during the 255 day period ($\sum_{i=1}^{n} X_i$) were calculated.

$$\sum_{i=1}^{n} N_i = \frac{N_1\,(1-Y^n)}{(1-Y)}$$

With

$N_1 = U_0\,pa\,(1\text{-}T\text{+}Tbp)$

and

$$\sum_{i=1}^{n} X_i = \left(U_0 + \frac{U_0 paR\,(1-T-Tb)(1-Y^{n-1})}{(1-Y)}\right) T$$

(See supplementary material for more detail)

2.3. Economic Models

The economic model was based on biological parameters relating to *S. aureus* infection dynamics and transmission ratios derived from previous studies (Lam et al. 1996, Lam et al. 1997, Zadoks et al. 2002, Deluyker et al. 2005,) and from the cost/benefit studies of antibiotic treatment of subclinical S. aureus mastitis (Swinkels et al. 2005).

Two scenario trees incorporating transmission cycles were built to model low risk and high risk scenarios. Each tree presented a certain number of potential outcomes that resulted from a succession of potential events. The succession of a treatment, its potential results, and the evolution of these results, the potential new infection and their potential evolutions represented a step. A cost index was calculated resulting in the addition of all the probable outcomes of the tree. Each outcome has a weighted cost which resulted from the addition of the product of each probability and cost for each transmission cycle (step). In the low risk scenario, the scenario tree had 36 steps with probability and cost associated to each of them resulting in 100 potential outcomes. In the high risk scenario, the scenario tree had 18 steps resulting in 46 potential outcomes. A stochastic approach was used in order to predict partial financial cost in dairy herds with different initial *S. aureus* prevalence using direct and indirect costs (Table 1). The model simulated and compared cost of treatments for 3 days, 8 days or no treatment of subclinical IMI with *S. aureus*.

Table 1. Economic parameters used in developing the cost/benefit indices.

Economical parameter	Cost / losses (ZAR)	References
General information		
Milkings /day	2	
Production (Litres/day)	General distribution Minimum = 0; Maximum = 50; 6 different levels of production (15; 20; 25; 30; 35; 50) with their associated probability (32%; 37.5%; 19.5%; 7%; 2%; 2%)	Lactodata, 2013
Milk price (ZAR)/litre	Triangular distribution Minimum = 3.6; Most Likely = 4.1; Maximum = 4.6	National data of South Africa
Production (ZAR/day)	Production * Milk Price	National data of South Africa
Cow price (ZAR)	Uniform distribution Minimum = 5000; Maximum = 10000	National data of South Africa
Duration for lactation (days) chosen for this study	255	
SCC >200,000cells/ml	Normal distribution Mean = 0.451986 SD = 7.65×10^{-4}	Petzer *et al.* 2017b
High Prevalence scenario: *S. aureus* positive if SCC >200,000cells/ml	Normal distribution: $[0.24 - 0.26]_{90\%}$	Data created for this scenario
High Prevalence scenario: *S. aureus* positive if SCC ≤200,000cells/ml	Normal distribution: $[0.045 - 0.055]_{90\%}$	Data created for this scenario
Low Prevalence scenario: *S. aureus* positive if SCC >200,000cells/ml	Normal distribution: $[0.0672 - 0.0695]_{90\%}$	Milk Laboratory data (2008 – 2012)
Low Prevalence scenario: *S. aureus* positive if SCC ≤200,000cells/ml	Normal distribution: $[0.01495 - 0.01595]_{90\%}$	Milk Laboratory data (2008 – 2012)
Costs (ZAR)		
3 days treatment (remedies)	-102	Intramammary syringes (Ampicillin + Cloxacilllin)
8 day treatment (remedies)	-272	Intramammary syringes (Ampicillin + Cloxacilllin)
Laboratory analysis	Somatic Cell Count = -5; Microbiology = -20	Milk Laboratory, Faculty of Veterinary Science Onderste-poort, South Africa
Teat dip /cow/milking	t1 = -0.32	Cost as calculated by Ecolab based on their products

Disinfectant used for backflush /cow/milking	t2 = -0.12			Cost as calculated by Ecolab based on their products
Disinfecting hands /cow	t3 = -0.05			Cost as calculated by Ecolab based on their products
Post milking teat disinfection / lactation	(t1+t2+t3)*2*255 = -249.9			
Losses				
Production loss (during and after treatment)	12 days (if 8 days treatment) 5 days (if 3 days treatment)			Results based on Delvotest (DSM) as guidelines to calculate additional days
Production loss SCC >200,000 cells/ml (L/day)	PL1= Triangular distribution Minimum = -25%*Prod; Most Likely value = -13%*Prod; Maximum = -2.5%*Prod			Giesecke *et al.* 1994
Production loss SCC >200,000 cells/ml (ZAR/day)	PL1*Milk price			
Production loss SCC ≤200,000cells/ml (L/day)	PL2 = Triangular distribution Minimum = -7.5%*Prod; Most Likely = -2.5%*Prod; Maximum = 0			Giesecke *et al.* 1994
Production loss SCC ≤200,000cells/ml (ZAR/day)	PL2*Milk price			
Clinical mastitis				
Level of clinical mastitis	Mild	Moderate	Severe	Adapted from Hollard *et al*. 2015,
Loss L/year (due to clinical mastitis)	-300	-400	-1000	
Loss L/day (Yearly loss/Duration lactation)	L1 = -1.176470588	L2 = -1.568627451	L3 = -3.921568627	
Production loss/day	Triangular distribution Minimum = L1; Most Likely = L1; Maximum = 0	Triangular distribution Minimum = L2; Most Likely = L2; Maximum = 0	Triangular distribution Minimum = L3; Most Likely = L3; Maximum = 0	

Probability of clinical mastitis	PC1=Uniform distribution Minimum = 0.6 Maximum = 1	PC2=Uniform distribution Minimum = 0 Maximum = 0.3	PC3=Uniform distribution Minimum = 0 Maximum = 0.1	Adapted from Bar *et al*. 2007
Probability to be culled	PD1=Uniform distribution Minimum = 0 Maximum = 0.1	PD2=Uniform distribution Minimum = 0 Maximum = 0.25	PD3=Uniform distribution Minimum = 0.2; Maximum = 0.95	Adapted from Bar *et al*. 2007
Production loss clinical (ZAR/day)	(L1*PC1+L2*PC2+L3*PC3)*Milk price			
Probability culled if clinical (PD)	IF (PC1*PD1+PC2*PD2+PC3*PD3) ≥1 then PC=1 IF NOT PC= PC1*PD1+PC2*PD2+PC3*PD3			

The probable severity of mastitis (change in milk appearance, clinical symptoms present in the udder and / or systemic signs) were incorporated in this model. The cost of the teat treatment was integer in the model for the low risk scenario but was removed for the high risk scenario. The model calculated cost derived from one cow for the 255 days study period. For simplification purposes we took into account the variation in the milk production along the lactation period (milk curve) using a general distribution (not considering different distributions for the different steps).

2.4. Statistical analysis – Economic model environment and software

The economic model was run for 10,000 times using a Latin Hypercube simulation using the software package @Risk (@Risk version 5.5.0 Professional edition, 2009,© Palisade Corporation, 31 Decker Road, Newfield, NY) add-in for Microsoft Excel (©Microsoft Office Professional Edition, 2010).

The results of the simulation were used to determine the mean, the 2.5^{th} percentile and the 97.5^{th} percentile of the resulting distribution of the cost index. A sensitivity analysis was run using rank order correlation coefficient, in order to avoid any assumption about the relationship between inputs and outputs (Vose 2000). This analysis provided coefficients that illustrate the impact of the variability or uncertainty of the inputs on the uncertainty of the output (the cost index). They illustrate also the direction of this impact, either increasing or decreasing the output. The inputs with the highest correlation coefficients (> [0.08]) (Figures 3a and 3b) were used for the sensitivity analysis of the model. This analysis were run for all scenarios.

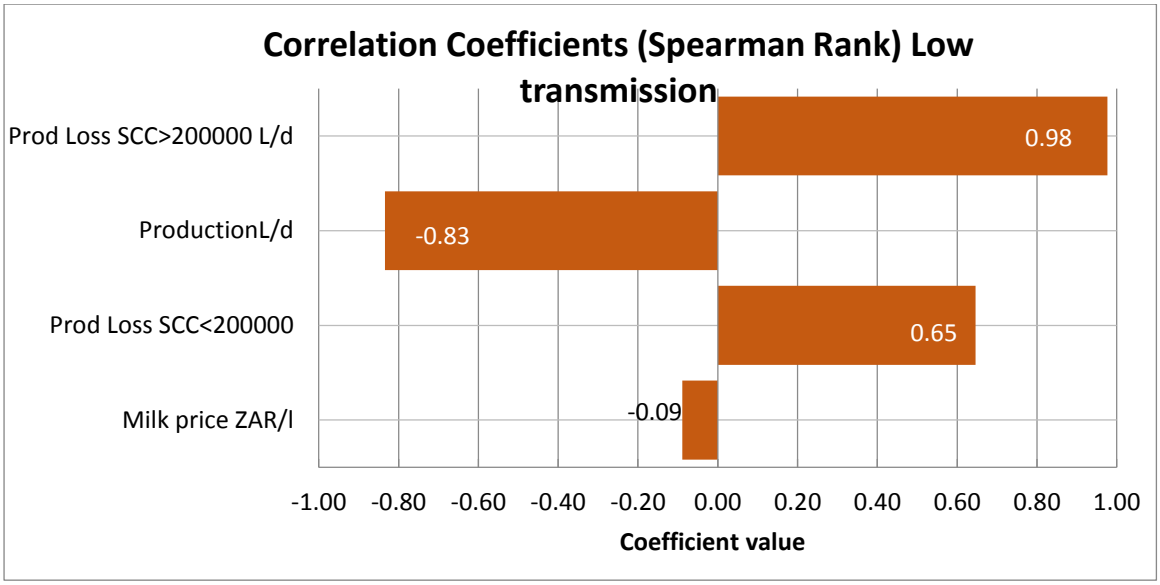

Figure 3a. Correlation coefficients (Spearman Rank) of low transmission ratio scenario.

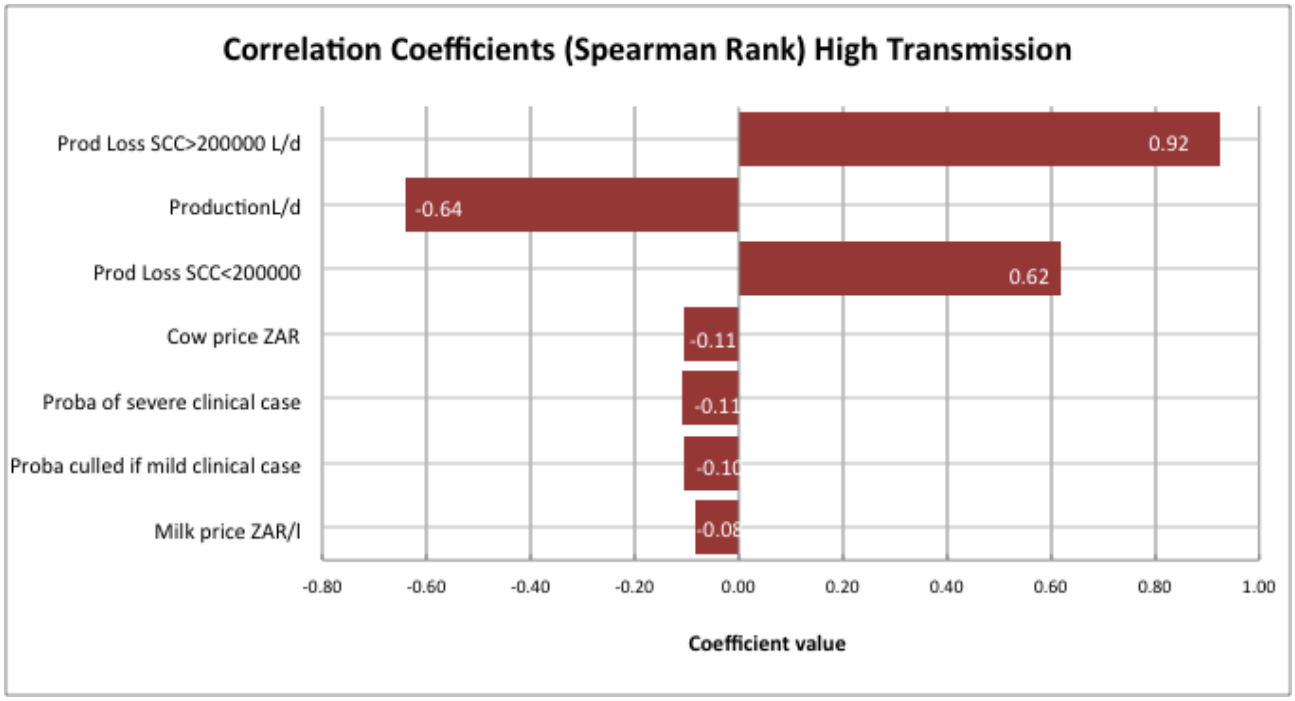

Figure 3b. Correlation coefficients (Spearman Rank) of high transmission ratio scenario.

3. RESULTS

3.1. Economic models

Estimated parameters from the economic model investigated the scenario of *S. aureus* IMI with a low transmission ratio, also referred to in this study as low risk, regardless of the initial infection prevalence, indicated that treating only *S. aureus* IMI with SCC >200,000

cells/ml for eight days cost the least while treating all IMI for eight days cost the second least (Tables 2 and 3). In the case of high transmission ratio, eight-day treatment of all IMI was indicated as the least costly option, while not treating at all cost the most (Tables 2 and 3).

Table 2. Results of the economic model comparing cost outcome (cost index) of the three treatment scenarios in herds with a low initial *S. aureus* intramammary infection (IMI) prevalence, (6.83% if SCC > 200 000 1.54% if SCC ≤ 200 000 cells/ml), with high or low transmission ratio.

Risk level for new	**Treatment duration of subclinical *S. aureus* IMI**		**Cost derived from 1 cow per lactation (ZAR)**		
***S. aureus* IMI**	**SCC >200 000 cells/ml**	**SCC ≤200 000 cells/ml**	**Mean**	**5th Percentile**	**95th Percentile**
Low transmission ratio	None	None	-3239	-6910	-1138
Low transmission ratio	3 days	None	-3590	-7443	-1264
Low transmission ratio	3 days	3 days	-3663	-7836	-1309
Low transmission ratio	8 days	None	-3179	-6536	-1186
Low transmission ratio	8 days	8 days	-3236	-6592	-1217
High transmission ratio	None	None	-6122	-12274	-2345
High transmission ratio	3 days	None	-4030	-8171	-1457
High transmission ratio	3 days	3 days	-3681	-7782	-1236
High transmission ratio	8 days	None	-3095	-6381	-1098
High transmission ratio	8 days	8 days	-2616	-5735	-764

Table 3. Results of the economic model comparing cost outcome (cost index) of the three treatment scenarios in herds with a high initial *S. aureus* intramammary infection (IMI) prevalence, (25% if SCC > 200 000 and 5% if SCC ≤200 000 cells/ml), with high or low transmission ratio.

Transmission cycle (step) duration)	***Risk level for new*** **S. aureus *IMI***	***Treatment duration of subclinical*** ***S. aureus* IMI**		***Cost derived from 1 cow per 255 days (ZAR)***		
		SCC >200,000 cells/ml	***SCC ≤200,000 cells/ml***	**Mean**	**2.5th Percentile**	**97.5th Percentile**
51 days	Low transmission ratio	None	None	-6387	-14063	-2025
51 days	Low transmission ratio	3 days	None	-7681	-16461	-2518
51 days	Low transmission ratio	3 days	3 days	-7915	-16642	-2600
51 days	Low transmission ratio	8 days	None	-6176	-12911	-2178
51 days	Low transmission ratio	8 days	8 days	-6357	-12963	-2232
115 days	High transmission ratio	None	None	-18042	-35161	-7426
115 days	High transmission ratio	3 days	None	-10402	-20216	-4215
115 days	High transmission ratio	3 days	3 days	-9259	-18650	-3506
115 days	High transmission ratio	8 days	None	-6982	-13539	-2857
115 days	High transmission ratio	8 days	8 days	-5433	-11043	-1932

The number of persistent *S.aureus* IMI were calculated per 51 day cycle in order to observe whether the different treatment options would shorten the duration on *S. aureus* infections in the herd (Table 4).

The number of clinical cases treated in the scenario where no subclinical cases were treated exceeded the number of treatments in all other scenario (Table 5). This meant that by initially treating the subclinical *S. aureus* IMI, less treatments were needed overall to achieve a better epidemiological outcome over the same period.

Combining results of the economic and epidemiological models allowed for a more informed decision, taking both the end point epidemiological status of the herd at the end of the measure and cost involved to achieve this end point. Only results of the two best economic options (Table 2 and Table 3) were selected (Table 6).

Table 4. Results of the three different treatment scenarios summarised to indicate the persistent *S. aureus* IMI per cycles and those still present after a period of 255 days in each case comparing scenarios of low and high initial *S. aureus* prevalence and transmission risks. (Calculation was rounded off to the nearest integer).

Prevalence and Risk Scenario		Duration (Days)	No treatment of subclinical *S. aureus* IMI	Treat all subclinical *S. aureus* IMI		Treat subclinical *S. aureus* with SCC >200 000	
	Treatment periods		0 Days	3 Days	8 Days	3 Days	8 Days
Low Prevalence	Low Risk	51	0	0	0	0	0
		102	0	0	0	0	0
		153	0	0	0	0	0
		204	0	0	0	0	0
		255	0	0	0	0	0
		Total cases	0	0	0	0	0
	High Risk	115	3	2	1	2	1
		230	4	1	1	2	1
		255	4	1	0	2	1
		Total cases	11	4	3	5	3
High Prevalence	Low Risk	51	1	1	0	1	1
		102	0	0	0	0	0
		153	0	0	0	0	0
		204	0	0	0	0	0
		255	0	0	0	0	0
		Total cases	1	1	0	1	1
	High Risk	115	17	11	7	12	9
		230	25	8	3	11	6
		255	27	8	3	11	5
		Total cases	69	27	13	34	20

Table 5. A comparison of expected outcomes of treatment scenarios of clinical flare-ups, new intramammary infections and number of subclinical treatments during the 255 day period in scenario with both low and high initial *S. aureus* prevalence and transmission ratio.

		Assumed scenario	No treatment of subclinical *S. aureus* IMI	Treatment of all subclinical *S. aureus* IMI		Treatment of subclinical *S. aureus* IMI with SCC >200 000	
		Duration of treatment	**0 Days**	**3 Days**	**8 Days**	**3 Days**	**8 Days**
Low Prevalence	**Low Risk**	Clinical flare-ups	1	1	0	1	0
		Total new IMI	1	0	0	1	1
		Subclinical *S. aureus* treated	0	4	4	3	3
	High Risk	Clinical flare-ups	14	2	1	3	2
		Total new IMI	47	11	4	14	5
		Subclinical *S. aureus* treated	0	12	7	9	6

		Assumed scenario	No treatment of subclinical *S. aureus* IMI	Treatment of all subclinical *S. aureus* IMI		Treatment of subclinical *S. aureus* IMI with SCC >200 000	
		Duration of treatment	**0 Days**	**3 Days**	**8 Days**	**3 Days**	**8 Days**
High Prevalence	**Low Risk**	Clinical flare-ups	3	2	1	2	2
		Total new IMI	4	1	1	1	1
		Subclinical *S. aureus* treated	0	16	15	13	12
	High Risk	Clinical flare-ups	53	7	3	11	6
		Total new IMI	180	42	16	53	20
		Subclinical *S. aureus* treated	0	45	27	36	21

Table 6. Combining economic and epidemiological outcomes of treatment scenarios with different *S.aureus* prevalence and transmission risk to determine option providing the best financial option for the optimal epidemiological outcome of *S. aureus* based on the farmers set goal.

Prevalence and Risk scenario			Cost index		*S. aureus* dynamics: during or at the end of 255 days				
			Lowest and 2nd lowest financial outcomes		Persistent (at the end)	Number new (during)	Clinical flare-ups (during)	Number S/C Rx (during)	Total treatments (S/C & clinical) (at the end)
Low Prevalence	Low Risk	1st	Treated 8days (>200 000cells/ml)	-3179	0	1	0	3	3
		2nd	Treated 8days (all subclinical)	-3236	0	0	0	4	4
	High Risk	1st	Treated 8days (all subclinical)	-2616	0	4	1	7	8
		2nd	Treated 8days (>200 000cells/ml)	-3095	0	5	2	6	8
High Prevalence	Low Risk	1st	Treated 8days (>200 000cells/ml)	-6176	0	1	2	12	14
		2nd	Treated 8days (all subclinical)	-6357	0	1	1	15	16
	High Risk	1st	Treated 8days (all subclinical)	-5433	0	16	3	27	30
		2nd	Treated 8days (>200 000)	-6982	2	20	6	21	27

3.2. Sensitivity analysis

The results were consistent when calculating the mean of each correlation coefficient and their standard deviation according to all the different scenarios. The three most important

coefficients were the same, regardless of their transmission ratios. They were related in decreasing order of importance (considering their absolute values) to the daily production loss when the SCC was >200,000 cells/ml, the daily production and the daily production loss when the SCC was <200,000 cells/ml. These three prominent coefficients were the most important in terms of their impact on variation of the cost index (range of the cost index confidence interval). This is to be related to their own variations, [-85%; +81%], [-100%; +172%] and [-126%; +100%] for daily production loss when the SCC was >200,000 cells/ml, daily production and daily production loss when the SCC was <200,000 cells/ml respectively (Tables 2 and 3). The influence of these inputs on the cost index were in inverse direction for production losses whatever the SCC is compared to milk production. The lower the milk production, the higher was the cost index. This meant that the less important the cost of the IMI and its treatment was due to the negative figures for the cost index. In the inverse direction, the lower the value for the production loss (given in negative value) the lower was the cost index (negative value also). This means that the higher the production loss was, the more costly it was in terms of economic impact of the IMI and its treatment. The rest of the coefficients were less prominent and for them important variations were observed between scenarios in high and low transmission ratios. The fourth most important correlation coefficient (milk price in case of low transmission scenario and cow price in case of high transmission scenario) showed more important variations then the three first ones, as its standard deviations varied from 12% to 41% of the means respectively compared to the three most important coefficients which standard deviations varied only between 0.66% and 6.73% from the means. Another input that impacted the variation of the cost index was the milk price, regardless of the transmission ratio, with a correlation coefficient of -0.09 and -0.08 for the low and the high transmission ratio respectively. For the low transmission ratio no other input impacted consistently with correlation coefficient higher than 0.08 in absolute value. In case of the high transmission ratio scenario, other inputs had some impact on the variation of the cost index. These inputs were the cow price, the probability of severe clinical cases and the probability of culling the cow in case of mild clinical cases with correlation coefficients of -0.11, -0.11 and -0.10 respectively.

4. DISCUSSION

The development of our economic and epidemiological models has assisted us to gain understanding concerning the effect of complex interactions between *S. aureus* IMI and management. Our findings corroborate that pro-active treatment of subclinical *S. aureus* IMI resulted in less total treatments over 255 days compared to only treating clinical S. aureus mastitis cases. Another contribution of the current study was the combined results of financial and epidemiological outcomes of various treatment selections over 255 days. According to Halasa et al. (2007) factors affecting the performance of dairy cows that are internal to a farm are more likely to motivate producers to change them than external factors over which they do not have much control. Producers may be motivated to upgrade

parlour hygiene more readily once they have insight into monetary and epidemiological advantage of such efforts.

4.1. Economic model

The option with the lowest cost in the low transmission risk scenario, regardless of the initial herd prevalence of *S. aureus,* was to treat IMI with SCC exceeding 200 000 cells/ml for 8 days, while the worst financial option was to treat all of these cases for three days. No treatment of subclinical cases was only marginally more expensive than treating all IMI for eight days.

Considering scenarios with a high transmission risk and low prevalence (LP) of *S. aureus* IMI, the option with the lowest cost was to treat all *S. aureus* IMI for 8 days and the highest cost was not to treat at all. The lowest cost predicted in the high transmission scenario was lower than the lowest cost predicted in the low transmission scenario. This could be explained by the cost involved to achieve a lower transmission in a herd that included that of teatdip, disinfecting of hands and backflushing of clusters used as preventative measures were built in to the models.

The most aggressive treatment scenario (treating all IMI for eight days) was also the best economic option in the HP scenario (Tables 2 and 3). In the HP scenario, when no subclinical IMI were treated, the gain in the cost index in the low transmission risk scenario was 49.3% (-3239 ZAR and -6387 ZAR) and in the high transmission risk scenario 66.1% (-6122 ZAR and -18042 ZAR) higher than in the LP scenario.

The impact of the production losses on the cost index is easily understandable, as they are involved in the calculation of the cost index at each step of the treatment cycles. For each subclinical case remaining after treatment, the production losses for the remaining time of lactation as for each new infection evolving in subclinical cases, was taken into account. The important individual variability of these losses, [-85%; +81] and [-126%; +100%] when SCC was >200 000 cells/ml and when SCC was <200 000 cells/ml respectively, is therefore responsible for the important variation of the cost index. It would be possible to obtain a more precise cost index in case of a known farm where intra-variability would be lower than the one given for a whole population (Giesecke et al. 1994). Concerning the milk production it is logical that it impacted meaningfully on the cost index as it is used in the calculation of the loss of revenue, in form of remaining production, in case of culling of clinical cases (evolution after inefficient treatment, or status of new infected animal), as well as the loss of production during the treatment and the milk withdrawal period. The incomes from the culling was also incorporated in the calculation of the cost index but not the replacement. The important variability of the milk production [-100%; +172%] is based on the variation of the production during the lactation that were modelled using 6 different levels of production associated with a certain level of probability. The individual variability is also incorporated in this modelling. This variability is therefore responsible for an important part

of the variation of the cost index. At the herd level, when taking the lactation period and production level of his herd into account, it would allow to reduce the variability of this parameter and therefore the variation of the cost index. The impact of the milk price on the cost index is explained by its combination with the milk production and the milk losses when calculating the cost index. Nevertheless its low variability [+/-12%] reflected in its lower impact in the variation of the cost index. In case of the high transmission ratio scenarios other inputs were also involved in the variation of the cost index. They are related to the culling of cows in case of clinical mastitis. Indeed when the transmission ratio is higher, the resulting probability of having cows culled is higher. This explains the more important impact of the variability of the cow price, of the probability of having severe clinical cases and the uncertainty of the probability to cull cows in case of mild clinical cases.

4.2. Epidemiological model

An important epidemiological finding of the present study was that treatment of subclinical *S. aureus* IMI effected the transmission risk of infections in all high risk scenarios. In both the low and high prevalence groups persisted cases at the end of the 255 days were more than 50% less compared to the untreated group. Persistent cases were reduced from 11 to 3, 4 and 5 in low prevalence and from 69 to numbers varying from 13 to 34 depending on the treatment in case of the high prevalence. This study highlighted that preventative treatment was only an additional tool in the control of staphylococcal infections in dairy cows. Elimination of infection from a herd would only be possible when no new infection from sources other than shedding herd mates might occur. In reality, infections from other sources are however likely (Barlow et al. 2013). Variation in a TR of *S. aureus* IMI has been observed before and was related to known infection risk factors (Lam et al. 1996, 1997).

4.2.1. Persistent *S. aureus* cases

The number of persistent *S. aureus* IMI can provide insight concerning the potential udder parenchyma damage that can be expected, with a consequent lower lifetime production and reduction of longevity of infected animals. The epidemiological outcome per cycle (the 51 days that *S. aureus* persist) can determine the and whether *S. aureus* IMI could be eliminated prior to 255 days test period when applying some of the treatment options. According to Halloran et al. (1997) a reduction in duration of *S. aureus* infections will also aid to reduce exposure (risk) of other cows.

All persistent IMI were eliminated by day 51 in the low transmission risk scenario (in LP) and by day 102 in the case of HP of IMI (Table 4). Treatments aimed at eliminating persistent infections in the low TR scenario therefore did not seem justifiable. *Staphylococcus aureus* persisted in all high transmission risk scenario till the test end point (day 255) although the numbers varied between scenarios. When subclinical IMI were left untreated less IMI persisted cases remained in the initial LP (11 cases) compared with the HP (69 cases) (Table

4). Less IMI were present at day 255 in the high transmission risk and HP scenario when treated for 8 days (13 and 20) then those treated for 3 days (27 and 34) (Table 4).

4.2.2. New IMI and flare-ups of clinical mastitis

The key advantage of treating subclinical *S. aureus* IMI was apparent in the marked reduction of new IMI, particularly in the high transmission risk scenarios. In LP, new infections were reduced from 47 when no treatment was carried out to only four cases after the most aggressive treatment. Similarly new infections dropped from 180 (untreated) to 16 cases (aggressive treatment) in the HP scenario. These findings resulted in substantial reductions of up to 77% after the three-day treatment and as high as 91% after the eight-day treatment scenario (Table 5). According to Zadoks et al. (2002) udder quarters that were previously infected with *S. aureus* and that recovered from these infections were more likely to be re-infected than those that were never infected with *S. aureus*, indicating the lack of acquired immunity (Zadoks et al. 2002).

Only small differences in the number of new IMI were found between groups that were treated and those not treated in the low TR herds. When all subclinical IMI were treated for three or eight days in LP situations, new IMI did not occur until the end of the study. This indicated that *S. aureus* would be eradicated from herds in these scenarios (Table 5). Prevalence of *S. aureus* IMI did not seem to be an important initiator of new IMI in this study nor in a study done by Zadoks et al. (2001). New IMI infections were more closely associated with the transmission risk, which represents management in this study. New infections also depended on the duration, severity and shedding patterns of the *S. aureus* IMI (Schukken et al. 2014). In this study severity was addressed by incorporating culling option in the case of clinical mastitis. New cases were less likely to persist than older IMI and shedding patterns was incorporated in the low and high transmission risk and the duration of persistant infection that varied in these risk categories, 51 and 115 days respectively.

Even though treatment and dynamics of clinical mastitis did not form part of the current study, the numbers of clinical mastitis cases anticipated in the treatment scenarios were noted because all cows with clinical mastitis must be treated for ethical and practical reasons. Prudent used of antimicrobial products are of utmost importance to help maintain antimicrobial sensitivity, or the development of antimicrobial resistance, and to prevent antimicrobial residues in milk (Davies and Davies, 2010). In the low transmission risk scenario, aggressive treatment options failed to prevent clinical mastitis regardless of the initial herd prevalence, while some success was predicted in high transmission risk herds (Table 5). When calculating the treatments per scenario, the predicted subclinical IMI treated and the incidence of clinical mastitis were added together to provide a more accurate reflection of a field situation (Table 6). Only in high transmission risk herds did the number of clinical flare-ups of *S. aureus* mastitis differ clearly between treatment groups. The model predicted that in the HP high transmission risk scenario, where no subclinical *S.*

aureus IMI were treated during the 255 study days, 53 cows would contract clinical mastitis and require treatment. Although almost the same number (52 cases in total) of treatments were predicted for the three-day treat- all scenario in a similar herd, only 7 of these 52 cases were likely to be clinical mastitis. For the eight day treatment scenario markedly less cows were expected to receive treatment: 30 (3 clinical) when all, and 27 (6 clinical) when those with only high SCC were treated. Although at first it may seem to be a contradiction to treat subclinical infections when aiming at lower the overall number of treatments, prudent treatment of subclinical IMI did lead to less treatments overall. Not only were less cows treated, but the potential risk of udder parenchyma damage decreased, because substantially less clinical mastitis cases were expected (Kitchen et al. 1980). Treatment shortened the duration of infections with consequently less shedding of *S. aureus,* which in turn was expected to lower the risk of new IMI (Schukken et al. 2014) as the study period progressed.

4.2.3. Combining cost/benefit and epidemiological outcomes

The two scenarios that were financially the most favourable for each transmission risk and prevalence scenario were compared after 255 days to the epidemiological outcomes. The economic model indicated that the eight-day treatment was effective in the control of this chronic udder disease. Cost index in high prevalence herds were 44.3% to 49.4% higher for infected animals than in low prevalence herd (Table 6).

With a low prevalence the number of new IMI, clinic cases and treatments required were almost similar and both options were viable. Nevertheless in the case of the low transmission risk the lower cost option required one less treatment and 57 ZAR less per animal (5 700 ZAR less per 100 animals) but one more new case occurred. If the farmer was aiming to clear the herd of *S. aureus* having less new infection may be worth the low extra cost of the second option. With a high transmission risk and low prevalence the same number of total treatments were required but the higher cost option had one more clinical case. Clinical cases may lead to more udder parenchyma damage and shedding (duration and quantity) (Kitchen et al. 1980) and treatment in this cases would be a reactive action compared to treatment of subclinical cases where the action is pro-active. The cost difference in this case was 47 900 ZAR per 100 cows and the lowest cost option would therefore be preferred.

In the high prevalence low risk scenario for 18 100 ZAR less in a 100 cows herd, there is a tossup between having to treat 2 less cases and having 1 less clinical case. In the worst cases scenario (high prevalence and high risk) although the lowest cost scenario have 3 more treatments it have half of the clinical mastitis cases and a fifth less new cases plus the advantage of saving 154 900 ZAR and therefore will be preferred by the farmers.

Management remains the key factor in order to control and eradicate *S. aureus* IMI from a herd. Prudent treatment of subclinical IMI was however indicated as another tool to aid in this process mainly in HP herds.

5. CONCLUSION

Models are an innovative approach for simulating epidemiological and economic situations and these outcomes were combined as an aid in better decision making.

Costs in high prevalence scenario were almost double than that seen in the LP scenario while cost in the case of high transmission risk scenario were always higher. The most viable financial and epidemiological options in all cases were the 8 day treatments duration.

In the LP scenario, the total number of treatments of the viable financial situation were similar. In the HP scenario and high TR, although there were three more treatments required in the most viable financial option, less of these cases were expected to result in clinical mastitis.

Treatment had overall effect on lowering the number of persistant cases but even a more important effect in preventing new IMI and clinical flare-ups in all high risk scenario. Treating all *S. aureus* positive cases, regardless of their SCC, is mainly justified in the high risk scenario.

Global concern and new legislation regarding antimicrobial use due to resistance in bacteria is currently evident. Prudent use of antibiotic treatment does not mean withholding treatment. Opting to treat subclinical *S. aureus* may therefore seem at first a contradictory decision but this study indicated that treatment of subclinical IMI with *S. aureus* in high risk herd scenario resulted in less total treatments (clinical and sub-clinical) during the 255 days. Not only were fewer cows treated, but when treating subclinical cases the probability of bacterial cure was likely to be better than in clinical mastitis cases, and less parenchyma damage could be expected. The best case scenario nevertheless remains to create a low TR environment within a *S. aureus* positive herd where the focus will be on excellent management rather than treatment.

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ADDENDUM 1 (Persistant *S. aureus* cases)

Calculation of persistant *S. aureus* cases:

$U_1 = U_0 (1-T)\ paR + U_0 TbpaR$

$U_2 = U_1 (1-T)\ aR + U_1 TbpaR$

$U_{n+1} = U_n (1-T)\ aR + U_n TbpaR$

$U_{n+1}/U_n = (1-T)\ aR + TbpaR$

$U_{n+1}/U_n = aR - aRT + TbpaR$

$U_{n+1}/U_n = aR\ (1 - T + Tbp)$

So we can write

$U_n = U_1\ [aR\ (1-T+Tbp)]^{n-1}$

$U_n = U_0 (1-T)\ paR\ [aR\ (1-T+Tbp)]^{n-1} + U_0\ Tb\ paR\ [aR\ (1-T+Tbp)]^{n-1}$

$U_n = U_0\ paR\ [aR\ (1-T+Tbp)]^{n-1}\ (1-T+Tb)$ formula(a)

Where

U_n = number of persistent cases of *S. aureus* IMI at end of the period

U_0 = initial number of *S. aureus* IMI prevalence at the start of the simulation

T = percentage of the *S. aureus* IMI in the herd that was treated (all)

p = percentage *S. aureus* IMI that persistent form old cases (78%)

R = percentage persistent *S. aureus* IMI form new IMI (62%)

a = percentage of new IMI with *S. aureus* (RR)

b = percentage *S. aureus* IMI that was not cure (65% or 40%)

n = number of cycles

ADDENDUM 2 (Clinical cases of *S. aureus*)

Calculation of clinical cases (Cl) of S. aureus IMI.

Cl_1 = Clinical Flare-up + New clinical cases

= $U_o\,(1\text{-}T)\,C_0 + U_0\,Tb\,C_0 + U_0\,(1\text{-}T)\,pa\,C_n + U_0\,TbpaC_n$

Cl_2 = $U_1\,Tb\,C_0 + U_1\,(1\text{-}T)\,aC_n = U_1\,(TbC_0 + aC_n - aC_nT)$

Cl_3 = $U_2\,(Tb\,C_0 + aC_n - aC_nT)$

Cl_{n+1} = $U_n\,(Tb\,C_0 + aC_n - aC_nT)$

Then we replace U_n using formula (a)

Cl_{n+1} = $U_0\,paR\,(1\text{-}T + Tb)\,[aR\,(1\text{-}T+Tbp)]^{n-1}\,(TbC_0 + aC_n - aC_nT)$ formula (b)

We want to calculate the total number of clinical cases and therefor need to add all the clinical cases that developed during the different cycles:

$$Sn = \sum_{i=1}^{n} Cl_i$$

In order to simplify the equation we preferred a change in the variables using A, ß, and Y as new variables as follows:

with A = Cl_1

ß = $U_0\,paR\,(1\text{-}T+Tb)\,(TbC_0 + aC_n - aC_nT)$

Y = $aR\,(1\text{-}T+Tbp)$

So $Cl_{n+1} = ßY^{n-1}$

$S_n = A + ß Y + ß Y^2 + ... + ß Y^{n-2}$

$YS_n = AY + ß Y^2 + ... + ß Y^{n-1}$

$S_n - YS_n = A + ß Y - AY - ß Y^{n-1}$

$S_n = \frac{A(1-Y)+ßY(1-Y^{n-1})}{1-Y}$

$S_n = A + \frac{ßY(1-Y^{n-1})}{1-Y}$ formula (c)

Where

C_n = clinical onset

C_o = % clinical flare-up

Cl_i = number of clinical cases developed during the i^{th} cycle

U_0 = initial number of *S. aureus* IMI prevalence at the start of the simulation

T = percentage of the *S. aureus* IMI in the herd that was treated (all)

p = percentage *S. aureus* IMI that persistent form old cases (78%)

R = percentage persistent *S. aureus* IMI form new IMI (62%)

a = percentage of new IMI with *S. aureus* (RR)

b = percentage *S. aureus* IMI that was not cure (65% or 40%)

n = number cycles

ADDENDUM 3 (Numbers treated)

Calculation of the total number of treatments (X) of subclinical S. aureus IMI.

$X_1 = U_0T$

$X_2 = U_1T$

$X_2 = U_0\,Tbp\,aRT + U_0\,(1\text{-}T)\,p\,aRT$

$X_3 = U_2\,T$

$X_n = U_{n-1}\,T$

$\sum_{i=1}^{n} X_i = U_0\,T + U_1\,T + \ldots\ldots\ldots + U_{n-1}\,T$

$= (U_0 + \ldots\ldots\ldots\ldots + U_{n-1})\,T$

We first need to calculate

$U_0 + \ldots\ldots\ldots\ldots + U_{n-1}$

$U_{n-1} = U_0\,paR\,(1\text{-}T + Tb)\,(aR\,(1\text{-}T + Tbp))^{n-2}$ formula (a)

$U_1 = U_0\,paR\,(1\text{-}T + Tb)\,(aR\,(1\text{-}T + Tbp))^{0}$

$U_2 = U_0\,paR\,(1\text{-}T + Tb)\,(aR\,(1\text{-}T + Tbp))$

$U_3 = U_0\,paR\,(1\text{-}T + Tb)\,(aR\,(1\text{-}T + Tbp))^{2}$

and we had

$Y = aR\,(1\text{-}T+Tb)$

$U_{n-1} = U_1\,Y^{n-2}$

$$\sum_{i=1}^{n} X_i = \left(U_0 + \frac{U_1(1 - Y^{n-1})}{(1 - Y)}\right) T$$

ADDENDUM 4

Results at SCC threshold of 150 000 cells/ml in composite milk samples.

Table 7. Comparing the number of persistent *S. aureus* cases in herd with initial low and high *S. aureus* herd intramammary infections and risk of infections, for the various treatment scenario over 3 and 8 days, in quarter and composite milk samples at SCC thresholds of 200 000 cells/ml and composite samples at 150 000 cells/ml.

RX Scenario Persistent at end of 255d							
Treatment duration	Risk level	Herd STA prevalence	No treatment	Treat all subclinical STA	Quarter samples: Treat subclinical STA with SCC >200 000	Composite samples: Treat subclinical STA with SCC >150 000	Composite samples: Treat subclinical STA with SCC >200 000
3 days	Low	High	0.0541	0.0000	0.0000	0.0000	0.0000
		Low	0.0142	0.0000	0.0000	0.0000	0.0000
	High	High	26.8534	0.2129	6.897	6.1892	14.2918
3 days		Low	7.0356	0.6321	1.8070	2.7812	3.7444
8 days	Low	High	0.0541	0.0000	0.0000	0.0000	0.0000
		Low	0.0142	0.0000	0.0000	0.0000	0.0000
	High	High	26.8534	0.1310	1.3313	1.2116	5.0523
8 days		Low	7.0356	0.03433	0.3488	0.7813	1.3237

Table 8. Comparing the number of new, clinical and total number of subclinical *S. aureus* cases that required treatment in herds with initial low and high *S. aureus* herd intramammary infections and risk of infections, for the various treatment scenario over 3 and 8 days, in quarter and composite milk samples at SCC thresholds of 200 000 cells/ml and composite samples at 150 000 cells/ml.

A) Low Risk / High prevalence	No Rx	Rx all		Rx quarters SCC > 200 000 cell/ml		Rx composite SCC > 150 000 cell/ml		Rx composite SCC > 200 000 cell/ml	
	115d	3 Days	8 Days	3 Days	8 Days	3 Days	8 Day	3 Days	8 Days
Persist End	0.012	0	0	0	0	0	0	0	0
Tot New	4.374	1.137	0.687	1.272	0.914	1.340	1.029	1.382	1.100
Tot Clinical	4.106	2.133	1.289	2.386	1.715	2.513	1.929	2.593	2.064
Tot Rx	-	15.705	15.426	12.484	12.262	10.865	10.672	9.849	9.674
B) High Risk / High prevalence	No Rx	Rx all		Rx quarters SCC > 200 000 cell/ml		Rx composite SCC > 150 000 cell/ml		Rx composite SCC > 200 000 cell/ml	
	115d	3 Days	8 Days	3 Days	8 Days	3 Days	8 Day	3 Days	8 Days
Persist End	54.596	3.271	0.289	8.383	6.012	10.953	8.889	12.565	10.695
Tot New	308.288	53.132	19.715	79.046	52.482	92.071	68.953	100.246	79.290
Tot Clinical	81.202	14.549	5.399	21.645	14.371	25.212	18.882	27.451	21.712
Tot Rx	-	44.671	26.934	35.509	21.410	30.903	18.633	28.013	16.891
C) Low Risk / Low Prevalence	No Rx	Rx all		Rx quarters SCC > 200 000 cell/ml		Rx composite SCC > 150 000 cell/ml		Rx composite SCC > 200 000 cell/ml	
	115d	3 Days	8 Days	3 Days	8 Days	3 Days	8 Day	3 Days	8 Days
Persist End	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Tot New	1.146	0.298	0.180	0.605	0.511	0.351	0.270	0.362	0.288
Tot Clinical	1.076	0.559	0.338	1.135	0.959	0.659	0.506	0.679	0.541
Tot Rx	-	4.115	4.042	3.271	3.213	2.847	2.796	2.580	2.535
D) High Risk / Low Prevalence	No Rx	Rx all		Rx quarters SCC > 200 000 cell/ml		Rx composite SCC > 150 000 cell/ml		Rx composite SCC > 200 000 cell/ml	
	115d	3 Days	8 Days	3 Days	8 Days	3 Days	8 Day	3 Days	8 Days
Persist End	14.304	0.857	0.076	2.053	1.563	2.870	2.329	3.436	2.815
Tot New	80.772	13.921	5.165	18.374	12.884	24.123	18.066	28.600	21.641
Tot Clinical	21.275	3.812	1.414	5.031	3.528	6.606	4.947	7.832	5.926
Tot Rx	-	11.704	7.057	7.339	4.425	8.0966	4.882	9.303	5.610