ALINE CALLEGARI SILVA

Epidemiology and economics of mastitis in smallholders dairy farms in southeast of Brazil.

São Paulo 2020

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Epidemiology and economics of mastitis in smallholders dairy farms in southeast of Brazil.

Thesis submitted to the Postgraduate Program in Experimental Epidemiology Applied to Zoonoses of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Doctor's degree in Sciences.

Department:

Preventive Veterinary Medicine and Animal Health

Area:

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Advisor:

Prof. Ph.D., Nilson Roberti Benites

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Faculdade de Medicina Veterinária e Zootecnia

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CERTIFICADO

Certificamos que a proposta intitulada "A INCIDÊNCIA DA MASTITE E SEU IMPACTO ECONÔMICO EM DIFERENTES SISTEMAS DE PRODUÇÃO BOVINA LEITEIRA.", protocolada sob o CEUA nº 9901091216 (ID 004945), sob a responsabilidade de Nilson Roberti Benites e equipe; Aline Callegari Silva - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovada pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 23/05/2018.

We certify that the proposal "THE INCIDENCE OF MASTITIS AND ITS ECONOMIC IMPACT ON DIFFERENT DAIRY BOVINE PRODUCTION SYSTEMS.", utilizing 400 Bovines (400 females), protocol number CEUA 9901091216 (ID 004945), under the responsibility of Nilson Roberti Benites and team; Aline Callegari Silva - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was approved by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 05/23/2018.

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Área: Medicina Veterinária Preventiva E Saúde Animal

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Local do experimento: Propriedades rurais na região de Campinas.

São Paulo, 18 de fevereiro de 2019

400

N١

Anneliese Tcalar

Profa. Dra. Anneliese de Souza Traldi Presidente da Comissão de Ética no Uso de Animais Faculdade de Medicina Veterinária e Zootecnia da Universidade Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

idade: 2 a 5 anos

Peso:

400 a 600 kg

Roseli da Costa Gomes Secretária

de São Paulo



Comissão de Ética no Uso de Animais Faculdade de Medicina Veterinária e Zootecnia Universidade de São Paulo

> São Paulo, 17 de abril de 2019 CEUA N 9901091216

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Parecer Consubstanciado da Comissão de Ética no Uso de Animais FMVZ (ID 004562)

A Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, no cumprimento das suas atribuições, analisou e APROVOU a Notificação (versão de 25/fevereiro/2019) da proposta acima referenciada.

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hah, film

Prof. Dr. Marcelo Bahia Labruna Coordenador da Comissão de Ética no Uso de Animais Faculdade de Medicina Veterinária e Zootecnia da Universidade Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Camilla Mota Mendes Vice-Coordenador de São Paulo

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Date: ____/___/____

Committee Members

Prof	
Institution:	Decision:
Prof	
	Desision
	_ Decision;
Prof	
Institution:	_ Decision:
Prof	
Institution:	_ Decision:
Prof	
Institution:	_ Decision:

I would like to dedicate this thesis to my family, who supported me during my studies with love and companionship.

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Thank my daughter Pilar Callegari Fillietaz Balcao for the love and smiles everyday, and my husband Lucas Fillietaz Balcao for the love, support and companionship.

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"Somewhere, something incredible is waiting to be discovered". Carl Sagan

RESUMO

SILVA, A. C. **Epidemiologia e economia da mastite em pequenas propriedades leiteiras no sudeste do Brasil.** 2020. 85p. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2020.

Os objetivos específicos deste estudo foram a) analisar os fatores de risco associados à mastite em pequenas propriedades leiteiras do Sudeste do Brasil, b) apresentar os principais métodos econômicos aplicados para analisar o custo da mastite em rebanhos leiteiros, c) analisar o total custo da mastite em pequenas propriedades leiteiras convencionais e em transição para o orgânico. A tese está estruturada em 3 capítulos referentes a 2 estudos e 1 revisão. Nos capítulos 1 e 3, os estudos foram desenvolvidos a partir de dados obtidos de 10 pequenas propriedades leiteiras (7 convencionais e 3 em transição para o orgânico), visitadas 4 vezes, durante o período de maio/2018 a junho/2019. Na primeira visita foi aplicado um questionário que continha informações sobre dados gerais da fazenda e detalhes individuais das vacas. Em todas as visitas, uma combinação de exame clínico do úbere e o teste de Tamis, foi aplicada para diagnosticar mastite clínica (CM) e o Calfornia Mastitis Test para determinar mastite subclínica (SCM). Capítulo 1, uma análise de regressão logística foi aplicada para investigar associações, entre fatores de risco a nível de vaca e de rebanho com a presença de CM e SCM. Do total de 4.567 quartos foram testados, 107 (2,3%) tinham CM, enquanto 1.519 (33,2%) tinham SCM. A nível de vaca, o risco de CM foi maior no meio da lactação (50-150 DIM), enquanto o SCM foi maior no final da lactação (> 150DIM) e menor em vacas primíparas do que multíparas. A nível de rebanho. o uso de tratamento de vaga seca (OR 4,23, IC 95% 1,42-12,62) foi associado ao risco de CM. Orrdenha casos clínicos (OR 0,37, IC 95% 0,24-0,56) e os casos subclínicos por último (OR 0,21, IC 95% 0,09-0,47) e a sala de ordenha limpa (OR 0,27, IC 95% 0,15-0,46) tiveram reduzido risco para SCM, enquanto rebanhos com ração otimizada tiveram maiores riscos para SCM (OR 9,11, IC 95% 2,59-31,9). No capítulo 2, apresentamos uma revisão que identificou os diferentes métodos e modelos matemáticos utilizados na literatura para realizar o cálculo do custo da mastite a fim de fornecer informações para nortear trabalhos futuros. No capítulo 3, o custo total da mastite (falhas e prevenção) foi calculado para fazendas leiteiras convencionais e em transição. As fazendas convencionais apresentaram média de U\$ 434,07 por vaca (U\$ 68,76 - U\$ 931,76) e as fazendas em transição apresentam média de U\$ 580,42 por vaca (U\$ 362,48 - U\$ 1010,83). Os custos médios totais por produção de leite por ano foram de U\$ 0,1018 (U\$ 0,03 - U\$ 0,23) para fazendas convencionais e U\$ 0,1231 (U\$ 0,07 - U\$ 0,18) para as fazendas em transição. Em conclusão, identificamos alguns fatores de risco para mastite em pequenas propriedades, todavia mais pesquisas com uma maior abrangêcia geográfica é requerida para desenvolver um programa de controle da mastite no Brasil. As análises econômicas da mastite demonstraram que seu custo total é influenciado por uma combinação de fatores como o status da saúde do animal, produção de leite e manejo da fazenda.

Palavras-chave: Fatores de risco. Custo da Mastite. Economia da saúde animal. Medidas preventivas. Transição para orgânico.

ABSTRACT

SILVA, A.C. **Epidemiology and economics of mastitis in smallholders dairy farms in southeast of Brazil.** 2020. 85 p. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2020.

The specific objectives of this study were a) analyze the risk factors associated with mastitis in smallholders dairy farms in the Southeast of Brazil, b) present the main economic methods applied to analyze the cost of mastitis in dairy herds, and c) analyze the total cost of mastitis in conventional and in transition to organic smallholders dairy farms. The tese are structured in 3 chapters referent to 2 studies and 1 review. In chapters 1 and 3, the studies were developed from data collected and provided by 10 smallholders dairy farms (7 conventional and 3 in transition to organic), visited 4 times, during the period of May 2018 to June 2019. At the first visit were applied a questionnaire that contained information about general farm data and individual cows details. In all visits, a combination of udder clinical examination and the Tamis (screened mug) test were applied to observe clinical mastitis(CM), and California Mastitis Test to determine subclinical mastitis (SCM). Chapter 1, a logistic regression analysis was applied to investigate associations between cow and herdlevel risk factors with the presence of CM and SCM. The total of 4567 quarters was tested, 107 (2.3%) had CM, while 1519 (33.2%) had SCM. At the cow level, CM risk was highest in mid-lactation (50-150 DIM), while SCM was highest in late lactation (>150DIM) and lower in primiparous than multiparous cows. At the herd level. using drying off treatment (OR 4.23, 95% CI 1.42-12.62) was associated with CM risk. Milking clinical (OR 0.37, 95% CI 0.24-0.56) and subclinical cases last (OR 0.21, 95% CI 0.09-0.47) and clean milk parlor (OR 0.27, 95% CI 0.15-0.46) had decreased odds for SCM, while herds with optimized feed had greater odds for SCM (OR 9.11, 95% CI 2.59-31.9). In chapter 2, we presented a review that identifies the different methods and mathematical models used in the literature to perform the calculation of the cost of mastitis, allowing future reflections on methodological standardization and to provide information to guide future work. In chapter 3, the total cost of mastitis(failure and preventive) was calculated for conventional and transition's smallholders dairy farms. The total cost of mastitis identified was variable in conventional with a mean of U\$ 434.07 per cows (ranging from U\$ 68.76 - U\$931.76) and in organic transition with a mean of U\$ 580.42 per cows (ranging from U\$362.48 – U\$ 1010.83) for the period of 2017 to

2018. The mean total costs per milk production per year were U\$ 0.1018 (U\$ 0.03 - U\$ 0.23) for conventional farms and U\$ 0.1231 (U\$ 0.07 - U\$0.18) for the transition to organic`s farms. In conclusion, we identified some risk factors for mastitis on smallholder farms, but further research on more farms across more areas of Brazil is required to develop targeted control programs for mastitis on smallholder farms. Also, the analyzes resulting from mastitis cost showed that its total cost is influenced by a combination of factors such as the animal's health status, milk production and farm management.

Keywords: Risk factors. Mastitis cost. Animal health economics. Preventive measures. Transition to organic.

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ABBREVIATION LIST

CMT	California mastitis test
СМ	Clinical Mastitis
FAO	Food and agriculture organization
MAPA	Brazilian ministry of agriculture, livestock and food supply
PCR	Polymerase chain reaction
SCM	Subclinical mastitis
UFOAM	International federation of organic agriculture movements

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1 INTRODUCTION

The bovine mastitis is one of the most important diseases of dairy herds in the world (STEVENS et al., 2016). Losses are not only in the animal health, but also economic, milk quality, welfare and in bacteriological resistance due to excessive use of antibiotics (LAVEN, 2009).

Mastitis is characterized by being an inflammation of the mammary gland whose etiology can be traumatic, allergic, metabolic or infectious (RADOSTIS et al., 2007). The intramamary infectious cases can be classified as contagious or environmental mastitis. The contagious mastitis is the infection caused by pathogen from mammary gland affected by mastitis (FOX and GAY, 1993). Environmental mastitis is the one whose source of infection is the environment frequented by cow (KHAN and KHAN, 2006).

The main microorganisms that cause infectious mastitis are *Staphylococcus aureus*, streptococci (*S. agalactiae*, *S. uberis*), coliforms (*Escherichia coli*, *Citrobacter freundii*, *Enterobacter sp.*, *Klebsiella sp.*) (COSTA et al., 1995; BENITES et al., 2002; OLIVEIRA et al., 2015), more recent *Corynebacterium sp* (SOUT et al 2008).

The high occurrence of this disease is related to its multifactorial risk which involve characteristics of the animals, environment and/or management (VLIEGHER et al 2018). Among of the risk factors related to animal level, we can identify age, parity, lactation stage, amount of milk production, breed, previous mastitis, udder below the hock, lack of forage food support and body score (DOHERR et al., 2007; SARKAR et al., 2013; CARDOZO et al., 2015). At herd level, we can identify environmental and management factors and we can enumerate the humidity, temperature, housing systems, herd size, milking practices, residual milk and property management (COENTRÃO et al. 2008; CORDING, et al. 2013).

The longitudinal retrospective study developed in Brazil, analyzed a records from 9,789 dairy cows for 65 months (January 2009 to May 2014) and found that first month of lactation, high somatic cell count, rainy season and history of clinical mastitis cases were factors associated with CM for both primiparous and multiparous cows. (OLIVEIRA et al., 2015).

Other study, evaluate the risk factors for new and chronic subclinical intramammary infections (IMI) with data from 1700 cows in lactation located in 30 dairy herds in southern Brazil and identified that the factors: cows with more than 3 lactations, cows with a mean hyperkeratosis score above 3, cows with the udder below the hock, cows with the very dirty

udders and milking infected animals before health cows, presented significant association with cow developing a new case of subclinical mastitis with increased odds ratio (CARDOZO et al., 2015).

Preventive measures practices are a helpfull way to avoid the ocorrence of mastitis and/or minimizar their serius damage in the cow health. The measures as postmilking teat disinfection, dry-cow therapy, keeping cows standing following milking, the use of milkers' gloves, and back-flushing the milk cluster after milking a cow with (sub) clinical mastitis or milking them last, avoiding overcrowding, have been reported as a benefical measures in the control of this diseases (HALASA, 2007; VLIEGHER, et al., 2018). However need to be well implemented and the profile of farms need to be considered to develop an effective result.

The risk factors associated with the incidence rate of clinical mastitis were analysed in Britsh dairy herds with low bulk milk somatic cell counts, in a cross-sectional survey. The results found that incidence of clinical mastitis increased for some management measures as kept cows standing in a yard after milking (compared to cubicle houding) and always practiced postmilking teat disinfection, but also found a lower incidence of clinical mastitis in measures as gathering yard used before milking was scraped at least twice a day PEELER et al., 2000).

Parallel to these risk factors and responding for market demand, a model of management that has been introduced recently in Brazil, is the organic milk production system, which they follow specific regulations (IFOAM, 2011; FAO, 2015). Differing from a conventional production model, organic milk production systems recommend that animals have access to pasture or outdoor circulation, good health, well-being, coupled with some restrictions such as antibiotics, conventional food and some agricultural inputs (eg pesticides and chemical fertilizers) (BRASIL, 2011).

However, the restrict use of antibiotics in organic dairy herds resulting in one of the greatest challenges in the treatment of mastitis on these systems, but attend the concern from public health, food safety, and regulatory perspectives about the use of antimicrobials in food-producing animals (WHITFIELD, and LAVEN, 2018). The use of antimicrobials in animals poses a potential risk for public health as it contributes to the selection and spread of antimicrobial resistance (LAVEN, 2009; PETROVSKI et al. 2011). Also, contributes to increasing the cost of mastitis in the dairy herd.

The high prevalence and complexity of mastitis results in a disease of economic importance (DE VLIEGNHER ET ALL. 2018). Economically, the mastitis occurrence is associated with direct and indirect losses and expenditures. (PETROVSKI et al. 2011). To assess economic costs of mastitis, directs expenditure and losses have to be quantified and aggregated (PETROVSKI et al. 2011; HOGEEVENT et al 2011). Expenditures represent some economic effects of disease that are manifested as extra inputs into livestock production (such as treatment and prevention of mastitis.). Losses implies a benefit that is taken away as discarded milk and decrease in the milk yield (SEEGERS et al. 2003; PETROVSKI et al. 2011). Many factors composes the costs of mastitis, those most commonly addressed are reduction in milk production, disposal of animals, discarded milk, cost of treatment of clinical cases, increase in labor costs and decrease in milk sales price (JUNIOR et al., 2005; PETROVSKI, 2006; LOPES et al. 2016), costs with food (HALASA et al., 2007) and costs with preventive measures (HUIJPS et al., 2010, LOPES et al.2016), are the main economic losses and expenditure associated with the presence of mastitis in the herd.

The availability of accurate and opportun informations are an important subsidy for correct and effective decision making. Thus, researches have been evaluated the cost of mastitis and the factors of greatest economic impact on the final cost of mastitis (CHA et al., 2011; GUIMARAES et al. 2017). However, most mastitis cost studies only associate costs with loss of production, discarded and treatments (VAN SOEST et al., 2016), preventive measures also result in important expenses to control mastitis (HUIJPS et. al., 2010). Recent studies, have been suggesting the study of a total cost of mastitis, considering a model wih the sum of failure (losses) and preventive cost (HOGEVENT et al., 2011, VAN SOEST et al., 2016). Emerging questions related cost-effectiveness of some preventive measures have been enlightening the importance of knowing more about cost of some measures and their relation with distinct production systems.

In parallel to this facts, restricted studies have been reporting risk factors associated with mastitis and economic analysis of mastitis in Brazil, and none study, in our knowledge, reported the cost of mastitis in farms in conversion to organic, including failure cost (losses) and expenditure (preventive measures).

1.2. OBJECTIVE

1.2.1 General objectives

Evaluate the epidemiological and economics of mastitis in smallholder dairy farms in the Southeast of Brazil.

1.2.2 Specific objectives

a) Analyze the risk factors associated to mastitis in farms in conversion to organic and conventional farms

b) Present the main economic methods applyed to analyses the cost of mastitis in dairy herds.

c) Analyze the total cost (failure plus preventive measures) of mastitis in smallholders dairy farms in conversion to organic and in transition to organic.

CHAPTER 1

Aline. C. Silva^a, Richard. A. Laven^b, Nilson.R. Benites^a Manuscript submitted to Tropical Animal Health and Production. Submitted October, 2020.

2. RISK FACTORS ASSOCIATED WITH MASTITIS IN SMALLHOLDER DAIRY FARMS IN SOUTHEAST BRAZIL

ABSTRACT

A prospective, repeated cross-sectional study was carried out between May 2018 to June 2019 in 10 smallholder dairy farms. Potential risk factors for subclinical (SCM) and clinical mastitis (CM) at the herd and cow level were recorded through interviewing the owner and by observation. A combination of udder clinical examination and the Tamis (screened mug) test were applied to observe CM, and California Mastitis Test to determine SCM. The total of 4567 quarters was tested, 107 (2.3%) had CM, while 1519 (33.2%) had SCM. At the cow level, CM risk was highest in mid-lactation (50-150 DIM), while SCM was highest in late lactation (>150DIM) and lower in primiparous than multiparous cows. At the herd level. using drying off treatment (OR 4.23, 95% CI 1.42-12.62) was associated with CM risk. Milking clinical (OR 0.37, 95% CI 0.24-0.56) and subclinical cases last (OR 0.21, 95% CI 0.09-0.47) and clean milk parlor (OR 0.27, 95% CI 0.15-0.46) had decreased odds for SCM, while herds with optimized feed had greater odds for (OR 9.11, 95% CI 2.59-31.9). No effect of farm system (conventional or converting to organic) on mastitis risk was observed. This study has identified some risk factors for mastitis on smallholder farms, but further research on more farms across more areas of Brazil is required to develop targeted control programs for mastitis on smallholder farms.

KeyWords: Farm management. Mastitis. Milking management. Smallholder dairy farms. Risk factor.

2.1 INTRODUCTION

Although Brazil is the world's fifth largest dairy producer, its dairy industry is principally made up of smallholder dairy farms (FAO, 2010). This has impacts on animal health, especially mastitis, as the structure of the industry limits access to milk quality improvement programs, modern technologies and mastitis diagnosis (Busanello et al., 2017). The rates of both clinical and subclinical mastitis are high in Brazil. Oliveira et al (2015) reporting that ~30% of cattle had at least one case of clinical mastitis per annum, and that, including repeat cases the average number of clinical mastitis cases per lactation was 1.02. In regard to subclinical mastitis, Busanello et al., (2017) reported a prevalence of 46% with 18% of uninfected cows developing subclinical mastitis every month. The high level of mastitis on Brazilian dairy farms results in significant economic loss, reduced animal welfare and milk quality and increased use of antibiotics.

Risk factors for mastitis can be divided into factors related to animals, and factors related to environment and management (Halasa et al, 2007). The key risk factors at the cow-level are age, lactation stage, milk production, breed, body condition score and season (Doherr et al., 2007; Sarkar et al., 2013; Cardozo et al., 2015, Oliveira et al., 2015). Key environmental and management risk factors include humidity, temperature, housing systems, herd size, milking management and management of the environment (Coentrão et al. 2008; Huijps et al 2010, Cording, et al. 2013).

Relatively few studies have reported the risk factors for mastitis on Brazilian dairy farms (e.g. Cardozo et al., 2015, Oliveira et al., 2015; Da Cunha et al., 2016). These studies have generally focused on larger farms. The aim of this study was to provide data on risk factors for mastitis on smallholder dairy farms in São Paulo state in SE Brazil.

2.2 MATERIALS AND METHODS

All animal work was approved by The University of São Paulo ethics committee (Protocol no. 9901091216)

2.2.1 Description of the study area

The farms selected for the study were a convenience sample of dairy farms in the mesoregion of Piracicaba close to the University of São Paulo. Ten farms were selected; seven were conventional dairy farms and three were transitioning to organic status,(a process which takes18 months in Brazil; MAPA 2011). All of the transitioning farms were in the first year of transition.

2.2.2 DATA COLLECTION

Farm visits - Data were collected between May 2018 and June 2019. All 10 farms were first visited in May/June 2018. Nine of the farms were then visited four times over the subsequent 12 months at 3-4 month intervals by the first author. The remaining farm (which was a conventional farm) was visited again in September 2018 but subsequently stopped producing milk so was not visited again.

Questionnaire. At the first visit the farmers were interviewed about potential risk factors for mastitis at the herd and cow level using a prepared questionnaire (supplementary information). Prior to use in the study, the questionnaire had been tested on two farmers, who did not participate in the study. The questionnaire included questions on general farm data (e.g. farm and herd size, and milk production), farm level management (e.g. feeding

practices), and individual cow details (e.g. age and lactation stage). The questionnaire also contained more detailed questions on management measures related to mastitis, such as milking order of cows with confirmed mastitis (subclinical or clinical), teat disinfection pre- and post-milking, and use of gloves.

Milking procedure was then observed on each farm to confirm that the questionnaire answers were correct. During this milking all cows were checked for clinical mastitis, using a combination of udder observation and palpation and the Tamis (screened mug) test to observe changes in milk appearance (Radostis et al, 2007), and for subclinical mastitis using the California Mastitis Test (CMT) (Ramirez et al 2010). For each case of mastitis (subclinical or clinical) the cow and affected quarter(s) were recorded

2.2.3 STATISTICAL ANALYSIS

All statistical analyses were carried out using R version 3.1.1 (Rcore Team, 2014). Two separate analyses were carried out: one with clinical mastitis as the dichotomous outcome variable at the quarter level and one with subclinical mastitis as the dichotomous outcome variable at the quarter level. For both analyses data from quarters with the other type of mastitis were excluded from the model. Initially, for both clinical and subclinical mastitis, univariable multilevel logistic regression models (lme4; Bates et al, 2015) were created for all predictor variables at the cow level, and where there was variability between farms, at the farm level. Farm and cow were included as random effects in these models. Predictors with P-value< 0.25 were then put forward for testing in multivariable models (Dohoo et al., 2003). Potential predictors were assessed for collinearity and not included in the further models when the correlation was >0.6; where there was collinearity the variable with the lower p-value was selected for inclusion in the modeling process.

Three models were created for each outcome variable - cow level model (parity, lactation stage) herd level milking practices model (milking clinical cases last, milking subclinical cases last, use separate clothes, gloves, disinfecting cluster between milk, clean milk parlor) and environment and management model (production system, visit date, protocol treatment by veterinarian, drying off treatment, J5 vaccine, dry cow minerals, homeopathic salt, optimize feed before calving).

Parameters for the multivariable model, were selected (p < 0.05) using backward stepwise approach. To check confounding, the variables removed during the initial process

were added one by one. A variable was considered as a confounder if its removal resulted in changes of the remaining predictors by \geq 20%. Confounders were kept in the model.

2.3 RESULTS

2.3.1 FARM DESCRIPTION

All farms kept their cattle in a semiconfined housing system; one of the farms had no access to grazing, whereas the other nine had access to grazing. Main feeds were silage (mean 26.5 kg/cow/day; range 15-35 kg/cow/day) and concentrate (mean 3.8 kg/cow/day; range 0.2 to 6 kg/cow/day).

A range of breeds were present on all farms, with predominance of Holstein (52%) breed and Gyr x Holstein crossbreds (40%) being the commonest breeds . The remaining cattle were 100% Jersey (8%).

On nine farms, the cows were milked twice daily, while on the other farm the cows were milked once daily only. Mean farm size was 12.6 Ha (range 5 – 24 Ha), mean lactating herd size was 33 cows (range 12 – 46 lactating cows) and average total herd milk production was 13.56kg milk/day/cow (range 8 – 20.5kg milk/day/cow).

2.3.2 Clinical and subclinical mastitis prevalence

Over the period study 1165 cow observations were made for mastitis, with 4567 quarter observations where mastitis status (clinical, subclinical or absent) was recorded. Of the 1165 cow 83 (7.12 %) had clinical mastitis and 704 (60.43%) and of the 4567 quarter observations, 107 (2.3%) had clinical mastitis, while 1519 (33.2%) had subclinical mastitis. 2.3.3 Risk Factor analysis

For clinical mastitis at the individual cow level two risk factors remained in the model: parity and lactation stage. Multiparous cows had increased odds of clinical mastitis compared to primiparous cows odds ratio (OR) 1.9 but the 95% CI ranged from 0.88 to 3.8 (Table 1). Compared to cows with DIM<50, later lactation stages had a higher risk of mastitis (Table 1)

Table 1 - Final multivariable logistic regression model of the association between cow-level risk factors and clinical mastitis at the quarter level in 10 smallholder dairy herds in Piracicaba Mesorregrion, São Paulo, Brazil.

		Clinical mastitis	Subclinical Mastitis
Parity			
	multiparous	Reference	Reference
	primiparous	0.54 (0.26- 1.13)	0.54(0.4-0.7)
Lactation stage			
	<50 DIM	Reference	Reference
	50-150 DIM;	2.62 (1.03- 6.67)	1.62 (1.19-2.18)
	> 150 DIM	1.83 (0.75- 4.48)	2.74(2.05-3.6)

DIM = Days in milk

Both parity and lactation stage were also present in the final model for subclinical mastitis. Compared to primiparous cows multiparous had a higher odds of subclinical mastitis (OR 1.9; 95%CI 1.4 - 2.5) (Table 1). As for clinical mastitis, there was an effect of lactation stage with higher odds of subclinical mastitis (compared to cows with DIM<50) in cows at a later lactation stage (Table 1)

At the herd level, the model for milking management practices and clinical mastitis failed to converge, so no multivariate analysis was possible. Table 2 summaries the explanatory variables included in the final multivariable model of subclinical mastitis and milking management practices. Of these factors, all three (milking clinical or subclinical cases last, and having a clean milking parlour) were associated with reduced odds of subclinical mastitis (Table 2)

Table 2 - Final multivariable logistic regression model of the association between herd level Milking Management Practices risk factors and Subclinical mastitis (positive California Mastitis Test) at the quarter level as reflected by the t in 10 smallholder dairy herds in Piracicaba Mesorregrion, São Paulo, Brazil.

Variables	Category	Odds ratio (95% CI) (ref. category "no' in all cases)	
Milking clinical cases last	Yes	0.37 (0.24-0.56)	
Milking subclinical cases last	Yes	0.21(0.09- 0.47)	
Clean milk parlor	Yes	0.27(0.15- 0.46)	

Table 3 sumarizes the models relating herd level environment and management factors to clinical and subclinical mastitis. For the clinical mastitis model the variables included were drying off treatment, vaccination with a J5 vaccine and visit date. Using drying off treatment

or vaccination were both associated with an increased odds of clinical mastitis, although the confidence intervals were wide especially for the latter. The subclinical mastitis model included vaccination, optimizing feeding and visit date. In contrast to clinical mastitis, vaccination was associated with a reduced risk of subclinical mastitis, although the confidence intervals were wide. Production system (conventional or transitioning to organic) was not included in either model.

Table 3 - Final multivariable logistic regression model of the association between herd level Environmental and Management Practices risk factors and clinical and subclinical mastitis at the quarter level in 10 smallholder dairy herds in Piracicaba Mesorregrion, São Paulo, Brazil

Variable	Category	Odds ratio(95%CI) (ref. category "no' all cases except visit date)		
		Clinical Mastitis	Subclinical Mastitis	
Drying_off_ Treatment	Yes	4.23(1.42-12.62)	-	
Vaccine	Yes	2.44(0.74-7.97)	0.32(0.077 – 1.33)	
Optimize_feed	Yes	-	9.11 (2.59-31.9)	
Visit date	First (June-July)	Reference	Reference	
	Second (September)	1.28 (0.60 – 2.73)	2.07 (1.65 – 2.60)	
	Third (Dezember- January)	2.56 (1.25 – 5.24)	1.82 (1.42 – 2.33)	
	Forth (April- June)	3.81 (1.93 – 7.52)	2.62 (2.04 -3.36)	

2.4. DISCUSSION

This is, as far as the authors are aware, the first study of the risk factors associated with mastitis (clinical or subclinical) that has focused on smallholding dairy farms in Brazil. In addition, many previous studies of risk factors for mastitis in tropical smallholders dairy farms (e.g. Tolosa et al 2013 and Ndahetuye et al 2020) have not been longitudinal studies with multiple measurements on the same farms. Thus, although relatively small, this dataset does provide useful information on the risk factors for mastitis in an understudied part of the Brazilian dairy industry.

The proportion of quarters identified as having clinical mastitis over the period of the study was 2.3%. This is not directly comparable to the figures reported by Oliveira et al (2015) as the data from the current study are at the quarter level not the cow level and a

prevalence rather than lactational incidence, but the figures from this study are consistent with the very high mean lactational incidence (~100%) reported by Oliveira et al (2015). The 2.3% figure is higher than the 1.3% reported in smallholder farms in southern Ethiopia by Abebe et al (2016), and 7.12 % is higher than the 4.8% figure reported (at the cow level) in smallholder farms in Zimbabwe (Katsande et al.,2013).

The proportion of quarters identified as having subclinical mastitis over the period of the study was 33% and at cow level 60.43% . This is not directly comparable to the figure reported by Busanello et al., (2017) of 46% at the cow level, as that was a single point prevalence. However, it is compatible with that figure and could indicate even higher rates of subclinical mastitis in these farms than reported by Buasnello et al (2017) in larger Brazilian dairy farms. For example, the 33% figure reported in this study is very similar to the prevalence of subclinical mastitis at the quarter level of 36% reported by Abebe et al (2016), 33% reported by Mekonnen et al (2017) and 37% reported by Ndahetuye (2020), all of whom reported cow-level prevalences of 62%.

Larger scale studies across more farms in more districts are required to better characterize mastitis risk in smallholding dairy farms in Brazil, but the results of this study suggest that clinical and subclinical mastitis are as much a problem on Brazilian smallholdings as they are on larger dairy farms and may be even worse.

At the cow level, parity and lactation stage were the only risk factors included in the final model for both clinical and subclinical mastitis. The parity (an increased risk of mastitis for multiparous compared to primiparous cows) is a consistent effect found in many studies of risk factors for mastitis (e.g. Ramirez et al., 2014; Cardozo, et al., 2015; Oliveira et al., 2015) including those that have focused on smallholders (e.g. Mekonnen et al (2017). For both clinical and subclinical mastitis, later lactation stages were associated with higher odds of occurrence. This is consistent with many previous studies looking at mastitis in smallholder dairy farms (e.g. Kivaria et al 2007; Tolosa et al 2013; Mekonnen et al 2017; Ndahetuye et al 2020) though not all (e.g. Abebe et al 2016) and in larger farms (e.g. Cardozo et al 2015) . The effect of lactation stage on subclinical mastitis is likely to be related to the accumulation of chronic infections which have not been identified during lactation. In contrast the reason why clinical mastitis is more common in later lactation is less clear, especially as in larger Brazilian dairy herds, clinical mastitis appears to be commoner in early lactation (Oliveira et al 2015).

We were not able to identify the association between herd level milking management practices and clinical mastitis because the model did not converge, but for subclinical mastitis the practices remining in the final model were milking clinical cases last, milking subclinical cases last and having a clean milk parlour. All of these practices have been associated with reduced mastitis in large commercial dairy herds (Hogeveen et al 2011), and on smallholdings (e.g. Kivaria et al 2004; Abebe et al 2016). The number of milking management practices that this study identified as being associated with subclinical mastitis in this study is relatively low, but this may be because the study lacked power and, for many potentially important practices, there was very little variation between farms. Thus, the absence of a management practice in the final model should not be taken to indicate that practice is not a risk factor for subclinical mastitis .

The herd-level environment and management practices which remained in the final model for clinical mastitis were dry cow therapy, use of a J5 vaccine and time of year. The first two practices were both associated with increased risk of clinical mastitis, despite both being intended to reduce clinical mastitis (Laven and Lawrence, 2008; Denis et al, 2009). Details were not collected on either the vaccination or the dry cow regime, but it is likely that the positive association was because farms that used vaccination or dry cow therapy were responding to a clinical mastitis problem so were likely to have a higher prevalence of mastitis than farms which did not use vaccination or dry cow therapy. This paradoxical effect may have been exacerbated by incorrect use of the treatments (e.g. poor timing of vaccination or inadequate hygiene during dry cow therapy) which would have resulted in them having no or limited impact on mastitis. Subsequent studies should look more closely at the use of these therapies by smallholders and identify why and how they are being used.

Clinical mastitis was at its lowest prevalence at the first visit in June/July and highest in the last visit in April/June. In south-eastern Brazil, there are two seasons: the cool dry season (April -September) and the warm rainy season (October – March) (Embrapa 2012). These data thus reflect likely environmental mastitis risk, with a lower risk in the dry period and higher risk in the rainy season. This is consistent with the findings of Oliveira et al (2015) on larger dairy farms. Subclinical mastitis was also apparently affected by season in a similar way. Further investigation of the role of season in mastitis on smallholder farms in Brazil may assist the development targeted control programmes for use at high risk periods of time. In addition to season, vaccination with a J5 vaccine and the optimization of feed were also in the final model relating herd-level environment and management practices to the prevalence of subclinical mastitis. It is unclear why J5 vaccination was related to reduced prevalence of subclinical mastitis as that vaccine targets E. coli which is not an important cause of subclinical mastitis (Denis et al, 2009). Optimization of feed was associated in this dataset with a very large increase in the odds of a quarter having subclinical mastitis (OR 9.1). This may reflect that farmers who optimize feed are generally higher producers which may increase mastitis risk (Lievaart et al 2007). Further information is required on the association between feeding, production level, body condition score and the risk of mastitis (both clinical and subclinical) on smallholder dairy farms in Brazil.

No effect of farm system on mastitis risk was observed on this study. This suggest that smallholder farms which transition to organic farming do not necessarily have to have increased mastitis risk, but further research is required to confirm that this will be the case on most farms.

In conclusion, the risk of mastitis on smallholders dairy farms in Brazil, both clinical and subclinical is very high. This study has identified some risk factors for mastitis on such farms, but further research on more farms across more areas of Brazil is required to develop targeted control programmes for mastitis. This research should be combined with identifying the knowledge gaps of smallholders in regard to mastitis and the barriers to implementing mastitis control on such farms.

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CHAPTER 2
3 ANIMAL HEALTH ECONOMICS: MASTITIS AND ITS ECONOMIC MODELING

ABSTRACT

The economic study of animal health aims to promote health and profitability of farms by directing decision making and optimizing animal health management. Among the most economically important diseases in dairy production systems, mastitis is one of the most costly diseases. The economic study of mastitis provides information that enables the producer to make decisions and implement new measures that empower the productive system. However, the choice of methodology suited to each reality and the development of methodological standardization still a challenge. In this context, this article aims to identify the different methods and mathematical models used in the literature to perform the calculation of the cost and/or economic impact of mastitis. Also, this review will allow future reflections on methodological standardization and will provide information to guide future work.

keywords: Animal Health. Dairy. Economic Methods. Mastitis.

3.1 INTRODUCTION

The economic analysis of animal health is a valuable tool to support animal health management and guide planning at the individual level and national level, resulting in better decisions make (Morris 1999). The importance of economic analysis is growing, being more consolidated in developed countries (Otte and Chilonda 2000). The prevalence of the disease, integrated with market exigence and economic penalties, has been stimulating the economic health analysis in Brazill. However still a lack of studies on this subject in Brazil and in developing countries. (Hogeveen et al 2019). Most of the challenge in economic analysis in developing countries is the availability of information, level of technical knowledge of farmers, habit to apply animal health economic analysis in the routine.

In order to develop economic strategies for animal diseases, different methods have been used (Yalsin et al 1999; Huijps et al 2010; Lopes et al., 2011; Geary et al., 2012). The choice of the appropriate methodology varies for each reality and research objective., being a challenger the methodological standardization especially in countries in which studies of economic analysis of diseases aren't yet consolidated.

Among the livestock diseases of greater economic relevance, mastitis is one of the most expensive diseases in dairy farms, due to the losses (eg. decrease milk production) and expenditure (additional inputs to reduce the level of mastitis) (McInerney, J., 1996), interfering with animal welfare (Heikkilä et al., 2012). Thus, this article aimed to identify the

different methods and mathematical models used in the literature to calculate the cost and/or economic impact of mastitis.

3.2 ECONOMIC ANALYSIS OF ANIMAL HEALTH

Economic studies of animal health aim to promote the health and profitability of farms by directing decision making and optimizing animal health management (Marsh, 1999). Research in this field mainly addresses three interrelated aspects: a) analyzing the economic effects of animal diseases, b) directing the appropriate allocation of resources, c) contributing to determining the profitability of controlling specific diseases, programs and procedures for managing health (Dijkhuizen and Morris, 1997; Rushton, 2009).

The economic analysis of animal health is developed by a diversity of methods. The cost of the disease varies according to the productive system (milk, meat) and the diseases evaluated (Bennett, 1992; Otte and Chilonda, 2000). The assessment of the economic effects generated by the disease individually for animals or farms tends to be more direct compared to wider systems such as regional and national levels, due to a large number of variables to be considered. (Dijkhuizen and Morris, 1997). Thus, the most common of them are partial budget analysis, enterprise budget, decision tree analysis, linear programming and dynamic programming, simulation, cost–benefit analysis, cost–effectiveness analysis. (Dijkhuizen and Morris, 1997; Otte and Chilonda 1999, Shim et al, 2004). The choice of economic methods for a specific analysis will be influenced by several factors (Bennett., 1992), such as the nature of the problem, the complexity of the system involved, and the availability of data (Dijkhuizen e Morris, 1997).

The most economic framework used to analyze production diseases consists of an expanded, but also simple and useful approach. The example of this simple framework, consisted in the total cost disease (C) which evolve the sum of production losses (L) and control expenditure (E) that can include resource to prevent and control diseases as veterinary services and hygiene measures (McInerney, 1996, Rushton et al., 1999).

3.2.1 Main methods of animal health economic analysis

Partial budget analysis, is an appropriate tool to analyses endemic diseases or retrospective analysis of disease outbreaks (Dijkhuizen and Morris, 1997). It is the simplest

economic analysis, which is used to estimate cost/benefits, direct and indirect cost per disease cases in deterministic or stochastic simulation model (Rushton et al., 1999, Swinkels, et al., 2005; Rollin et al., 2015 Ahmedet al., 2020).

Enterprise budget uses gross margin analysis to assess the effects of disease control in the entire livestock company (Morris et al., 1999). However, it is not used when intending to analyses the economic impact of individual disease due to the possibility of the result to be overshadowed by some other factor or disease (Smith 1995; Rushton et al 1999).

Linear programming, this mathematical method allocate scarce resources to competing activities in an optical manner using a linear objective function (Bennet et al., 1992), it is useful in farm planning (Annetts and Audsley et al 2002), can be adapted to assess risks in decision-making, however, it is little used in economic analyzes of animal health (Zepeda et al., 1998; Stott et al, 2003).

Decision analysis models clarify the decision-making process, by prescribing the course of action in alternatives ways, using a structured graphical as decision trees (Dorsthorst et al., 2006). Decision tree evaluates the probability of information and monetary values built in different possible outcomes in a set of sequential decisions (Jarvis and Valdes-Donoso, 2018).

The cost-benefit and cost-effectiveness, are widely used to analyses economic strategies doing support decision-making, mainly in disease prevention or treatment and eradication programs (Gussmann et al., 2016; Kaniyamattam et al., 2020).

Some of these methods make use of a simulation approach which can be performed in different ways. For a further understanding, simulation is an advanced mathematical model method to predict the economic effects of the diseases, using different techniques such as: static (does not take time into a count), dynamic (considers time), deterministic (defined quantity predictions), or stochastic (model the probability of the various outcome, under different conditions) (Nielsen, 2009). Stochastic simulation has been suggested to be the most relevant method, however, the complexity and the need for a large amount of information limit his wide use (Thornley, 2001; Dijkhuizen and Morris, 1997).

In this way, there are different models of economic calculation of animal health, they are often performed in different combinations. Partial budget in deterministic model and simulation (Swinkels, et al 2005; Ahmed, et al., 2020). Cost-benefit in an dynamic and deterministic model (Kaniyamattam, et al 2020). Decision made in static and simulating model (Dorshorst et al. 2006). Monte Carlos Simulation in Dynamic and Stochastic (Liang et al., 2017).

The application of these methodologies and their varied combinations have been used in

some studies of economic analysis of mastitis.

3.3 COST OF MASTITIS

3.3.1 MASTITIS

Mastitis is characterized by being an inflammation of the mammary gland whose etiology can be traumatic, allergic, metabolic, or infectious (Radostis et al., 2007). The infection origin of mastitis is the most important from the economic point of view and public health. In addition to the visible losses generated by the mastitis clinic, there are silent cases caused by subclinical mastitis, which are often performed chronically and results in reduced production of milk.

3.3.2 MASTITIS AND FACTORS IMPACTING IN THE COST

Among the factors that impact the cost are: the reduction in milk production, culling, milk disposal, cost of treating clinical cases, increase in the cost of labor, decrease in the sale price of milk are the main economic losses conditions associated with the presence of mastitis in the herd (Petrovski et al., 2006; Halasa et al, 2007). Also, expenses with preventive measures compose the total cost. (Huijp et al., 2008). Factors that can affect the final cost of mastitis may vary over time and should be considered for each situation assessed (Lopes et al., 2016). Reduction milk production, culling, and discarded milk are the more impacting economic factors (Rollin et al., 2015, Doehring and Sundrum, 2019).

The most of mastitis economic analysis considers only losses (direct or indirect). The direct losses include discard of milk (treatment and withdrawal time of drug), veterinary expenses, medication and extra labor, culling, and indirect loss include reduction in milk production, effect in the fertility of cows during lactation (Rollin et al 2015). Few studies included preventive measures in the cost and calculated as a total cost of mastitis. (Huijps et al., 2008, Van Soest et al., 2016, Lopes et al., 2016). The most preventive studies analyzed the cost and benefits of treatment protocols (Hogeveen et al 2019).

Reduction in milk production caused by clinical and subclinical mastitis are significant being considered an indirect economic loss and it is hardly estimated by producers (Petrovski et al., 2006). Clinical mastitis result in a mean of 5 % reduction in total milk production. (Huijps et al., 2008). In subclinical mastitis, in each SCC duplication above 50,000 cells/ml can result in a milk production loss of 0.4 kg of milk/day for primiparous cows and 0.6 kg of milk/day for multiparous cows (Hortet and Seeger 1998). The effects of mastitis in the reduction of milk production varies with lactation stage, parity, and pathogens involved during the infecction (Gröhn et al., 2004, Gonçalves., et al 2016).

Culling is more associated with chronic mastitis and severe clinical cases, resulting in a great impact in the cost. The calculaton of culling is not easy to calculation if consider the influence of other diseases in the health of cows. However, in general the culling cost is calculating considering the market value of the cow and the net income received from premature culling and replacement (Rollin et al., 2015).

Medication and veterinary expenses vary by country. Some authors do not consider veterinarian fees for mastitis control. Labor costs are considered difficult to measure (Halasa et al., 2007). The disposal of milk is done on days of treatment with antimicrobials and withdrawal time of the drug, it can result in a huge impact on the cost of mastitis (Petrovski et al, 2006), and varies with the price received for the milk (Doehring and Sundrum, 2019).

3.3.3 Preventive Measures in mastitis Cost

Mostly study related to the economic cost of mastitis used to calculate the economic losses, some of them analyze cost benefits or/and cost-effectiveness, and a restrict number of studies include preventive measures in the analysis (Hogevent et al. 2019). The diversity approach and preventive measures included in mastitis economic analysis can be visualized in table 4. The main preventive measures calculated were pre and pos-dipping disinfection, dry cow therapy.

Preventive Measures	Method	Level and Description	Cost \$ of preventive measures	Reference
Premilking udder preparation; Post- milking teat disinfection; Milking- machine test; Dry-cow therapy; Use of individual-cow recording service	Deterministic parcial budget	BTSCC of 623 farm records of their mastitis- control procedures	\$29.87 cow/year mastitis-control expenditure *	Yalcin et al., 1999
Milking clinical cases last; Milking subclinical cases last; Separate cloth;Wash dirty udders;-Prestripping; Milkers' gloves; Postmilking teat disinfection; Rinse clusters clinical; Rinse clusters subclinical; Replace teat	Partial budget, Stochastic simulation	Herb level simulation Default Dutch Farm with 65 dairy cows	Range from \$ 38 to \$ 8,981.01 **	Huijps et al., 2010

Table - 4. The main preventive measures and mathematical methods and model used to modelling mastitis cost

cup liners; Treatment protocol; Drying off; Keep cows standing; Dry cow minerals; Prevent overcrowding;-Clean stalls; Clean yards; Optimize feed				
Monitoring (culture, BMSCC and individual SCC); Pre-milking teat disinfection;Post dipping;Vaccination; Dry cows therapy; Maintenance of milking machine	Partial budget, Operactional effective cost (COE)	Herb level simulation Defalt brazilian farm with 100 cows	Range from \$244.13 and \$ 392.75 per lactating cow , corresponding to BMSCC de 250.000 and 1.000.000 cells/mL respectively. ***	Lopes et al 2011
Diagnostic testing;-Veterinary attention;- Machine maintenance;-Teat disinfectant; Machine washing -Dry cow therapy	Stocastic budgetary simulation model (Moorepark Dairy Systems Model- MDSM)	BMSCC 4 source	Total farms cost increased by BMSCC increased from \$167.22 at a BMSCC<100,000ce lls/mL to \$155.68 at a BMSCC>400,000 cells/mL **	Geary et al., 2012
Clean lanes ; Clean cubicles; Fixate cows after milking; Pre-strip ; Milkers' gloves;Wash dirty udders;Teat disinfectant;Milk high SCC cows last; Wash dirty udders;Teat disinfectant; Milk high SCC cows last;Rinse cluster after clinical case; Dry off	Deterministic partial budget	Test-day milk record data were collected from all dairy cows 108 Dutch	Total of \$134.82/lactating cow per year**	Van Soes et al., 2016
pre-milking teat disinfection; post- milking teat disinfection; wearing gloves during milking; mastitis vaccine; dry cow therapy * exchange rate June 2020 is £ 0.8069 for	Deterministic Partial budget U\$1.00	Herb level ,questionnaire 145 dairy producers	\$14.22/ 100 cows year	Aghamoha mmadi et al., 2018
Cheminge rule Julie 2020 15 2 0.0000 101	0.00			

** exchange rate June 2020 is €0.8901 for U\$1.00

*** exchange rate June 2020 is R\$5.4638 for U\$ 1.00

The cost and effectiveness of the management measures practices is not easy to measure, and some studies that analyses the trade-off failure cost (direct costs associated with disease) versus prevention (Van Soest et al 2016) or losses versus mastitis control expenditure (prevention) (Yalcin et al., 1999) found that not necessary, the highest expenses with preventive measures result in the lowest losses (Van Soest et al., 2016). However any study segregates the preventive measures in a) milking management, b) farm management, and c) dry cow management. Considering that each sector of milk production have different risk factors, segregating preventive measures could become more possible to find the critical risk point and result in indicator to better decision making. This could interesting way to analyses this economic effect of management measures.

3.3.4 Methods of economic analysis of mastitis

To carry out the quantification of losses and expenses caused by a disease such as mastitis, researchers have been using different methods of analysis.

The deterministic partial budget model, for example, was used to estimate the economic impact of clinical mastitis in the first 30 days of lactation on farms in the USA. Direct and indirect costs was included in the calculation and resulted in a total economic cost of US\$ 444, including US\$ 128 in direct costs and US\$ 316 in indirect costs. The indirect cost, in the long-term, represented 71% of the total cost per case of mastitis and the future loss of milk production represented 28% of the total cost per case of mastitis (Rollin et al., 2015).

Another study used the "decision tree" analysis method to assess the economic impact of mild and moderate clinical mastitis in the first case in the current lactation, in different durations of intramammary treatment and pathogen, using on-farm culture. They concluded that the ideal economic strategy would be to avoid treating clinical cases caused by gramnegative pathogens and treat CM caused by gram-positive for 2 d instead 5 or 8d (Pinzón-Sánchez, et al., 2011).

A dynamic program built on a specially structured optimization and simulation model was developed in a scenario based on data from 5 large herds in the state of New York. The simulation included a detailed representation of repeated episodes of clinical mastitis and the effects of various factors on its cost. In the basic scenario, 92% of CM cases were recommended to be treated. The average cost of a case of CM was US\$ 179. It was composed of US \$ 115 due to lost milk yield, US\$ 14 due increased mortality. and US\$ 50 due to costs associated with treatment (Bar et al. 2008)

A linear program model was developed to identify a scenario with the lowest cost for mastitis associated with the dry period, based on data from the large field trial. The economic optimal use of antimicrobials treatment (100 to 0% of cows) was determined, concluding that the optimal percentage of cows to be dried off with antimicrobials depends on the udder health situation and for all scenarios on the three different type of herds, the selective dry cow treatment was economically more beneficial compared to blanket dry cow treatment (Scherpenzeel et al 2018).

The estimated cost of mastitis in heifers in initial lactation using the Monte Carlo simulation model in stochastic molds, found that the average costs of heifer mastitis (somatic cell count> 200,000 cell/ ml) were \notin 332 at the farm level (\notin 85 - \notin 1657) and \notin 31 (\notin 4.29 - \notin 82.86) per heifer present on a farm (Huijps et al., 2009).

The cross-sectional study compared the cost-effectiveness of diagnostic strategies (bacterial culture and /or quantitative real-time qPCR) to detect contagious mastitis caused by *Mycoplasma bovis, Staphylococcus aureus* and *Streptococcus agalactiae* in infected cows in two California dairy herds, concluding that the most cost-effective alternative to the reference strategy was whole herd milk culture for all pathogens (Murai et al., 2014).

3.4 . DISCUSSION

The economic cost of diseases has been developed for a long time, but still being a restrict discipline in veterinary courses. The different methods have been developed and improved, however some of them still in improvement, as stochastic simulations. Between the economic methods, partial budget is one of the mostly used method being applyied in stochastic and deterministic model.

The use o economic analysis of animal health has been a useful tool to surveillance and monitory at individual level to international level. In recent years, the interaction of animal health, economic resources, and social perspective has been asked for to be included in the decision make (Rushton et al., (2017). The adaptations to particularities of each production system can influence the effectiveness of the decision-making.

Regarding mastitis, the economic framework including the indices considered in the analysis is well consolidated but still in upgrading, with the intesion of find the result with more accuracy and specifics details, especially with inclusion and the improvement in preventive measures analysis cost.

It is known that the cost of mastitis are mainly impacted by reduction on milk production and culling factors. However, there isn't a consensus in some factors calculation, as an example, should or not include the expenses with food in the reduction of milk production. The decision in include or not food expenses can interfere in the final result but also depend on which food management the cows are submitted. The grazing system can result in complex calculations, different from the free-stall system.

In parallel, in developing countries as Brazil, restrict studies have been analyses the cost of mastitis (Guimaraes et al., 2017) some of them published only in the Portuguese language (Holanda Junior et al., 2005, Demeu et al. 2015, Lopes et al., 2016). But, with the expansion of the milk market, the necessity of milk qualities, has being a demand for economic analysis of mastitis studies and application of this economic analyses in the Brazilian farms by tropical perspectives. Some adaptations on the decision-make in Brazilian dairy farms and control programs, considering the distinct breed characteristics and production system could be necessary.

Also, in the last years have been increasing the concerned with antibiotics resistance. Could be interesting that studies of economic mastitis approach the restrict use of antibiotics with use of a selective treatment by pathogen identification, since there is a demand for reducing the use of antibiotics in production systems as expressed by Speksnijder et al., (2015) and European legislation (European Union, 2003).

3.5. CONCLUSION

The economic study of mastitis is an important tool that allows measuring and guiding decision-making on farms, enabling a better economic return on milk production activity as well as improvements in animal welfare. There are few studies that summarize the methodologies of economic analysis of diseases, including preventive measures in the calculation and that characterize their particularities. In addition, in some countries the economic study of animal health still considered a challenge to be overcome. Numerous are the methods and combinations used to carry out this economic analysis. The cost results of mastitis are varied, making comparisons between regions and countries difficult. Denoting thus, the need to establish international guidelines in relation to the methods of economic analysis of animal health, especially of the economic analysis of mastitis in dairy herds.

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CHAPTER 3

4 THE TOTAL COST OF MASTITIS: AN EMPIRICAL STUDY IN CONVENTIONAL AND IN TRANSITION TO ORGANIC SMALLHOLDER DAIRY FARMS IN BRAZIL.

ABSTRACT

Mastitis is a great concern in dairy herds worldwide due to its effect on the profitability of the farm. The economic aspect of this disease has been reported by several studies, but few of them on tropical conditions, and approach preventive measures and empirical data. This study aimed to quantify the total cost of mastitis including management measures in smallholder farms in conventional and in transition to organic tropical conditions. Date were collected from 10 selected smallholders' dairy farms during 4 visits in the period of May 2018 to June 2019. Seven dairy farms were conventional (represented by letter A to G) and three were transition to organic ones (represented by letter H to J), located in the mesoregion of Piracicaba, São Paulo. The total cost of mastitis (faliure cost plus preventive cost) was calculated for each farm considering the average of a 305 day milk yield period. The mean total cost of mastitis identified were variable in conventional with mean of U\$ 434.07 per cows (ranging from U\$ 68.76 – U\$931.76) and in organic transition with mean of U\$ 580.42 per cows (U\$362.48 - U\$ 1010.83) smallholders dairy farms. The farm B and J presented the highest cost per cow per year and E and F the lowest total cost per cow per year. The mean failure cost of mastitis were U\$ 371.89 (ranging from U\$39.08 - U\$ 865.05) and U\$ 522.44 (ranging from U\$298.32 - U\$ 954.18) for conventional and transition's farms, respectively. The mean preventive cost were U\$ 62.18 (ranging from U\$29.68-U\$118.14) for conventional farms and U\$57.98 (ranging from U\$53.14- 64.16) for transition's farms. Overall, in the total cost of mastitis, preventive measures is a fixed cost and failure cost is a variable cost, influenced main by subclinical cases, preventive measures and culling. However the economic cost and their proportion can not be extrapolated to the other herbs, in our study, the smallholders dairy farms in transition to organic showed to be competitive compared to conventional ones, even with challenger in follow the organics regulation. Also, the differences between farms showed that the total cost of mastitis per lactating cow per year is influenced by a combination of factors as animal health status, milk productivity per cow, farm management.

Keywords: Dairy Farms. Failure Cost. Mastitis Cost. Preventive Cost.

4.1 INTRODUCTION

Mastitis still a great concern in dairy herds worldwide due to its effect on the profitability of the farm. The economic aspect of this disease has been reported by several studies, but few of them on tropical conditions, and especially in Brazil (Guimarães et al., 2017, Demeu et al., 2015). The economic assumptions attached to mastitis cost's include treatment, losses (eg. reduced milk production, discarded milk and culling), and preventive measures (Halasa et al.,

2007). However, restricted studies approach preventive measures and empirical data (Huijps et al., 2010).

The importance of preventive measures to control mastitis is well known (Dufour et al., 2011; Stevens et al., 2017) but one perceived that some measures can be too expensive or difficult to implement (Van Asseldonk et al., 2010). Decisions need to be made, and the economic assessment of management associated with the benefits on the production and animal health can contribute to set priorities on the farm (Dijkhuizen et al., 1995; Huijps, 2010). For the appropriate approach of preventive measures in the farms, the cases number of mastitis, available resources of labor, farm structure, and finance, would be considered (Hogevent et al., 2011)

The increasing demand of the market for food safety (with reduced use of antibiotics) and from production system that considers the health and welfare of the animals (FAO 2012), organic dairy farms are getting more attention in Brazil. The transition stage for farms became organic is a crucial moment to determine the success of organic farms. Since the beginning of conversion, farmers implement management practices according to organic specifications (Bouttes, 2019; MAPA, 2011). The adequate health management procedures, especially related to mastitis, can determine the good and adequate standard of production, with expected net benefits (Blanco-Penedo et al., 2019).

This study aimed to quantify the total cost of mastitis including management measures in smallholder farms in conventional and in transition to organic tropical conditions.

4.2 MATERIALS AND METHODS

The present study was conducted between May 2018 and June 2019 in 10 selected smallholders' dairy farms located in the mesoregion of Piracicaba, São Paulo. Seven dairy farms were conventional (represented by letter A to G) and three were transition to organic ones (represented by letter H to J).

The farms were visited four times during this study period, with an interval of 3-4 months, the exception of one conventional farm left the study after 2 visits. At the first visit, it was applied a survey about general farm data (e.g. farm and herd size, and milk production), farm-level management (e.g. feeding practices), and individual cow details (e.g. age and lactation stage). Management measures related to mastitis such as subclinical mastitis monitoring, teat disinfection pre and post-milking, use of gloves, vaccination, use of

homeopathic, salt dry of treatment, protocol treatment by veterinary were detailed questioned in each farm.

In all visits, lactating cows were checked for clinical mastitis during the milking using a combination of udder observation, palpation, and the Tamis test (screened mug test) to observe changes in milk appearance (Radostis et al., 2007). Subclinical mastitis was checked using the California Mastitis Test (CMT) (Ramirez et al., 2010). For each case of mastitis (clinical or subclinical) the cow and affected quarter (s) were recorded. The mean prevalence of mastitis was obtained from all visits on each farm. Descriptive statistics were performed to identify prevalence and significance differences of clinical and subclinical mastitis between means of the production systems.

4.2.1 COST OF MASTITIS

The total cost of mastitis was calculated for each farm considering the average of a 305 day milk yield period. The values of expenditures and services were gathered by farmers, literature, and at the regional market. The estimate of total costs of mastitis was considered the failure costs and preventive costs (Van Soest et al., (2016; Huijps et al., 2008). Failure costs include cost of milk loss, discarded milk, treatment, drugs, veterinary services, extra labor, replacement of culling. Preventive costs included preventive measures in the farm management and milking practices (eg. pre and pos milking). The calculation formulas is in the appendices A.

The expected reduction in 305 day-milk yield was calculated as 5% per clinical case of mastitis (Seeagers et al., 2003). The SCM milk production losses were estimated at the level of each quarter/cow considering the mean stage of lactation at 155 days in milk (DIM) and obtained for each CMT analysis with the expected reduction in 305d milk yields in CMT1, CMT2 and CMT3 equivalent to 8%, 12 and 14 % respectively (Seegers et al., 2003).

In discarded milk due to treatment, we used the average daily milk production per cow on farm to estimate the amount of milk discarded. The duration of treatment and corresponding withdrawal for allopathic treatment in conventional farm period was 6 days (3 days of treatment and 3 days of milk disposal), and considering organic regulation (MAPA, 2011) it was 9 days (3 days of treatment and 6 days of milk disposal) for farms in transition to organic. The mild and moderate clinical mastitis in conventional farms were treated with the intramammary antibiotic. Farms in transition to organic the mild and moderate mastitis were treated by a combination of massage, stripping out by hand in the milking parlor, and homeopathic treatment. In the clinical cases, 30% of the cases were considered severe (Tomazi et al., 2018), and needed to use systemic antibiotics associated with intramammary antibiotic and non-steroidal anti-inflammatory treatment. The severe clinical mastitis in transion's farms were also treated with allopathic medication in consideration of animal welfare. The daily dose was calculated for a cow with 550 kg body weight.

Extra labor and salary were estimated by information provided by farmers and observations, corresponding to 5 minutes per day treatment for each clinical case. In culling, were consider that 12% of the lactating cows with mastitis were expected to be culled due to information provided by farmers. The culling cost were calculated by the difference of the average value of a replacement cow in the herd and the value of culled cow for beef (Petrovisk et al., 2006; Halasa et al., 2007). The milk price (U\$/kg milk) was considered a mean of he period of July 2019 until June 2020. The technical data from the questionnaire were representative for farms in 2018, however, the cost estimates in this study were based on 2020 price levels. The parameters inputs and the values used to estimate mastitis cost for conventional and in the transition to organic smallholder`s dairy can be observed in Table 5.

Parameters inputs ^a		Conventional	Transition to Organic	Source
Milk price (U\$/kg)		0.3186 ^b	0.4779 ^c	CEPEA, 2020
Price of concentrates (U\$/Kg) ^d		0.3189	0.3345	Local market
Cost of antibiotics for treatment(U\$/case)	Mild and Moderate ^e Severed ^f	8.0291 28.6519	0.0864 28.6519	Local market
Cost of labor (U\$/hour)		1.7842	1.7842	Local Market
Other Assumptions				
Milk production loss per case of CM (%)		5	5	Seegers et al., 2003
Milk production loss per case of SCM (%)	CMT1 CMT2 CMT3	8 12 14	8 12 14	Seegers et al.,2003
Duration of treatment for clinical mastitis (days)		3	3	Farmers
Duration of total withdrawal time of clinical mastitis treated with allopatic medication (days)	2	6	9	Recommendati on by pharmaceutical companies
Time expend for treatment of clinical mastitis (min /case/day)		5	5	Farmers
Culling (% of clinical cases)		12%	12%	Compiled herd data ^g

Table 5 - Parameters inputs and values to estimate total mastitis cost in conventional and in transition to organics smallholders dairy farms

a Mean dollar exchange rate July 2019 to June 2020 is R\$4.4837 for U\$ 1.00.

b Value reference from period July 2019 to June 2020.

c Value reference is 150 % of conventional milk price from mean of July to June 2020 (CEPEA, 2020).

d The concentrate were the mean of 4kg/cow/day for farms, except farm F that used only 2 kg/cow/day and Farm E did not provided concentrate.

e Mild and Moderate CM - sub-acute clinical mastitis with grume and grume with inflammation respectively. Treatment considered were antibiotic intra-mammary for 3 d.

f Severe CM - acute clinical mastitis with grume, inflammation and septicemic symptoms (eg. anorexia and fever). Treatment Antibiotic intra-mammary and antibiotic systemic for 3 d and , ant-inflammatory for 2 d.

g Compiled herd date provided by 5 smallholders dairy farms (3 conventional farms and 2 farms in transition to organic).

Prevention measures mastitis were based in the main management measures applied by farmers. The management measures taken into consideration on this study including milking management, farm and environment management. For each measure will be considered: labor (\$/min) and consumable item (U\$/year) (Huijps et al., 2010) (Table 6).

Eastava Cast	Amount	Unit/cow or	Mean Price ^b	A	
Factors Cost	Amount	farm/year ^a	(U\$/Unit)	Amount	
Screened mug (U\$/year)	1	unit/farm/year	18.9576	1 unit	
CMT - rakel (U\$ /year)	1	unit/farm/year	2.8994	1 unit	
CMT - reagent (ml/milked cow)	8	ml/cow	2.6764	500ml	
Pre and Pos-dipping cup	1	unit	14.4970	1 unit	
Pre-dipping disinfectant	2	ml/cow	26.7636	20 L	
Post-dipping detergent	4	ml/cow	64.2327	20 L	
Paper packet	4	sheet/cow	14.9430	6000 unit	
Teat cup linears (sets of 4) ^c	8	set of 4/farm/year	20.0727	4 unit	
Clean Milk Parlor ^d					
Acid disinfectant	250	ml/cleaning	22.3030	5 L	
Alkaline disinfectant	250	ml/cleaning	52.8581	20 L	
Gloves	2	unit	5.1297	100 unit	
Antimastitic dry off	4	unit/quarter	1.9850	1 unit	
Vaccine Rottatec J5 45ml	3	dose(15ml)	19.8497	45ml	
Homeopathic salt (U\$/cow)	1.7	g/cow/day	2.5381	600g	
Water price (U\$/m3)	500	lL/cleaning	2	10m ³	

Table 6- Parameter inputs and market price of consumable to estimate preventive costs of mastitis in conventional and organic smallholders dairy farm in Brazil

a Assumption based manufacture and farmers information.

b Estimated from local market price.

c Farms A, E and F had used 2 milking cluster (set of 4 teat cup linear).

d Farms A, E and F had bucket milking systems consuming only alkaline disinfectant (170ml/cleanning), not using acid disinfectant and less water (100ml).

4.3. RESULTS

The herd of farm studied kept their cattle in a semiconfined housing system, with average size 33 cows (range 12 – 46 cows/day), and average 305d-milk yield 14.0 kg milk/day/cow (range 8 - 20.5kg milk/day/cow), the most productive farms were C, D, G, I and J and the less productive were E and F. The herds were milked twice a day except for one conventional farm (Fam E). The predominant breed where Gir X Holstein Crossbred (60%), followed by Holstein (40%), Jersey(10%).

Over the period study, 1.165 cow observations were made for mastitis, with 4.567 quarter observations where mastitis status (clinical, subclinical, or absent) was recorded. The prevalence of mastitis over the period study varied between farms with a mean 2.3% clinical mastitis and 33.2% subclinical mastitis for all farms. The prevalence of clinical mastitis for the conventional farm was 2.69% (\pm 0.3) and in transition to organic 1.7% (\pm 0.3) respectively. The mean prevalence of subclinical mastitis for conventional farms was 33.9% (\pm 1.16) and for farms in transition to organic were 34.15 (\pm 0.99), non-significant statistical differences in the mean prevalences of mastitis were observed between production systems.

From all tests, day records a mean of clinical and subclinical mastitis were calculated for one year with 305milk days.

4.3.1 TOTAL COST OF MASTITIS

The total cost of mastitis identified was variable in conventional with the mean of U\$ 434.07) per cows (ranging from U\$ 68.76- – U\$931.76) and in organic transition with a mean of U\$ 580.42 per cows (U\$362.48 – U\$ 1010.83) smallholders dairy farms for the period of 2018 to 2019 (table 7). The mean total costs per milk production per year were U\$ 0.1018 (U\$ 0.03 - U\$ 0.23) for conventional farms and U\$ 0.1231 (U\$ 0.07 – U\$0.18) for the transition to organic`s farms. Farm B and J presented the highest cost per cow per year and E and F the lowest total cost per cow per year. The failure cost of mastitis were U\$ 371.89 (ranging from U\$39.08 - U\$ 865.05) and U\$ 522.44 (ranging from U\$298.32 - U\$ 954.18) for conventional and transition`s farms, respectively. The preventive costs were U\$ 62.18 (ranging from U\$29.68-U\$118.14) for conventional farms and U\$57.98 (ranging from U\$23.14- 64.16) for transition`s farms.

Table 7 - Average cost of mastitis and percent distribution of economic factors of conventional and in transition to organic smallholder's dairy farms. located in the macrorregion of Piracicaba. São Paulo. Brazil

Economic and Production Indices	А	В	С	D	Е	F	G	Η	Ι	J
Mean of number of cows in lactation	19.0	27.0	46.0	31.0	17.0	12.0	35.0	25.0	40.0	41.0
Mean 305 day production (kg)	13	13	17	20.5	8	9	15	10	17	19
Production system model ^a	Conv.	Conv.	Conv.	Conv.	Conv.	Conv.	Conv.	Org.	Org.	Org.
Mean of CM cases per year (quarter)	24	114	21	33	0	0	42	6	12	69
Total mean SCM cases	255	666	837	300	66	252	639	456	396	822
Animals culled due CM (cows/year)	3	14	3	4	0	0	5	1	1	8
Failure Cost (\$/cow/year)										
Production losses CMl (U\$/cow/year)	13.03	48.18	6.93	21.33	0.00	0.00	16.46	3.12	7.72	49.46
Production losses SCM (U\$/cow/year)	154.66	339.71	325.95	219.14	39.08	190.05	292.11	269.98	273.56	718.17
Discarded milk (U\$/cow/year)	18.93	66.72	8.50	23.68	0.00	0.00	21.03	2.86	5.58	31.53
Veterinarian (U\$/cow/year)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Medicines (U\$/cow/year)	20.81	69.55	7.52	17.54	0.00	0.00	19.77	2.91	3.63	18.63
Labor (U\$/cow/year)	0.56	1.88	0.20	0.47	0.00	0.00	0.54	0.18	0.22	1.27
Culling (U\$/cow/year)	101.42	339.01	36.65	85.47	0.00	0.00	96.35	19.27	24.09	135.12
Failure Cost/year (U\$/cow/year)	309.42	865.05	385.75	367.63	39.08	190.05	446.26	298.32	314.81	954.18
Preventive measure (U\$/cow/year)	59.98	66.70	54.16	118.14	29.68	41.33	65.24	64.16	53.14	56.65
Total Costs Mastitis(U\$/cow/year)	396.40	931.76	439.92	485.77	68.76	231.38	511.50	362.48	367.95	1010.83
Total Costs per milk (U\$/cow/year)	0.10	0.23	0.09	0.08	0.03	0.08	0.11	0.12	0.07	0.18

a Conv - Conventional farms, Org -Farms in transition to organic

Subclinical mastitis losses were the main component of total cost for both production system. followed by culling, preventive measures, and clinical mastitis losses (Table 8).

The mean of subclinical mastitis losses, culling, and preventive measures for conventional farms accounted for 51.4%, 21.69%, 14.3% respectively in the total costs. The mean subclinical mastitis losses, preventive measures, and culling for farms in organic transition`s farms accounted for 72.46%, 10.25%, and 9.99% respectively. Subclinical losses were proportionally and numerically lower in conventional farms (U\$ 222.96 vs U\$ 420.57 cow/year). Culling and preventive measures were proportionally higher in conventional farms corresponding to mean value of U\$94.13 and U\$62.18 respectively per cow per year in transition's farms compared to U\$ 59.49 and U\$57.98 respectively per cow per year in conventional ones.

Clinical mastitis losses were proportionally low in farms in transition to organic (3.46%) compared to conventional farms (3.48%), but numerically higher in transition's farms (U\$ 20.10 per cow per year) compared to conventional farms (U\$ 15.13 per cow per year). Discarded milk and medication and were proportionally and numerically higher for the conventional farm (4.57% and 4.45%) compared to farms in transition to organic (2.30% and 1.45%). Veterinarians were not consulted for mastitis cases. Extra labor had a lower weight of economic impact on both production system models.

Production and Economic factor		ional's Farm	Transition to organics Farm		
Nr cows in lactation/month	27	(12-46)	35	(25-40)	
Mean 305 day production (kg)	14	(13 - 21)	15	(10 - 19)	
Milk Price (\$/kg)	0.3186		0.4780		
Average of clinical cases per year (quarter)	33	(0 - 114)	29	(6 - 69)	
Average of subclinical cases per year (quarter) ^a	431 ^a	(66 – 837)	558	(396 - 822)	
Failure Cost					
Production losses clinical (\$/cow/year)	15.13	3.48%	20.10	3.46%	
Production losses subclinical (\$/cow/year)	222.96	51.36%	420.57	72.46%	
Discarded milk (\$/cow/year)	19.84	4.57%	13.32	2.30%	
Veterinarian (\$/cow/year)	0.00	0.00%	0.00	0.00%	
Medicines (\$/cow/year)	19.31	4.45%	8.39	1.45%	
Labor (\$/cow/year)	0.52	0.12%	0.56	0.10%	
Culling (\$/cow/year)	94.13	21.69%	59.49	10,25%	
Failure cost of mastitis (\$/cow/year)	371.89	85.68%	522.44	90,01%%	
Preventive measure cost (\$/cow/year)	62,18	14.32%	57,98	9,99%%	
Total Costs Mastitis (\$/cow/year)	434,07		580,42		
Total Costs per kg of milk (\$/cow/year)	0.1018		0.1231		
- Assessed a formation of the CMTE > 1					

Table 8 - Average cost of mastitis and percent distribution of economic factors for conventional and in transition to organic smallholder's dairy farms, located in the macrorregion of Piracicaba, São Paulo, Brazil

a Average of quarter with CMT \geq 1.

4.3.2 Cost-effectiveness relationship of preventive measures and failure Cost

The cost with preventive measures related to failure cost of mastitis in each farms can be visualized in Figure 1. The high cost with prevention not necessarily were related with low failure cost. We can notice that farm J and B even investing in preventive measure presented the highest cost with mastitis. Farm E and F were the farm that less expended with prevention and had less failure cost. The farm D expend more with preventive measures but milk production compensating this cost. The other farms presented close value of preventive measure costs and failure cost.





smallholders dairy farms

4.3.3 PREVENTIVE MEASURES COST

The preventive measures cost were variable values between farms. Protocol treatment (Fam E), vaccine (Farm G and J) milkers' gloves (Farms I and J) were the preventive measures less used between farms. Because of the restriction on the use of antibiotics, farms in transition to organic was not allowed to use dry off treatment (figure 1). Any farm applied all preventive measures.



Figure 2 - Preventive measures applied in conventional (A - G) and in transition to organic (H - J) smallholders dairy farms located in the mesorregion of Piracicaba, São Paulo during period of June 2018 to June 2019.

The mean of preventive measures cost were higher to conventional farms accounted in U\$ 62.18 per cow per year (U\$29.69-U\$118.14) compared to transition's farms accounted in U\$ 57.98 per cow per year (53.14 - 64.16) (Table 8). The main component in preventive measures cost for conventional and in the transition to organic farms were clean milk parlor, pre-dipping, post-dipping. Clean milk parlor had the highest weight in preventive cost for transition's farms (39.03%) followed by pre-dipping (19.39%) and post-dipping (18.54%). In conventional farms, the clean milk parlor accounted for the mean of 37.71% of the preventive cost, followed by 12.92% of pre-dipping and 12.43% of post-dipping. Clean milk parlor was proportionally higher in transitions farms but the financial value was little increased in conventional farms (table 9).

The other measures were more variable between conventional and farm in transition to organic. Monitoring subclinical cases (0.39% vs 1.9%), milker' gloves (0% vs 2.10%), replace teat cups (8.01% vs 8.25%) vaccine (0.95 % vs 2.38%) and homeopathic salt (2.38% vs 4.46%) had decreased weight in the preventive cost in conventional compared to the transition's ones. Pre-striping was higher in conventional farms (U\$ 2.56 vs \$U 2.28 cow /year) corresponding to 4.12% of preventive measures. Protocol treatment and dry off treatment were only applied by conventional farms being in mean responsible for 11.90% and 9.19% of the preventive cost respectively. Protocol treatment was only applied in farm D, resulting in the greatest economic impact on this farm (figure 1).

Preventive Measures	Farms					
	Conventional				nic	
	U\$/cow/ year	Min. and Max.	%	U\$/cow/y ear	Min. and Max.	%
Monitoring subclinical cases	0.24	(0 - 1.68)	0.39	1.10	(0 - 1.66)	1.90
Pre-stripping	2.56	(2.12 - 3.29)	4.12	2.28	(2.18-2.47)	3.93
Pre-dipping	8.03	(0 - 11.58)	12.92	11.24	(11.17 - 11.39)	19.39
Pos dipping	7.73	(0 - 11.08)	12.43	10.75	(10.67 - 10.90)	18.54
Milkers' gloes(\$/pair)	0.00	(0.00)	0.00	1.22	(0 - 1.85)	2.10
Replace teat cup liners	4.98	(3.49 - 6.69)	8.01	4.78	(3.92 -6.42)	8.25
Clean milk parlor	23.45	(16.51- 31.35)	37.71	22.63	(18.53 - 30.38)	39.03
Protocol treatment	7.40	(0.00 - 94.46)	11.90	0.00	0	0.00
Drying off Treatment	5.71	(0 - 8.00)	9.19	0.00	0	0.00
Vaccine	0.59	(0 - 4.15)	0.95	1.38	(0 - 4.15)	2.38
Homeopathic salt	1.48	(0 - 2.59)	2.38	2.59	(2.59)	4.46
Total	62.18	(29.69-118.14)		57.98	(53.14 - 64.16)	100.00

Table 9 - Mean of preventive cost and percent distribution of each management measures in Conventional and in transition to organic smallholder's dairy farm located in mesorregion of Piracicaba.

4.4. DISCUSSION

In Brazil smallholders dairy farm is responsible for great percent of milk production in Brazil (FAO 2010), however, none study has previously estimated the total cost of mastitis in conventional and in the transition to organic smallholders dairy farms in Brazil. In our study, the prevalence of clinical and subclinical mastitis is in corroboration with literature (Langoni H. and Troncarelli 2011; Bueno et al, 2002), and had nonstatistical differences between production systems. The similar prevalence of production diseases in organic dairy farm compared with conventional farm have been observed in other studies demonstrated that organic farms not necessarily had been impacted by certification requirements (Blanco-Penedo et al 2019).

In the mean of the total cost of mastitis, conventional farms presented the lowest cost of mastitis per cow per year compared to farms in transition to organic. The mean of failure cost was lower (U\$ 371.89 vs U\$ 522.44) and preventive cost was higher for

conventional smallholders dairy farms (U\$ 62.18 vs U\$57.98) compared to transition's one.

The highest total cost of mastitis in transition farms are mainly in the reason of the highest cases of subclinical mastitis and the highest price of milk received by these farms compared to conventional ones, resulting in more absolute mean value. However, when considering the margin gross of milk production, the farm in the transition to organic receipted advantage economic input, compensating these economic losses.

In our study, milk loss of subclinical mastitis is the most impacting component in the cost of mastitis, corroborating with other surveys (Seegers et al., 2003, Van Soest et al., 2016, Guimarães 2017, Doehring and Sundrum 2019). However, the greatest weight of subclinical mastitis losses mostly of the time is neglected by farmers because of the difficulty to notice and knowledge to calculate the losses instead of more visible losses as discarded milk and culling (Guimarães et al., 2017).

The preventive measures had higher economic weight in conventional farms compared to transition farms, in reason to the highest weight of milk losses in farms in transition to organic and also due to the use of drying off and protocol treatments only in conventional farms. The culling had higher weight in the conventional farm in reason of increased mean of absolute clinical cases, compared to transition's farms. However, the impact of the mean of milk losses of clinical mastitis were close between production systems, numerally they were higher in farms in transition to organic because of the higher price of milk in these farms.

Transition farms, proportionally, discarded less milk compared with conventional farms in reason of the use of homeopathic medication in the mostly of clinical mastitis cases, don't being necessarily withdrawal time after treatment. However, we noted that organic farms need to have more carefully in keep low prevalence of clinical mastitis and avoid acute cases which can result in more expensive discarded milk and remotion of the animal from herb due to recurrent cases.

Medication was higher in conventional farms owing to the increased absolute value of clinical mastitis cases in conventional farms. Also, medication was less expensive to farms in organic transition for made use of homeopathic, which has a lower price compared to allopathic medication. Veterinary visits were not used to be calling for mastitis cases. The labor time expended for massage and stripping mild and moderate cases of clinical mastitis in farms in transition, was not available, being not included in the economic analyses.

The relationship of cost and effectiveness between preventive measures and failure cost demonstrated that there aren't straight relation between the use of preventive measures and reduced mastitis prevalence, a similar result was found by Van Soest et al., (2016). Some preventive management can have better net benefits compared to others (Huijps et al., 2010). Also, the prevalence of mastitis is influenced by the environment, animal health status and, management (Rushton, 2019).

Farms in transition had a particularity of the restricted use of antibiotics and appropriated environment conditions, nevertheless not necessarily implied in the use of all preventive measures allowed. In general, the farms presented a closing cost with preventive measures per cow in the year, except for farm E and F that presented low failure and preventive cost, however, these farms have particularity in to be less productive farms.

The most expensive measures were clean milk parlor, pre, and post-dipping. The more weigh of these preventive measures cost is due to the daily use in each animal expending more consumable items and labor. Monitoring subclinical cases where doing the CMT test were more used by transition's farms, probably for avoid the increasing on somatic cell account in the bulk tanker. Pre-stripping and replace teat cup liners even being performed in all farms had a low impact on the mean prevention cost for has low expenses. Measures as vaccine, use of gloves were not many common measures, vaccines were applied in the conventional and transition farms but gloves only in transition farms. Homeopathic were used in both production systems as preventive, provided mixed with salt.

Dry off treatment is not allowed in organic systems (MAPA, 2011) but the most conventional farmers used this preventive measure except by two farms. Protocol treatment depend on payment of monthly veterinary professional and farmers consider this practice expensive, farmers used to have veterinary assistance occasionally. Only farm conventional D applied these measures. However, when a farm receives a mastitis expertise visit, as veterinary, it can increase knowledge about the milking process and in the identification of sources of infection caused by this diseases (Van den Borne, 2014), consequently, can contribute to the reduction of mastitis cases and increase milk yield in the herd, offsetting the expenses with this preventive measure at the long term.

The variable effect of preventive measures on mastitis controls still a challenger because of the complexity of mastitis diseases. However, many studies confirmed the importance of preventive measures in this disease (De Vliegher et al., 2018). Thus, economic analysis of mastitis can bring a better perception of critical points in the dairy systems related to these diseases. The combination of mastitis cost, effectiveness analysis of management measure and improvement in the way that the management is performed could result in a better net benefit of preventive measures.

4.5 CONCLUSION

Overall, in the total cost of mastitis, preventive measures is a fixed cost and failure cost is a variable cost, influenced main by subclinical cases, preventive measures and culling. However the economic cost and their proportion can not be extrapolated to the other herbs, in our study, the smallholders dairy farms in transition to organic showed to be competitive compared to conventional ones, even with the challenger in following the organics regulation. However, farms in transition to organic and organic farms need to have carefully with subclinical, and clinical mastitis. The price received for milk, food security and sustainability confer to farms in transition to organic an advantage production system, compared to conventional systems despite to mastitis cost. The differences between farms showed that the total cost of mastitis per lactating cow per year is influenced by a combination of factors as animal health status, milk productivity per cow, farm management.

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5 GENERAL CONCLUSIONS

In the first chapter we concluded that the risk of mastitis on smallholder dairy farms in Brazil, both clinical and subclinical is very high. This study has identified some risk factors for mastitis on such farms, at cow level, CM presented increased association with middle stage of Lactation (50-150 DIM). The SCM had significantly decreased association with primiparous and late lactation stage (>150DIM) had the greater odds. At herd level drying off treatment (OR 4.23, 95% CI 1.42-12.62) and visit date were significant associated with CM. The third (January 2019) and forth (April-June 2019) visit date presented a greatest odds for CM. Milking clinical (OR 0.37, 95% CI 0.24-0.56) and subclinical cases last (OR 0.21, 95% CI 0.09-0.47) and Clean milk parlor (OR 0.27, 95% CI 0.15-0.46) had decreased odds for SCM and had greater odds for optimized feed (OR 9.11, 95% CI 2.59-31.9). The last three visit dates had significantly high odds compared to the first visit (June), with the highest one in forth visit (April-June). Further research on more farms across more areas of Brazil is required to develop targeted control programmes for mastitis. This research should be combined with identifying the knowledge gaps of smallholders in regard to mastitis and the barriers to implementing mastitis control on such farms.

The second chapter, in the review, we concluded that there are few studies that summarize the methodologies of economic analysis of diseases, include preventive measures in the calculation and characterize their particularities. In some countries the economic study of animal health is still considered a challenge to be overcome, specially in Brazil which have only a few economic health studies. Numerous are the methods and combinations used to carry out this economic analysis. However, the results of mastitis cost are variable, making comparisons between regions and countries difficult. Denoting thus, the need to establish international guidelines in relation to the methods of economic analysis of animal health, especially of the economic analysis of mastitis in dairy herds.

In chapter 3 we concluded the total cost of mastitis identified were variable in conventional with mean value of U\$434.07 per cows (ranging from U\$ 68.76 – U\$931.76) and in organic transition with mean value of U\$580.42 per cows (U\$362.48 – U\$ 1010.83) smallholders dairy farms. The farm B and J presented the highest cost per cow per year and E and F the lowest total cost per cow per year. The mean failure cost of mastitis were U\$ 371.89 (ranging from U\$39.08 - U\$ 865.05) and U\$ 522.44 (ranging from U\$298.32 - U\$ 954.18) for conventional and

transition's farms, respectively. The mean preventive cost were U\$ 62.18 (ranging from U\$29.68-U\$118.14) for conventional farms and U\$57.98 (ranging from U\$53.14-64.16) for transition's farms. Overall, in the total cost of mastitis, preventive measures are a fixed cost and failure cost is a variable cost, influenced main by subclinical cases, preventive measures and culling. However the economic cost and their proportion can't be extrapolated to the other herbs, in our study, the smallholders dairy farms in transition to organic showed to be competitive compared to conventional ones, even with challenger in follow the organics regulation. Nevertheless, farms in transition to organic and organic farms need have carefully with subclinical and clinical mastitis. The differences between farms showed that the total cost of mastitis per lactating cow per year is influenced by a combination of factors as animal health status, milk productivity per cow, farm management.
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APPENDIX

APPENDIX A. Preventive measures cost for each smallhouder dairy farm located Southeast of Brazil during period of june 2018 to june 2019.

APPENDIX B. Equations used to ocalculate total cost cost of mastitis.

APPENDIX C. Questionnaire

APPENDIX A – Table of preventive measures cost for each smallholder dairy farms.

Table 10 - Preventive measures Cost for each smallhouder dairy farm located mesorregion of Piracicaba, Sao Paulo, Brazil during period of June 2018 to June 2019.

	А	В	С	D	Е	F	G	Η	Ι	J
Preventive Meaures					\$/cow/	year				
Monitoring subclinical cases	0,00	0,00	0,00	1,68	0,00	0,00	0,00	0,00	1,66	1,66
Pré strip	2,71	2,42	2,12	2,32	2,83	3,29	2,25	2,47	2,19	2,18
Pre-dipping	11,58	11,35	10,81	11,28	0,00	0,00	11,23	11,39	11,17	11,17
Pos dipping	11,08	10,86	10,64	10,79	0,00	0,00	10,73	10,90	10,68	10,67
Milkers' gloes(\$/pair)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,85	1,80
Replace teat cup liners	4,23	5,95	3,49	5,18	4,72	6,69	4,59	6,42	4,01	3,92
Clean parlor	19,80	28,13	16,51	24,50	22,13	31,35	21,70	30,38	18,99	18,53
Protocol treatment	0,00	0,00	0,00	51,80	0,00	0,00	0,00	0,00	0,00	0,00
Drying off Tratament	8,00	8,00	8,00	8,00	0,00	0,00	8,00	0,00	0,00	0,00
Vaccine	0,00	0,00	0,00	0,00	0,00	0,00	4,15	0,00	0,00	4,15
Homeopathic salt	2,59	0,00	2,59	2,59	0,00	0,00	2,59	2,59	2,59	2,59
Total	59,98	66,70	54,16	118,14	29,68	41,33	65,24	64,16	53,14	56,65

APPENDIX B – Total cost of mastitis formulas.

Total Cost of mastitis : Failure Cost + Preventive Cost

Failure Cost : F $_{ML}$ + F $_{Dis}$ + F $_{Med}$ + F $_{Labor}$ + F $_{C}$

Table 11 - Equations used to calculate failure cost of mastitis

Failure Cost Factors		Formula
Milk production losses due to mastitis	F_{ML}	$F_{MLCM} + F_{MLSC}$
Clinical mastitis	F _{MLCM}	(average 305d milk yield/quarter * 5%) * number of cases * (milk price/kg – food price/kg)
Subclinical Mastitis	F _{MLS}	(average 305 d milk yield/quarter/day * 150d * 8%) *N _{CMT1} * (milk price/kg – food price/kg) + (average 155 d milk yield/quarter * 12%) *N _{CMT2} * (milk price/kg – food price/kg) + (average 155 d milk yield/quarter * 14%) *N _{CMT3} *(milk price/kg – food price/kg)
Discarded Milk:	F_{Disc}	(average days of treatment + average days of withdrawal) * number of cases/year * average daily milk yield/quarter (based on average 305d milk yield) * (milk price/kg + feed price/Kg)
Medication ^a	F_{Med}	Price of medication * clinical case/year
Labor for treat clinical mastitis	F_{Labor}	number of clinical mastitis case/year * (Treatment Time in minutes /60) *treatment days * Labor cost per hour (\$/hour)
Cows culled due to mastitis	F _C .	U\$ 223.03 * number of slaughtered cows – U\$ 8921,20 * number of replacement

a Dose for 550kg lactating cow

Preventive Measures Cost : $P_{mon} + P_{préstrip} + P_{predip} + P_{posdip} + P_{glo} + P_{rep} + P_{clean} + P_{treat} + P_{dryoff} + P_{vac} + P_{Hom}$

Table 12. Equations used to calculate failure cost of mastitis.

Preventive Measures Factors		Labor time (min//cow)	Frequency	Formula (\$ /cow/year)
Monitoring SCM	\mathbf{P}_{mon}	3 min	Monthly	Labour ((\$/min/test-day/cow) + CMT reagent (\$/ml/cow/))*12 + CMT raquel (\$/year)
Pré stripping	P _{Préstrip}	0.08 min	Twice daily	Labor(\$/min/cow/montly)) *12 + screened mug (\$/year)
Pre-dipping	P _{Predip}	0.08 min	Twice daily	((Labor(\$/min/cow/montly) + Disinfectant (\$/ml/cow/montly) + paper(\$/sheet/cow/montly)) *12 + Cup(\$/year)
Pos-dipping	P_{posdip}	0.05min	Twice daily	(Labor(\$/min/cow/montly)+ Disinfectant (\$/ml/cow/montly)) *12+ Cup(\$/yeat)
Milkers' gloes	P_{glo}	-	Twice daily	Gloves(\$/pair/montly) * 12
Replace teat cup liners	P_{rep}	-	Semester	Cup (\$/set of 4) * 8
Clean parlor	P_{Clean}	-		$P_{dalk} + P_{dac}$
Detergent alkaline	P_{dalk}	10 min	Twice daily	Labor(\$/min/montly) + Water (\$/600L/montly) + Disinfectant (\$/250ml/montly)
Detergent acid	P_{dac}	10 min	Weekly	Labor(\$/min/montly) + Water (\$/600L) /montly+ Disinfectant (\$/250ml/montly)
Protocol treatment	$\mathbf{P}_{\text{treat}}$	240min	Monthly	Vet Labor (\$/min * 240)* 12
Drying off Treatment	P_{Dryoff}	2min	annually	Labor(\$/min/cow/year) + Dry of Antibiotics (\$/cow/year)
Vaccine	P_{vac}	2min	annually	Labor(\$/min/cow) + Vaccine (\$/cow/year)
Homeopathic salt	P_{Hom}	-	daily	Homeopathic salt(\$/g/cow/ montly) *12

a. Refer to 2 times in 30 days (monthly frequency).

APPENDIX C – Questionnaire of characterization of productive systems.

General Data

Date: Address:: Farmer name: Contact:: E-mail:

Characterization of family or contracted labor.

1.Indicate who conducts the animals, time spent, monthly salary (R\$/Mês):

2. Indicate who milks the cows, time spent, montly salary (R\$/Mês):

3. Indicate who does the administratioin, time spent, montly salaty (R\$/Mês):

4.Indicate who does the administration, time spent, monthly salary (R\$/Mês):

5. Indicate who does the health management time spent, monthly salary (R\$/Mês):

Property Information

1. What is the size of the property (ha)?

2. What is the area of permanent protection area (APP) and Legal Reserve (RL) in ha?

3. What total area, how much is destined for dairy activity (ha)?

5. What is the available grazing area (ha)?

6.Type of system

_ grazing _semiconfined _ confined _ other

7. Production Model

_Conventiona

- _ Convertion to organic
- _ Organic

Herd Characteristics

1.Number of animals in dairy farming:

- 2.Number of lactating cows:
- 3. Number of Primiparous lactating cows:
- 4.Number of multiparous lactating cows:

5.Number of dry cows:

- 6.Number of heifers (12-24 months):
- 7. Number of heifers (> 24 months):
- 9.Number of calves weaned:

10.Number of calves suckling:

- 11.Age of weaning calves
- 13. Number of bulls:
- 14. Stoking rate of total milk area:
- 15. Stoking rate of pasture area:

Reprooduction and milk production

- 1.Strategy used for breeding the herd:
- Artificial insemination

-Use of bull

- -Transfer of embryos
- 2. What interval between deliveries?
- 3. What is the main breed in the farm?
- -Holstein
- -Jersey
- -Crossbreed Gir X Holstein (Grolando) -Crossbreed

Milk Production

 Total milk production (liters / month): Average productivity of cows (liters / cow): Average milk price: 	Mastitis in dairy cows		
4. Bonus on the value of milk (R \$ / liter):	1.Perform treatment of cases with:		
5. Quantity of milk discarded in the last month / day or month:	 Clinical Mastitis Subclinical Mastitis 		
6. Quantity of milk supplied to calves in the last month / day or month:	 Variable e 2. How many clinical cases did not treated if the last 3 month and why? 		
7. Milk quality in the last month (tank milk):			
Somatic cell count (CCS):	3. In which situation does not treat clinical		
Total bacterial count in milk (CBT):			
Total fat without milk (%):	a. which medications utilizes to treat mastitis:		
% Protein:	-Antibiotic tube:		
ESD (1000cl / ml):	- Systemic Antibiotic:		
NAKED:	-Anti-inflamatory:		
PL (liters):	- Dry cow antibioti:c		
Total milk solids (%)	-Homeopathy:		
	-Other:		
Herd Health	5.Describe mean of treatment time per case and disposal milk days per case;		
1.Major diseases affect the herd in recent	6.Mean of time (min) and expenses with		
months?	veterinary per clinical cases (R\$/mastitis		
2. Performs vaccination for:	cases)?		
-Aftosa	7.How many cases have you had Veterinary assistance in the last 3 months?		
-Brucellosis	8.How many cases did the animals dry up due to mastitis in the last 3 months?		
-Carbuncle			
-IBR / BVD	9. Did you have animal disposal?		
- Bovine Pasteurellosis/ Paratyphoid	10. Did you treat subclinical mastitis?		
Enterotoxemia / colibacillosis	11. Value in milk price penalty for high CCS		
-Rabies	10001.		
-Mastitis	Preventive Measures		

1. What are the preventive measures used in	Food management			
the property and in the time spent (min / day)?	1. There is a specific nutritional balance for			
-Milking clinical cases in the end	your property?			
-Milking subclinical cases in the end	2. What foods are provided to the animals			
-Pré dipping	and quantity (kg / animal / day) in summer and winter.			
-Post-dipping	-Pasture			
-Wash dirty teat	- Ration			
-Wash dirty udder	-Silage			
-Wash milking liners between milking	-Ray			
-Wash milking liners end milking	-Barley			
-Use separate clothes or paper	-Sugar Cane			
- Clean milking parlor	-Other type:			
- Milking Maintenance	3. Time of pasture access:			
- Gloves	-Summer:			
-Treat dry cows	4. Perennial grazing area(ha) (fixa):			
-Vaccine	- Total cost of pasture formation and/or			
- Protocol treatment by veterinary	annual maintenance (R\$/anual):			
-Keep cows standing after milking	5. Annual summer pasture area (ha):			
-Provide mineral supplement specific to dry cows	- Total cost of pasture formation and/or annual maintenance (R\$/anual):			
-Homeopathic salt	6. Annual winter pasture area (ha):			
-Optimize feed before calving (how many days before calving)	- Total cost of pasture formation and/or			
-Reduce overcrowding	annual maintenance			
-Clean stables	7. Silage production area (ha)			
	Total production cost (R \$ / annual).			
2. Time and expenses with preventive measures (R\$/Mês):	8. Hay production area (ha)			
3. What mastitis control measures are used on	Total Production Cost (R \$ / annual)			
the property and frequency (weekly, biweekly	9. Production area of another crop (ha)			
or monthly): - Antibiogram and culture.	Observations regarding the area and cost of production and / maintenance;			
- Somatic cells account (SCC), bacterial total	10. Machine time used for feeding			
analysis	(hour / day):			
- Individual SCC - CMT	11. Mineral supplement offer. Quantity (grams / day) in lactating cows.			
4.Average of expenses with control				
measures(R\$/ Monthly):	Dry cow Management			

1. The decision to dry cow is based in:	- One week pre partum				
-Gestation time (previsão de dias faltantes para	- < one week prepartum				
o parto)	- > one week prepartum				
-Diecrease in milk yield	- NA				
-Score condition of animal	3.Which food is provided to prepartum				
-Lactation time	cows?				
	Pasture				
2. How many days before calving dry the	Silage				
cows?	Ration				
- 60 dias	4. After calving the cow's food change?				
- 45 dias	-Yes				
- <45 dias	-No				
-Don't use any criteria	5. What changed in the food of post postpartum cows?				
3. What strategy do you use to dry cows?	- Increase the ration (Kg/cow/month)				
-Abrupta	-Increase silage (Kg/cow/month)				
-Gradual					
-Use of intramamarine antibiotic	6.How long does the calves stay with the				
-Use of parenteral antibiotic	cow?				
-Use of sealant					
-Stop doing milking only	Milking Management				
-Stop doing milking only -Remove the feed	Milking Management 1. What is the average distance that cows go				
-Stop doing milking only -Remove the feed -Does nothing (wait for the cow to dry on its	Milking Management 1. What is the average distance that cows go through from stalls to milking parlor (m)?				
-Stop doing milking only -Remove the feed -Does nothing (wait for the cow to dry on its own)	Milking Management 1. What is the average distance that cows go through from stalls to milking parlor (m)? 2 Time spend to arrive at milking parlor: 4. Time for start milking				
-Stop doing milking only -Remove the feed -Does nothing (wait for the cow to dry on its own) 4Reduces the supply of food to dry cows?	Milking Management 1. What is the average distance that cows go through from stalls to milking parlor (m)? 2 Time spend to arrive at milking parlor: 4. Time for start milking 5. Time for start milking				
-Stop doing milking only -Remove the feed -Does nothing (wait for the cow to dry on its own) 4Reduces the supply of food to dry cows? -Yes	Milking Management What is the average distance that cows go through from stalls to milking parlor (m)? Time spend to arrive at milking parlor: Time for start milking Time for finalize milking: 				
-Stop doing milking only -Remove the feed -Does nothing (wait for the cow to dry on its own) 4Reduces the supply of food to dry cows? -Yes -No	 Milking Management 1. What is the average distance that cows go through from stalls to milking parlor (m)? 2 Time spend to arrive at milking parlor: 4. Time for start milking 5. Time for finalize milking: 6. Describe the waiting pen, clean, type of floors presence of cover and trough? 7 87 7 				
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-Stop doing milking only -Remove the feed -Does nothing (wait for the cow to dry on its own) 4Reduces the supply of food to dry cows? -Yes -No -Sim 5.What type of salt is offered to dry cows? -Specific salt for dry cow -The same as lactating cows -Other Pre and pos partum Management 1. Is there specifc location for prepartum ? -yes -No 2.When the gestation cows where conduced to his prepartum place?	Milking Management 1. What is the average distance that cows go through from stalls to milking parlor (m)? 2 Time spend to arrive at milking parlor: 4. Time for start milking 5. Time for finalize milking: 6. Describe the waiting pen, clean, type of floors, presence of cover and trough;7.87 7. 7. Describe the exit pen, cleanness type of floors, presence of cover and trough;				

Parallel without pit (conventional stable)3.How many cluster of liners are use in the milking:4.Number of milking/day:5. Mean of number of cows is milking

simultaneously:

ANNEX

Manuscript of Chapter 2 submitted to Tropical Animal Health and Production.

Tropical Animal Health and Production

Risk factors associated with mastitis in smallholder dairy farms in southeast Brazil. --Manuscript Draft--

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Corresponding Author:	Aline Callegari Silva Universidade de São Paulo Faculdade de Medicina Veterinária e Zootecnia Sao Paulo, SP BRAZIL				
Corresponding Author Secondary Information:					
Corresponding Author's Institution:	Universidade de São Paulo Faculdade de Medicina Veterinária e Zootecnia				
Corresponding Author's Secondary Institution:					
First Author:	Aline Callegari Silva				
First Author Secondary Information:					
Order of Authors:	Aline Callegari Silva				
	Richard. A. Laven, PhD				
	Nilson Roberti Benites, PhD				
Order of Authors Secondary Information:					
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Suggested Reviewers:	Marcos Bryan Heinemann, PhD, Professor, School of Veterinary Medicine and Animal Science, University of São Paulo (USP) marcosbryan@usp.br Prof. Marcos has expertise in veterinary epidemiology and mastitis. Camila Stefanie Fonseca de Oliveira, PhD, Professor, School of Veterinary Medicine. Federal University of Minas Gerais (UFMG) sfo.camila@gmail.com Profa. Camila has expertise in veterinary epidemiology and dairy productions system.				
Opposed Reviewers:					