José Alfredo Bran Agudelo

OCORRÊNCIA E FATORES EPIDEMIOLÓGICOS INDIVIDUAIS E DE REBANHO ASSOCIADOS À CLAUDICAÇÃO BOVINA EM REBANHOS LEITEIROS NO SUL DO BRASIL

Tese submetida ao Programa de Pós-Graduação em Agroecossistemas da Universidade Federal de Santa Catarina para a obtenção do Grau de Doutor em Agroecossistemas.

Orientadora: Profa. Dra. Maria José Hötzel.

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"Ocorrência e Fatores Epidemiológicos Individuais e de Rebanho Associados à Claudicação Bovina em Rebanhos Leiteiros no Sul do Brasil"

Por

JOSÉ ALFREDO BRAN AGUDELO

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The neo-Ionian defence of science against Eleatic metaphysics rests at bottom on their vindication of locomotion: if things can move, science is possible; if locomotion is impossible, science falls with it.

> The Logic of Locomotion, in: The Presocratic philosophers, Jonathan Barnes, 1982

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RESUMO

A presente pesquisa teve como objetivos avaliar a ocorrência e descrever os fatores epidemiológicos associados à apresentação de claudicação em vacas leiteiras, bem como analisar as perspectivas e ações dos agricultores em relação à ocorrência desse problema em bovinos leiteiros. Foram realizados três estudos em Paraná (50 granjas em confinamento visitadas uma vez) e Santa Catarina (44 granjas a pasto visitadas duas vezes). Todas as vacas lactantes no rebanho foram avaliadas para observar o escore de locomoção. Foram coletados dados em nível individual e de rebanho. Foi realizada uma entrevista com os agricultores com rebanhos a pasto. Foram descritos vários fatores epidemiológicos associados a claudicação bovina (baixo escore corporal, dias em leite, paridade). Outros fatores associados em nível do indivíduo só foram constatados em sistema a pasto (raça, lesões no casco) ou confinado (dias em leite, lesões na pele ao redor das articulações). Os rebanhos com predomínio de raça Holandês e os rebanhos que foram forçados a caminhar muito rápido, tiveram a maior incidência de claudicação nas granjas com acesso ao pasto. O uso de colchões como base do cubículo em freestall e instabilidade (escorregadio) do piso na área de alimentação das vacas foram os fatores associados com maior peso nas análises em rebanhos confinados. A través das entrevistas realizadas com agricultores foi observado que muitos agricultores não são treinados para identificar e fazer um adeguado manejo preventivo da claudicação, nem contam com assistência veterinária para resolver esse problema. Muitos agricultores pareciam não estar cientes da magnitude da ocorrência de claudicação nas suas propriedades. Existe uma grande oportunidade para diminuir a ocorrência de claudicação nesse tipo de rebanhos. Estrategias de manejo preventivo da claudicação, supervisão continua dos animais e provisão de conforto para as vacas são praticas que podem reduzir o problema em nível dos rebanhos. É recomendável aumentar o grau de conhecimento e habilidades que os agricultores das regiões visitadas têm em relação à etiologia, prevenção e manejo da claudicação.

Palavras-chave: Dor. Bem-estar Animal. Epidemiologia. Produção leiteira.

RESUMO EXPANDIDO

INTRODUÇÃO

A claudicação bovina é um sinal clínico caracterizado pela locomoção anormal. Esse problema é comum nos bovinos leiteiros e afeta seriamente sua saúde e bem-estar. As vacas claudicantes podem ter lesões dolorosas nas extremidades ou danos severos nos tecidos, redução no consumo de alimento, perda de peso, diminuição da produção e falhas reprodutivas que podem levar ao descarte prematuro. A alta ocorrência de claudicação afeta a rentabilidade dos rebanhos devido à diminuição da produção e aos custos dos tratamentos.

A claudicação pode ser causada por diferentes doenças, porém as doenças frequentemente associadas com a claudicação afetam principalmente os cascos ou os tecidos adjacentes às extremidades posteriores dos bovinos e são classificadas como desordens de origem infecciosa (dermatite digital, necrobacilose interdigital) e alterações produzidas por perturbações da formação do tecido córneo do casco (doença da linha branca, úlcera de sola).

Do ponto de vista populacional, diversos fatores têm sido associados à alta ocorrência de claudicação em bovinos leiteiros. Esses fatores são classificados como atributos dos indivíduos (idade ou raça, por exemplo) ou fatores em nível do rebanho como o manejo, a alimentação e o uso de protocolos de higiene e prevenção de afecções podais.

OBJETIVOS

A presente pesquisa teve como objetivos avaliar a ocorrência e descrever os fatores epidemiológicos associados à apresentação de claudicação em vacas leiteiras, bem como analisar as perspectivas e ações dos agricultores em relação à ocorrência desse problema em bovinos leiteiros.

MATERIAIS E MÉTODOS

Foram realizados dois estudos epidemiológicos do tipo transversal para avaliar a ocorrência e os fatores epidemiológicos associados à claudicação em bovinos leiteiros no oeste de Santa Catarina (SC) e na região de Castro no Paraná (PR). Adicionalmente, no oeste de Santa Catarina foram conduzidas entrevistas com os agricultores para conhecer suas opiniões, conhecimentos e práticas de manejo da claudicação em bovinos leiteiros. A amostragem das granjas foi realizada por conveniência.

Em Santa Catarina foram selecionadas 44 granjas de produção de leite com acesso a pasto (> 16 horas por dia). As unidades foram visitadas em duas ocasiões em 2015. Nessa região foram levantados dois indicadores de ocorrência: incidência acumulada no período entre cada visita e a prevalência em cada visita. No Paraná foi realizada uma visita em 50 granjas leiteiras em sistemas de Freestall e Compost-barn durante o ano 2016 e foi calculada a prevalência de claudicação nos rebanhos.

Em cada visita foram realizadas avaliações nos animais: escore de locomoção (1-5), registro de lesões superficiais de casco (SC) e lesões nas extremidades (PR), escore de condição corporal (1–5) e raça. Adicionalmente, foram realizadas avaliações no ambiente (salas de ordenha, corredores, áreas de alimentação e repouso dos animais, áreas de pastagem) usando uma lista de checagem e uma entrevista com os produtores para obter informações sobre manejo, produção e saúde dos animais. Os registros básicos de produção, reprodução e sanidade foram coletados nos rebanhos sempre que estiveram disponíveis. Em SC, a entrevista com os agricultores também incluiu um questionário com perguntas sobre os conhecimentos e ações que os agricultores tinham em relação à ocorrência de claudicação em seus rebanhos.

foram processados Os dados e analisados seguindo procedimentos comumente usados em epidemiologia para descrever e sintetizar informações de eventos de saúde de populações: elaboração de diagramas de causalidade, organização dos dados e eliminação de erros, descrição minuciosa de cada variável, análise lógica e matemática de vieses e de variáveis de confundimento e de intervenção, modelagem estatística com análise univariável (cada fator em relação a cada desfecho) e posteriormente multivariável (cada fator significante [P < 0.2] no teste univariável com cada desfecho). A redução dos modelos estatísticos foi realizada manualmente, descartando uma a uma as variáveis que não foram significantes (P < 0.05) e atualizando o modelo a cada vez. As análises estatísticas de inferência realizadas foram, na sua maioria, modelos mistos: regressões logísticas para análises em nível individual e regressões lineares para análises em nível do rebanho.

Em SC o principal desfecho utilizado nos modelos foi a incidência acumulada no período entre cada visita; em nível individual foi verificado se existia associação entre as variáveis explicativas e a

ocorrência de um novo caso de claudicação; em nível de rebanho foi analisada a associação entre as variáveis explicativas e a incidência acumulada em cada rebanho. Cada rebanho foi usado como fator aleatório na análise em nível individual para controlar o efeito de medições realizadas em grupos e para informar ao modelo a estrutura hierárquica do dado; o município onde cada granja estava localizada foi usado como fator aleatório em nível de rebanho para controlar o efeito de agrupação espacial das granjas.

A prevalência de claudicação em cada indivíduo e no rebanho foram os desfechos utilizados para cada nível de análise nos modelos estatísticos ajustados para os dados do PR. Nos modelos em nível do indivíduo foram usados os grupos (baias) de animais aninhados dentro de cada granja como fator aleatório para controlar o efeito da pseudorepetição, modelar o efeito de distribuição espacial e da organização hierárquica do dado. Foram ajustados múltiplos modelos em nível individual: um usando a população total avaliada e três diferentes modelos (por categorias de paridade) usando uma subamostra da população com dados de produção de leite, dias em leite e número de partos. Em nível do rebanho foi realizada uma regressão linear simples. Os modelos estatísticos foram construídos usando o software estatístico R e o pacote lme4.

RESULTADOS E DISCUSSÃO

Claudicação em vacas leiteiras em sistemas com acesso a pasto no Oeste de Santa Catarina

Os rebanhos tiveram, em média, 42 vacas em ordenha, variando de 28 a 74. A incidência acumulada de claudicação (1.110 vacas em 41 rebanhos) foi 29.6% e a prevalência (44 rebanhos) foi 31% (1.633 vacas) e 35% (1.836 vacas) na primeira e segunda visita, respectivamente. A ocorrência de claudicação foi relativamente alta nos rebanhos visitados, o que sugere que a claudicação é um problema presente em rebanhos a pasto e que estratégias de prevenção e controle em nível da população deveriam ser implementadas.

Os casos incidentes foram 4 vezes mais comuns em vacas Holandês do que em vacas Jersey e foram mais comuns em vacas com maior número de lactações (1.4 a 13.1 vezes mais em vacas multíparas do que em vacas de primeiro parto). Vacas com escore corporal baixo (2-2.75 e 3) e com lesões superficiais nos cascos apresentaram 1.1 a 4.7 vezes maior incidência de claudicação do que vacas com escore corporal

maior e vacas sem lesões. A alta incidência de claudicação em vacas com baixo escore corporal, lesões superficiais nos cascos e com idade avançada sugere que medidas de prevenção e controle devem ser aplicadas nesses indivíduos que são mais susceptíveis. O uso de casqueamento, a alimentação adequada das vacas em cada estágio da lactação e o tratamento oportuno são ações que podem ajudar a diminuir o impacto negativo da claudicação nesses rebanhos.

Os rebanhos de raça Holandês apresentaram, em média, 13.5% maior incidência acumulada de claudicação e para cada 1 km/h de aumento na velocidade de condução das vacas por parte dos agricultores a incidência de claudicação aumentou, em média, 5%. A presença de claudicação em animais e rebanhos de raça Holandês sugere que essa raça pode ser mais suscetível a doenças que produzem claudicação quando manejadas em sistemas de pastoreio. Igualmente, fatores de manejo do rebanho podem estar vinculados à alta ocorrência de claudicação nessa raça: o nível de produção e as estratégias de alimentação do rebanho e especialmente das vacas recém paridas, podem ser fatores que intermeiam a relação entre raça e claudicação. A condução do rebanho a alta velocidade pode estar associada a maior probabilidade de lesão nas extremidades dos animais, mas também pode ser um fator que reflita o cuidado no manejo dos animais por parte de cada agricultor.

Claudicação em vacas leiteiras em sistemas com acesso a pasto: perspectivas e ações dos agricultores no Oeste de Santa Catarina

Nos sistemas de produção com acesso a pasto, os agricultores praticaram poucas medidas preventivas e de controle (casqueamento, uso preventivo de pedilúvios, registro de casos e tratamentos) para doenças que causam claudicação. A maioria dos agricultores (43 em 44) reportou ter tratado em algum momento animais que apresentavam claudicação no seu rebanho. Foi mencionado frequentemente o uso de antibióticos, casqueamentos e produtos de aplicação direta no casco (spray, unguentos) para tratar animais claudicantes. A abordagem terapêutica parece estar baseada preferencialmente no tratamento de casos isolados e em menor medida em estratégias de prevenção e controle em nível de população. Muitos dos agricultores visitados tiveram acesso a serviços e orientação veterinária (pública, da indústria de laticínios, ou privada), porém em nenhum caso mencionaram ter participado em processos de formação ou ter assistência técnica maneira geral, os agricultores subestimaram a prevalência de claudicação em seus rebanhos, porém, na segunda visita reportaram valores de prevalência de claudicação severa similares aos reportados pelos pesquisadores que avaliaram a locomoção das vacas. Dezessete agricultores reportaram o mesmo número de vacas com claudicação que foi estimada pelo veterinário como claudicação severa (ICC = 0.8). O fato dos agricultores subestimarem a claudicação pode refletir pouca motivação ou interesse no problema, ou mínimo conhecimento dos impactos do problema na saúde e bem-estar dos animais, talvez como reflexo de pouco acesso a treinamento, informação e orientação técnica nessa área. Poucos agricultores mencionaram que a claudicação fosse o primeiro problema de saúde em seus rebanhos, porém, mencionaram frequentemente que era um dos três problemas de saúde animal mais comuns nos seus rebanhos. Além da claudicação, mastite e falha reprodutiva foram mencionados como importantes problemas de saúde nos rebanhos. Esse achado pode refletir a ordem de priorização dos problemas de saúde do rebanho; assim, se múltiplos problemas de saúde acontecem no rebanho, as estratégias de controle podem ser focadas em problemas mais urgentes e prioritários para cada agricultor. Dessa maneira, um problema menos conhecido e pouco enxergado, pode ser negligenciado. Por outro lado, isso pode sugerir a falta de uma estratégia global de prevenção e controle de doenças nos rebanhos, o que acaba impedindo a visualização das relações entre cada problema de saúde e o bem-estar no rebanho (claudicação e falha reprodutiva podem estar relacionadas) e a aplicação de medidas preventivas padrão (higiene, adequada alimentação, tratamento oportuno de casos, por exemplo) que podem ajudar no controle de diferentes problemas.

Claudicação em vacas leiteiras em sistemas confinados no Paraná

Foram visitadas 38 granjas usando sistema freestall e 12 que utilizavam compost-barn, totalizando 13.706 vacas. Cada granja teve, em média, 274 vacas em lactação, variando entre 41 a 901. A prevalência média de claudicação nos rebanhos foi 41%. Os fatores associados com alta frequência de claudicação nas vacas foram o baixo escore corporal, a presença de lesões de pele nas extremidades (região do carpo e do tarso) e o estágio da lactação.

Os seguintes fatores estiveram associados à maior prevalência de claudicação (acima da média estimada pelo modelo: 17.32%) nos rebanhos: a presença de pisos escorregadios nas áreas de alimentação das vacas (6.6% a 12.4%), o uso de colchões nas camas das vacas em

Freestall (14%), o tamanho do rebanho (8.5% a 11.6% maior em rebanhos com mais de 141 vacas) e o período de secagem das vacas (6.8% a mais em rebanhos com período < 60 dias). Realizar alimentação adequada das vacas para evitar o baixo escore corporal, controlar os fatores que geram lesões superficiais nas extremidades (material da cama, tamanho dos cubículos e limpeza das camas), e a aplicação de medidas preventivas para claudicação, especialmente em novilhas e vacas primíparas, são importantes medidas para reduzir o impacto negativo da claudicação nas vacas. O uso de camas confortáveis nos cubículos e o desenho adequado dos pisos são aspectos relacionadas à estrutura da granja que podem reduzir a prevalência de claudicação nos rebanhos visitados. O tamanho de rebanho é um fator que pode refletir a intervenção de outros fatores na prevalência de claudicação; consequentemente, não deve existir relação causal nessa associação. As diferenças na prevalência de claudicação em vacas com diferentes períodos de secagem podem ser reflexo do manejo das vacas secas, bem como o nível de produção de cada granja; assim, ambos fatores podem ter grande impacto na prevalência de claudicação nos rebanhos.

CONCLUSÕES

A claudicação parece ser um problema comum nos rebanhos e regiões estudadas. A grande variação da ocorrência de claudicação observada entre granjas, municípios e regiões evidencia que práticas de manejo realizadas pelos agricultores em cada lugar podem influenciar a ocorrência do problema e que há uma grande oportunidade para prevenir, controlar e diminuir os impactos negativos desse problema na saúde e bem-estar dos animais e na rentabilidade dos estabelecimentos leiteiros.

Palavras-chave: Dor. Bem-estar animal. Epidemiologia. Produção leiteira. Manqueira.

ABSTRACT

Lameness is a clinical sign characterized by locomotion disturbance in dairy cows. Lameness affects the health and welfare of dairy cattle. The aim of this study was to assess the occurrence and associated risk factors to lameness in smallholders grazing dairy herds and in freestall and compost-bedded barns. Additionally, we explore the farmers' perspectives and actions regarding lameness in their (grazing) herds. The three studies had an epidemiological approach. The studies were conducted in two different geographical regions: forty four grazing dairy farms located in the south of Brazil were visited twice in 2015 and 50 freestall and compost-bedded pack farms were visited once in 2016. All lactating cows present at the moment of the visits were gait scored by a single researcher. Individual-level attributes (e.g., body condition score, milk yield, days in milk, superficial hoof lesions) were collected. A face to face interview was conducted with farmers at the time of the visits. We found some associations between cow- and herd-level variables and lameness that were similar between different dairy systems (confined and grazing), i.e., low body condition score, parity. Other cow-level variables such as breed, and some leg and hoof abnormalities were detected in specific systems, in part due to the sample characteristics. Due to the greater sample size that we explored in the confined dairies we modeled different patterns of lameness distribution in cows across days in milk and parity. We also identified herd-level variables associated with lameness that were different by each housing system. Holstein herds and herds where cows were forced to move to the milking parlor at excessive speeds were associated with the highest incidence of clinical lameness in grazing herds. The use of mattress on freestall cubicles as stall base and the presence of slippery surfaces at the cow-feed alley were the predictors with stronger association with lameness in confined herds. Through the survey conducted with the farmers we found that most of them had no training on lameness management, and cited an overall lack of veterinary support to control lameness on their farms. The farmers seemed unaware of the extent of lameness on their farms. Interventions aimed at reducing lameness in large confined and small scale herds in this region of Brazil should include a preventative veterinary assistance approach focused, in controlling lameness in high risk individuals, promoting cow comfort and the use of animal welfare protocols focused in improving supervision of cow health by the farmers and suitable actions to control and prevent lameness. In addition, to increase farmers' knowledge and awareness on lameness is a very important point to improve on this kind of herds.

Keywords: Pain. Animal welfare. Epidemiology. Dairy

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OVERVIEW

The present manuscript is composed by three chapters with data derived from two observational cross-sectional studies and one crosssectional survey. The studies were conducted in two different regions located in the south of Brazil (Paraná and Santa Catarina States). Both regions are important in terms of dairy industry development in Brazil. For our knowledge, this is the first great epidemiological study on lameness in dairy cows conducted in Brazil.

The fist chapter describes the results of a cross-sectional study designed to assess the occurrence and the associations with lameness in cows in small-scale grazing dairy herds. This chapter was published as a research paper on the Journal Preventive Veterinary Medicine (BRAN et al., 2018b). The data and R-code that was used to run the main analyses of the study have also been published (BRAN et al., 2018d).

The second chapter describes the results of cross-sectional survey that was designed to investigate the farmers' perspectives and actions regarding lameness occurrence, prevention and control at the same farms where the lameness occurrence was assessed (i.e., first study). This chapter has been published in the Journal Preventive Veterinary Medicine (BRAN et al., 2018a). The data and R-code that was used to run the main analyses of the study have been published (BRAN et al., 2018c).

The third chapter describes the results of a cross-sectional study designed to assess the occurrence and the associations with lameness in dairy cows and herds in small, medium and large dairies using freestall and compost-bedded barns, located in Paraná State. This chapter is in publication process.

During the study process, the research team also focused efforts in working together with farmers and dairy industry stakeholders (public and private). We were able to socialize the research with people involved in dairy industry at the regions and mainly with the farmers. Didactic material and several meetings were conducted at the regions in order to be coherent with the scientific principle of humanism and social retribution of science to the communities. On-line information for extension purposes was disclosed and a permanent link is active: http://cartilhaonline.wixsite.com/letaufsc/bemestaranimal.

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1 INTRODUCTION

Lameness is a common issue that impairs health and welfare of dairy cattle. Cows affected by lameness have low dry matter intake and milk yield (BACH et al., 2007; BICALHO; WARNICK; GUARD, 2008), low risk of pregnancy (ALAWNEH; LAVEN; STEVENSON, 2011) and high risk of being culled (BICALHO et al., 2007). Economic losses in the affected herds are associated with treatment costs and specially to reduction of productivity (BRUIJNIS; HOGEVEEN; STASSEN, 2013; HUXLEY, 2013).

Lameness is a clinical sign characterized by locomotion disturbance in dairy cows (<u>O'CALLAGHAN</u>, 2002). Gait pattern modification in lame cows results from a compensatory posture, or adjusts in weight distribution during gait, in response to inadequate corporal balance, lesions, or pain associated mainly with orthopedic origin (hooves, joints, muscles). Thus, visual indicators are commonly used in order to assess if an individual is affected: the symmetry of limb's movement, gait's rhythm and speed, weight bearing on extremities, or abnormal postures (FLOWER; WEARY, 2009).

Most cases of lameness originate in lesions of the lateral claws on the hind feet (BLOWEY; WEAVER, 2011; POTTERTON et al., 2012). This pattern might be explained partly by the overloading of the softer parts of the lateral hind claws on normal gait (VAN DER TOL et al., 2002), by the asymmetric nature of bovine toes (lateral toes are longer than medial and, on hard surfaces, receive more weight) (MUGGLI et al., 2011) and by the changing padding capacity of digital cushion in different ages and days of lactation (lower padding in old cows and around lactation peak) (RÄBER et al., 2004; LIM et al., 2015).

Lameness may be the consequence of multiple diseases and some infectious agents have been identified as causes of lameness (REFAAI et al., 2013), but also non infectious causes of lameness have been described. The action of traumatic forces might affect selectively the limbs of individuals in higher risk (i.e., old cows on weeks around calving) and result in foot lameness (GREEN et al., 2014; LIM et al., 2015; RANDALL et al., 2015). The etiology of lameness is multifactorial and complex, but, in general, the interaction of two main factors appears to strongly influence the dynamics of non infectious lameness related to claw horn disruption: cow comfort issues, mainly related to environmental variables (i.e., inadequate size of facilities, overstocking, inappropriate floor, paths and surfaces lying

characteristics) (BUROW et al., 2014; SOLANO et al., 2015, 2016; RANJBAR et al., 2016; WESTIN et al., 2016a) and the relationship between physiological changes during transition period and nutritional management of the herd (i.e., fast loss of body weight and deficient feeding of cows around calving) (DIPPEL et al., 2009; BICALHO; OIKONOMOU, 2013; ALAWNEH et al., 2014).

Access to pasture or loafing areas has been reported as protective factors for lameness in confined dairy cows (HERNANDEZ-MENDO et al., 2007; OLMOS et al., 2009a; GARD et al., 2015). However, studies assessing lameness in grazing dairy herds, are scarce. Possible protective effect of grazing for lameness, might be due to reduction of risk for specific lesions or diseases (i.e., sole ulcer), but, in turn, some factors in grazing systems may potentially increase the probability of lameness occurrence.

Condition of paths, heat stress, or other comfort-related issues could be trigger factors increasing the occurrence of lameness by enhancing risk factors for diseases like white line disease, foot root or digital dermatitis. In fact, hoof lesions such as white line disease, sole injury and axial disease, seems to be more common in grazing cows (LAWRENCE; CHESTERTON; LAVEN, 2011) and sole damage (i.e., sole ulcer, double sole) has been found commonly in housed systems, if compared with grazing systems (NAVARRO; GREEN; TADICH, 2013). Also, access to pasture in cows housed in tie stall was associated with higher presence of digital dermatitis, white line separation and interdigital fibroma (CRAMER et al., 2009). Hence the risk factors for lameness, or the relative importance of specific exposures might differ between housing systems.

1.1 STUDY OBJECTIVES

The present study aimed to a) investigate the occurrence of lameness in dairy herds in southern Brazil and b) to describe the associated risk factors both at the cow- and herd-level. Additionally, c) we explored the way that farmers deal with lameness (i.e., prevention and control) in dairy cows and how aware they were regarding lameness occurrence and impacts at their farms.

2 LAMENESS IN DAIRY COWS: A BRIEF REVIEW

Lameness in cattle is the manifestation of abnormal locomotion, that is often associated with tissue damage, pain and discomfort (O'CALLAGHAN, 2002). However, lameness is not always an issue restricted to pain and evident tissue damage. Although most cases of lameness might be associated with pain or tissue damage, in some instances there is no evidence of macroscopic lesions and pain is not always demonstrable in lame cows; also, claw lesions are present in non-lame cows (DYER et al., 2007; TADICH; FLOR; GREEN, 2010).

In the cases where pain or macroscopic tissue damage cannot be demonstrated, lameness is still a problem: postural or gait dysfunctions might be a primary problem which affects all the perceptual-behavioral repertory (dry matter intake, resting, socialization) of the animal and may lead to secondary problems derived from muscular or articular overuse and underuse in different body parts (the back, contralateral or ipsilateral structures to affected limb may be affected). Also, uneasy venous return on extremities, pain on different structures than affected limb (i.e., back pain), chronic degeneration of structures, atrophy and compensatory hypertrophy of muscles and articular structures might be dysfunctions resulting from postural abnormalities.

Pain is an important issue associated with lameness, but some physiopathological alterations of nociceptive threshold, the subjectivity associated with pain experience, the neural plasticity, or the multiple forms of pain (MILLMAN, 2013) (i.e., acute or chronic, nociceptive or inflammatory, pathological or physiological) might be also taken in consideration when assessing the relationship between lameness and pain. Allodynia and hyperalgesia, for example, might be present in a limping cow (O'CALLAGHAN, 2002; NAVARRO; GREEN; TADICH, 2013; SHEARER, 2017) and the health and welfare consequences for individuals with those problems are also as important responses of individuals with as the unaltered nociception (PRESCOTT; MA; DE KONINCK, 2014).

In addition, information about automatic or objective pain quantification in lame dairy cattle is still scarce and most of the information about lameness is derived from visual locomotion assessment. This is a main issue when assessing and interpreting the results of lameness studies derived from visual assessment since inferences about nociception are hardly extracted without using deep clinical exploration of the lame individual (DYER et al., 2007; BLACKIE et al., 2013). In addition locomotion scores are subjective and different scores are adopted by researchers which may limit the comparisons between studies conducted in diverse conditions and contexts.

The ongoing challenges associated with high rates of lameness in cows are of great concern for the global dairy industry. This malady negatively impacts animal health, animal welfare, milk production and the herds' economic performance (GREEN, 2012; GREEN et al., 2014). Considering the magnitude of this problem, and the fact that lame cows are in pain (COETZEE et al., 2017) this phenomenon constitute a serious animal welfare problem at the population level. Lameness is often used as a main indicator of dairy cow welfare status on farms in many assurance programs, since it is a multicausal issue (GREEN, 2012). Thus, any intervention that can control this problem should impact the overall status of a dairy cows' health and welfare.

The main causes of lameness in dairy cows are claw horn disruption diseases (e.g., sole ulcer or white line separation) and infectious diseases affecting the foot (e.g., digital dermatitis, interdigital necrobacillosis) (HUXLEY et al., 2012). Claw horn disruption diseases are affected by the physiological effects happening around calving, for instance, increased laxity of the hoof suspensory apparatus (TARLTON et al., 2002), lactogenesis and decreases in body condition score (BCS), and reduced thickness of sole soft tissues (digital cushion and corium) (NEWSOME et al., 2017a, 2017b).

Additionally, biomechanical issues affecting the horn and the suspensory apparatus of the third phalanx, or any factor that potentially increases friction by excessive hoof wear and by abnormal weight distribution can predispose cows to claw injuries. Claw lesions are strongly associated with contusions within the horn (BICALHO; MACHADO; CAIXETA, 2009) that may affect the germinal epithelium that produces the horn, causing a disruption in the normal horn formation. The lateral hind claws are commonly affected by lesions, given that these structures normally support greater pressure (VAN DER TOL et al., 2002).

The causal pathways linking the specific conditions with the occurrence of lameness are mediated by diverse environmental factors and specific characteristics of the individual cows, which are continuously changing over time and thus may be linked to greater susceptibility periods, or places with greater exposures to the risk factors. Some farm characteristics such as management practices and

individual features associated with the occurrence of lameness in dairy cows have been described in different populations (CHAPINAL et al., 2014; SOLANO et al., 2015; NEWSOME et al., 2017a). Overall, any environmental factor that may cause, or increase the risk of hoof infection (e.g., presence of the infectious agent, exposure of cows to dirt and wet floors), or causes excessive stress and overloading of hooves in the cows (e.g., exposure to hard lying or walking surfaces, hoof overgrowth) may potentially increase the occurrence of lameness.

2.1 LAMENESS IMPACTS IN DAIRY HERDS

Some of the secondary problems associated with lameness in dairy cows are: a) reduction of dry matter intake and milk yield (BACH et al., 2007; BICALHO; WARNICK; GUARD, 2008); b) decrease of the risk of pregnancy (BICALHO et al., 2007; ALAWNEH; LAVEN; STEVENSON, 2011); c) cows affected by lameness also have high risk of being culled prematurely (<u>BICALHO et al., 2007</u>) due to lower production, or due to animal welfare considerations; d) also, there is considerable increase in the cost of treatments of lame cows (BRUIJNIS; HOGEVEEN; STASSEN, 2013) in farms affected by lameness.

Lame cows reduce both feed intake and the time they spent feeding (NORRING et al., 2014). Milk losses due to lameness were estimated to be between 314 and 424 kg/cow per 305 days lactation (BICALHO; WARNICK; GUARD, 2008). Severe lame cows have their potential (305 days in milk) yield reduced by 350 kg or 650 kg if they are observed lame in the first or the first two consecutive months of lactation, respectively (ARCHER; GREEN; HUXLEY, 2010). In addition, it has been reported reductions of milk yield between 0.5 to 1.5 kg/day after lameness diagnosis (WARNICK et al., 2001). However, the decrease in milk yield is not easily observed immediately before or after the lameness event, which might mask the effect of lameness on production when assessed by the farmers or their advisers. The greatest impact of lameness in production is associated with milk yield reduction in the whole lactation (ARCHER; GREEN; HUXLEY, 2010) which requires an in deep exploration of herd performance. Treating lame cows, however, is associated with increases in milk yield (ARCHER; GREEN: HUXLEY, 2010).

The daily risk of conception for lame grazing cows decreased by a factor of 0.78, which means that lame cows take more 12 days for became pregnant than unaffected cows (ALAWNEH; LAVEN; STEVENSON, 2011). Additionally, lame cows are at a 15% lower risk of pregnancy than nonlame cows (BICALHO et al., 2007).

Some aspects of housing system, or different herd level characteristics ("herd management") are important modulators of lameness occurrence. Some individual features of dairy cows such as age, milk yield, body conformation, or the rate of body condition score loss, may be important risk factors for lameness. The dynamic interaction between different factors on those complementary levels of analysis (population and individual) determines the occurrence of lameness in dairy herds. Hence, both levels should be considered in order to better understand the dynamics of lameness in dairy herds.

2.2 LAMENESS CLASSIFICATIONS AND RISK FACTORS

There are different classifications of lameness in dairy cows, however, those categories are not necessarily exclusive or rigid. Given the multi causal, progressive and dynamic characteristics of some conditions that cause lameness, most definitions can be complementary, or may express the dynamics of progression of a specific disease; i.e, lameness in the foot may affect muscles, joints and nerves and so, a lameness case that was originated in the hoof might compromise extrahoof structures.

Most lameness events have a relationship with hoof disorders, thus, it is usual to classify lameness by an anatomical criteria as foot lameness and upper limb lameness (BLOWEY; WEAVER, 2011). Other common classification of lameness is based on their etiology, as infectious and non-infectious: most of those being associated with hoof lesions (HUXLEY et al., 2012; REFAAI et al., 2013). Infectious agents might grow on the hoof, or on the skin around it, and result in foot lameness. Environmental conditions such as high soil moisture and lack of hygiene on stalls or floors might be important reservoirs of pathogens that infects the hoof. On the other hand, non infectious agents like traumatic forces interact with metabolic and physiological status of the cows to induce foot lameness associated with claw horn disruption lesions (CHDL). Two main factors appear to strongly influence the occurrence of non infectious lameness: cow comfort issues, mainly related to environmental variables (SOLANO et al., 2016; WESTIN et al., 2016a) and the relationship between physiological changes that

occur during the transition period (four weeks around the parturition) and the nutritional management of the herd (DIPPEL et al., 2009).

2.3 COW COMFORT ISSUES: LYING, STANDING, AND FACILITIE'S DESIGN AND CONDITION

Excessive or prolonged stress induced by forces acting on the hooves or other body structures responsible for cows' movement (muscles, joints, nerves), might impact the normal locomotion of dairy cows. Lying behavior alterations are associated with lameness in dairy cows in two opposite ways: like a possible cause and as a consequence. Evidence for the last effect is strongly because information from crosssectional studies on lameness is found frequently, but analysis of lying behavior preceding the event of lameness is lacking. However is accepted that any affecting normal lying time or lying comfort (i.e., high temperature, uncomfortable surfaces for lying, overstocking) might enhance the stress over the hooves and, eventually, may result in CHDL and lameness.

Decreasing lying comfort and observation of abnormal lying postures are also associated with increases in lameness occurrence (DIPPEL et al., 2009). Lame cows have longer lying times and fewer and longer lying bouts than nonlame cows (ITO et al., 2010; SEPÚLVEDA-VARAS; WEARY; VON KEYSERLINGK, 2014; WESTIN et al., 2016a). Also herds with higher lameness prevalence have longer mean daily lying time (SOLANO et al., 2016). The prolonged lying time in lame cows might be a response to discomfort and pain associated with lameness and may be responsible for most part of the reduction in feeding and weight lost in lame cows.

Allowing the cows to access comfortable facilities seems to increase lying times in cows with limitations for lying, and this can be a protective factor for lameness, possibly by reducing the trauma or concussion of the hoof and by avoiding venous stasis or higher hydrostatic pressure on the feet of the cow. Offering more space on stall (\geq 114 cm wide), feed alleys (\geq 350 cm wide), as well as using sand (WESTIN et al., 2016a) or higher depth of bedding materials on stalls is associated with increases in the cows' lying time (SOLANO et al., 2016). The use of mattress on stalls is associated with higher lameness occurrence (ITO et al., 2010). Bedding material seems to be an important comfort issue for claw health in dairy cows. The prevalence of claw lesions was less common in compost-bedded packs barns (CB)

than on free-stall (F) housed herds for heel horn erosion (26.9% CB and 59.5% in F), white line disease (20.4% CB and 46.6% in F) and interdigital hyperplasia (0.2% in C and 3.1% in F) (BURGSTALLER et al., 2016). The authors of that study argue that lower lesions might be observed in compost barn herds as a result of softer, dried ground and subsequent higher cleanliness of hooves on that system. The lameness prevalence, however, was the same on both housing systems.

The relationship between body size and facilities dimensions seems to influence cow comfort on resting, moving, standing or lying. Prolonged exposition to uneven or uncomfortable facilities might influence the posture of cows and, eventually, enhance the occurrence of lameness. Not fitting the average stall width increases the odds of being lame 3.7 and 1.3 times in primiparous and multiparous cows, respectively (WESTIN et al., 2016a). Cows on this conditions might be in constant uncomfortable conditions and may be unable of lying and rest appropriately.

Overstocking may be a factor influencing the stress on the cows' foot. Increasing the available space for cows in holding yard has been associated with lower levels of lameness (RANJBAR et al., 2016). Inappropriate condition of structures or floors are also factors that might enhance the occurrence of lameness. Presence of damaged concrete on yards, or behaviors derived of inadequate fit of cows to facilities such as cows pushing each other or turning sharply near the parlor entrance, or exit, are associated with higher presentation of lameness (BARKER et al., 2010). The condition of paths or access areas to facilities might negatively affect the occurrence of lameness. The probability of severe lameness increased with no (4 times) or partly prepared (3.8 times) cover compared to prepared cover (BUROW et al., 2014).

2.4 EFFECTS OF NUTRITIONAL MANAGEMENT, MILK YIELD, BODY CONDITION SCORE AND PHYSIOLOGICAL STATUS OF THE TRANSITION COW ON LAMENESS

Two main risk factors for lameness associated with nutrition management of dairy cows have been identified: high milk production in early lactation and low body condition score (BCS) (BICALHO; MACHADO; CAIXETA, 2009; ALAWNEH et al., 2014; RANDALL et al., 2015). Apparently those factors may be intercorrelated. Cows that became lame produced more milk than control cows from the start of the lactation up to week 15 of lactation (<u>BICALHO; WARNICK;</u>

<u>GUARD, 2008</u>). In addition, cows that became lame also had loss of BCS in the first 4 (RANDALL et al., 2015) or between the 4-10 (HOEDEMAKER; PRANGE; GUNDELACH, 2009) weeks of lactation. Low BCS (<2, 3 weeks before) precedes repeated, or first lifetime (8-16 weeks after first parturition) lameness event in dairy cows (<u>RANDALL et al., 2015</u>). The association between BCS and lameness is present in different moments of lactation: cows with low BCS (2.75) at drying-off have greater occurrence of lameness (FODITSCH et al., 2016) and cows with BCS < 3 had greater occurrence of claw horn disruption lesions (CHDL), such as white line disease and sole ulcer (MACHADO et al., 2010).

Overall, the reduction in BCS is an indicator of body weight loss. In general, this association have two directions. Lame cows also lose weight (NORRING et al., 2014), due to the reduction in dry matter consumption, thus, body weight loss is, at the same time, an event that precedes the occurrence of new and chronic lameness cases, as well as a consequence of chronic cases of lameness. That fact constitutes a challenge for establishing causal relationships in cross-sectional studies. Loss of BCS increases the probability of lameness and decreases the likelihood of recovery over the next 15 days after the event (LIM et al., 2015), thus is not just the status of the BCS in a specific time, but also the dynamics of change of BCS (reflecting a measure of body weight loss and energy mobilization) (ALAWNEH et al., 2014) that contributes with the occurrence and persistence of lameness. That dynamics is also bidirectional: gain in BCS seems to be a protective factor for lameness (LIM et al., 2015).

The susceptibility of cows to loss BCS may be influenced by genetic selection, thus, cows predisposed to maintain good levels of body condition score seems to be less susceptible to have locomotion and lameness problems (KOUGIOUMTZIS et al., 2014). Selection for higher milk yield and protein production has been associated with specific claw lesions such as white line disease, sole ulcer and digital dermatitis (OIKONOMOU; COOK; BICALHO, 2013). This opens the possibility of thinking in genetic selection focused in hoof health in dairy cows as an interesting strategy to reduce lameness in dairy herds. Keeping an optimal BCS at critical periods of lactation curve, like dry off and transition period, seems to be important protective factors for non-infectious lameness.

Lactating cows experience radical metabolic changes throughout their productive life, specially on critical periods like the transition. Those metabolic changes seems to have an important role on the physiopathology of non-infectious lameness. There is a positive association of BCS and digital cushion thickness (DCT) because a cow that is losing body weight mobilizes fat from all tissues, including the digital cushion (GREEN et al., 2014). The diminution of the digital cushion thickness might reduce the mechanisms of hoof damping and facilitate the traumatic lesions of the foot (RÄBER et al., 2004, 2006). Although there is not a specific causal demonstration of the relationship between DCT and lameness, their association seems to be strong (BICALHO; MACHADO; CAIXETA, 2009). Also, it seems to exists temporary association between the two events. Cows are more likely to become lame between 91-120 days in lactation an this coincides with the period where DCT is lower (LIM et al., 2015).

This way, like happens with another health issues in dairy cattle, cows seems to be more susceptible to non infectious lameness in the period around the parturition. Assuming a standard lactation curve, cows are more susceptible to reduce BCS on the first weeks of lactation. Cows with higher milk yield (on peak of lactation or high producer cows) demand more energy and mobilizes more fat from body reserves than other animals in order to supply mammary gland production. The risk of being lame was 4.4 times greater in high yielding grazing cows that lost live weight on first 50 days in milk, than in cows with lower yield. Thus, it has been proposed that high milk production on first weeks of lactation is a risk factor for lameness (OIKONOMOU; COOK; BICALHO, 2013; GREEN et al., 2014).

The association between milk yield and lameness is also bidirectional and dynamic (time-dependent): cows with greater milk yield are prone to became lame, and after became lame, milk yield is reduced, but the daily production increases again after the cow is treated early (LEACH et al., 2012). Because the BCS loss and higher milk yield are both indicators that reflect the metabolic status of the cow, lameness events seems to result as a consequence of the interaction between those and other risk factors such as parity, age, days in milk, genetic selection, comfort issues like individual daily lying time or inadequate lying behavior.

Given that multiple diseases or lesions may cause lameness, is important to know the risk factors for specific diseases or hoof lesions causing lameness (LAWRENCE; CHESTERTON; LAVEN, 2011). The risk factors for single lesions are more specific than the broad spectrum of lameness risk factors and thus are easier to identify (DIPPEL et al., 2009). Studies assessing those specific risk factors for diseases causing lameness are scarce, but it seems that the relationship between milk production, BCS and digital cushion thinness is specifically associated with hoof horn dysfunction (i.e., CHDL) (MACHADO et al., 2010). Low BCS (< 2.5) is a risk factor for the principal non-infectious claw diseases: sole ulcer, white line disease and sole hemorrhages (GREEN et al., 2014).

Most lame cows are affected by hoof lesions located in the lateral claw of hind feet (Color Atlas of Diseases and Disorders of Cattle, 2011; HUXLEY et al., 2012). Thus, lameness associated with claw horn dysfunction seems to have a strong relationship with postural and mechanical forces acting on the skeleton and soft tissues of the cows. An important issue for understanding this dynamics is the asymmetry artiodactyls' toe. Lateral toes are longer than medial toes in hooves of wild (KELLER et al., 2009) and domestic even-toed ungulates (MUGGLI et al., 2011). This might be a possible adaptation that helps to stabilize the body of the animal on soft floors, like natural habitats of bovines (MUGGLI et al., 2011). However, when bovines stand for a long time in hard floors, this anatomical configuration may lead to compression and reduced perfusion of the corium and may increase the occurrence of claw horn diseases such as sole ulcer (MUGGLI et al., 2011) as well as hypertrophy and deformation of the outer hoof (KELLER et al., 2009). Thus, dairy cows with access to comfortable floor surfaces to stand and with provision of suitable areas and conditions to lying in a proper manner should be at least risk of having claw disorders and lameness.

2.5 GRAZING, OUTDOOR ACCESS AND LAMENESS

Let the cows having access to pasture or to outdoor loafing areas in confined herds might be a protective factor for lameness (DIPPEL et al., 2009; ADAMS et al., 2017). Dairy cows housed in free-stall had improvement in gait score when had access to pasture for four weeks (HERNANDEZ-MENDO et al., 2007). Also, in a cross-sectional study was noticed that cows with access to pasture had less severe lameness prevalence than animals housed in free-stall or open dry lots (ADAMS et al., 2017). Additionally, the lameness occurrence seems to be lower in some studies conducted in populations of grazing dairy cows, if compared with the observed prevalence in some confined systems. A study on dairy cows (one farm) in New Zealand reported a lameness incidence of 13% (ALAWNEH et al., 2014). The average lameness prevalence in grazing dairy herds in Australia was 19% (RANJBAR et al., 2016). However, lower lameness prevalence has been also reported recently in confined dairies in North America: 15% (WESTIN et al., 2016b) 14% (FODITSCH et al., 2016) 9.6% (ADAMS et al., 2017). Moreover, the variation in lameness occurrence between farms is high in the same study and studies in grazing systems are less common than studies conducted in confined systems which may be a source of bias for judging the relationship between lameness and housing.

The presence of many biological interactions and the multiple factors that contribute to the occurrence of lameness difficult to make inferences on housing systems (VON KEYSERLINGK; WEARY, 2017); thus, in order to make this comparison valid, it is important to consider potential confounders or intervening variables such as herd size, management, specific aspects of facility design and the use of effective preventive practices for lameness. Inferring from studies when comparisons are done at the population level, without considering the individual factors that contribute to the occurrence of lameness, may also be difficult, in large part, because there are multiple sources of bias mediating the associations at different levels of analyses (e.g., "ecological fallacy"). In addition, if the structure (e.g., parity, days in lactation of cows), spatial distribution (e.g., the hierarchical and heterogeneous division of population in herds and pens), and size of the study population is not considered, additional biases may exist further limiting the validity of the analyses; minimizing the value of the results to inform the public on practical implications of the study.

Thus, a direct comparison between confined and pasture housing, might not be fair, appropriate, or useful in terms of animal welfare and health improvement. On the other side, dairy grazing systems are not standardized as can be other housing systems and strong differences on weather, seasonal variation of feed supply, feeding practices and management, breeds, herd size and other factors might be present under the same classification of grazing housing system.

Possible protective effects of grazing for diseases causing lameness, might be mediated by the reduction on risk for specific conditions (i.e., sole ulcer), but, in turn, some factors that are common in grazing systems may potentially increase the probability of lameness occurrence. In fact, claw lesions such as white line disease, sole injury and axial disease, seems to be proportionally more frequent in grazing cows (LAWRENCE; CHESTERTON; LAVEN, 2011) while hoof abnormalities such as sole damage (i.e., sole ulcer, double sole) has been found commonly in housed systems, if compared with grazing systems (NAVARRO; GREEN; TADICH, 2013). Access to pasture in cows housed in tie stall was associated with higher presence of digital dermatitis, white line separation and interdigital fibroma (CRAMER et al., 2009). Thus, the condition of paths, heat stress, or other comfortrelated issues could be trigger factors increasing the lameness occurrence by enhancing risk factors for diseases like white line disease, foot root or digital dermatitis in grazing dairy cows.

Lying comfort has been mentioned as a protective factor for lameness in grazing cows. When compared with confined systems, higher (OLMOS et al., 2009a) but also lower (HERNANDEZ-MENDO et al., 2007) lying times have been reported in grazing cows under experimental conditions, but information about lying behavior in cows at pasture is limited. A study assessing lying time in grazing and confined herds showed that cows on both housing systems had equal lying times (NAVARRO; GREEN; TADICH, 2013). A mean daily lying time of 11 h (VON KEYSERLINGK et al., 2012), 10.6 h (SOLANO et al., 2016) and 11.4 h (WESTIN et al., 2016a) have been reported in dairy cows housed in confined systems. The mean daily lying times reported for grazing cows were 7.5 h (for primiparous cows), 8.5 h (for multiparous cows) (SEPÚLVEDA-VARAS; WEARY; VON KEYSERLINGK, 2014) and 15.2 h (NAVARRO; GREEN; TADICH, 2013). Thus, data on grazing dairy cows seems to be more variable, but studies are still limited regarding number of herds, cows and time repetitions. Knowing and appropriately measuring the average normal lying behavior of grazing dairy cows may be important indicator of cows' welfare and health.

Cows of the same herd that were managed on pasture had less severe hoof lesions, better locomotion and reduced occurrence of lameness compared to confined cows (OLMOS et al., 2009b), however, the evidence on this issue is not completely conclusive, due to lack of more studies on the issue. In other similar study the access to pasture did not reduce the presence of sole hemorrhages, sole ulcers or heel-horn erosion, but seemed to be associated with a reduction of digital dermatitis (HAUFE et al., 2012). On this regard, analysis of the long term effects of access to pasture on limbs, joints, hooves and muscles development might be important, especially when the animals are exposed to grazing at early stages of life. Having access to an open space and exercise in calves affects the characteristics of the digital cushion, hence, potentially increasing the shock absorbing capacity of this structure. Mean digital cushion volume and surface were higher (37% and 18% respectively), in calves with access to pasture and exercise area than in a control group (GARD et al., 2015). More conclusive studies on this issue should take into account the temporary dynamics of the foot development in grazing calves and heifers, as well as the specific causes of lameness on each situation and a feasible physiopathological paths to explaining the patterns in lameness causes in grazing systems.

3 COW- AND HERD-LEVEL FACTORS ASSOCIATED WITH LAMENESS IN SMALL-SCALE GRAZING DAIRY HERDS IN BRAZIL

3.1 INTRODUCTION

Lameness is a common issue that impairs the health and welfare of dairy cattle. Lameness negatively affects dairy herds by reducing the reproduction rate and milk yield. The cost of treatments and also the economic losses derived from involuntary culling affects seriously the dairy industry. The risk factors for lameness, or the relative importance of specific exposures, might differ for cows managed in grazing systems. For this reason, exploring lameness prevalence and incidence and risk factors in grazing herds may help to identify specific recommendations for the control and prevention of lameness in pasturebased systems. The aim of this study was to assess lameness occurrence in small-scale grazing dairy herds and to identify the associated cowand herd-level risk factors.

3.2 MATERIALS AND METHODS

The present cross-sectional study was carried out in 2015, in the western part of Santa Catarina State in Brazil. It was part of a larger study with multiple objectives including the identification of risk factors for peripartum diseases (DAROS et al., 2017) and stakeholder views of lameness in grazing dairy herds (OLMOS et al., 2018). The study report was conducted in compliance with the STROBE Veterinary Statement for reporting observational studies in epidemiology (SARGEANT et al., 2016). All procedures outlined below were approved by the Ethics Committees on Research on Humans (Protocol # PP1237779) and Animals (Protocol # PP00949) of the Federal University of Santa Catarina, Brazil and the University of British Columbia Animal Care committee (Protocol # A15-0082).

The sample of herds was selected by convenience, with farmers recruited based on information provided by people working in the dairy sector in the region. To minimize selection bias, informants were only aware of the general objective of the study. Farms were selected based on the following criteria: a) herd size of at least 40 cows, b) farms with good accessibility from main urban centers in the region, c) cows housed on pasture for at least 16 h/d, c) use of dairy production,

management and health records and d) farmer consent to participate in the study. From the initial group of 61 farmers that were invited to participate in the study, 8 declined and an additional 9 were excluded due to challenges associated with failing to identify a location where cows could be locomotion scored. The remaining 44 farms, located in 12 municipalities, were visited twice by two researchers accompanied by research assistants, to assess animal and environmental measures. The visits took place approximately 4 months apart during the summer/autumn and winter/spring months, respectively. For additional description of farm and cow management practices typical of this region, see (BALCÃO et al., 2017) and COSTA et al., 2013.

3.2.1 Animal-based evaluations

The same investigators visited each farm at the first and second visit, with each investigator responsible for taking the same measures on each of the visits. All lactating cows present in the farms at the time of each visit were examined. Cows were individually identified at the time of assessment. The hooves were inspected visually in the milking parlor and the presence of the superficial abnormalities was recorded, including interdigital skin hyperplasia, stage 4 digital dermatitis, scissor claw, horn cracks, horizontal and vertical fissures (BLOWEY; WEAVER, 2011). Body condition score (BCS) was measured during milking using a categorical scale (1-5 points with 0.25 unit increments) (EDMONSON et al., 1989). Locomotion scoring was done when the cows exited the parlor and were walking along a straight flat hard surface, using a five point scale, where 1 was sound and 5 extremely lame (FLOWER; WEARY, 2006).

The average speed (km/h) of herd movement was assessed when the farmer was moving cows to or from milking. Distance walked was determined using a digital pedometer (Onstep 400-Geonaute, Oxylane, France) held by one of the researchers walking behind the herd; the time when the first cow left pasture and the last cow arrived at the milk holding area or, alternatively, the first cow left the feeding area and the last cow entered the pasture was recorded. The researcher also recorded how the cows were moved, i.e., walking, motorized vehicles (e.g., motorcycle) or dogs, and also if the farmer pushed the cows when moving the herd (i.e., the farmer walked briskly behind the herd, made sounds or shouted, used sticks, or performed strong body movements intended to make the cows walk faster). For this predictor, data from the first visit were used to develop and test the method and data from the second visit were used for analytical purposes. Information on milk yield by herd, parity, and days in milk of cows were collected from farm records, when available. Data on milk yield per herd was obtained from dairy company records at the farm.

3.2.2 Management and environment based evaluations

Data on routine management practices were collected through a face-to face interview conducted with farmers at the first visit. Openended questions were asked of the farmers and their answers were recorded with a smartphone using a predefined form built for this project (PHAM et al., 2014).

Information was collected on milking routine, total farm area, grazing management, land area dedicated specifically to dairy production (perennial and annual pasture, or area planted with corn for silage). Given that records of specific feeding practices (amount of silage and concentrate fed per cow) and daily milk yield by cow were not routinely kept on the majority of farms, estimates of mean values per cow were obtained using the responses given by the farmers to the questionnaire.

Potential environmental risk factors for lameness were assessed through inspection of the milking area, feed bunk, paths and grazing areas. Use of any preventive measure for lameness was checked by reading through any available farm records to identify any event and treatment, hoof trimming, routine use of foot-baths. Questions were posed directly to the farmers to ascertain what sorts of lameness preventative management practices they had implemented on their farm.

3.2.3 Data Analyses

Data management and unconditional associations

Most variables tested were categorized. Information about the variables considered in the multivariable models, categories and the number of scores per level are presented in Table 1. Unconditional associations between pairs of predictors, and between predictors and outcomes were assessed in order to identify potential confounders or intervening variables and to select the predictors to build the multivariable regressions. Predictors associated with the outcome in the univariable analysis (P-value < 0.2) were tested in multivariable models

(DOHOO; MARTIN; STRYHN, 2007). All statistical analyses were performed using R (R CORE TEAM, 2018).

The prevalence of clinical (locomotion score \geq 3) and severe lameness (locomotion score \geq 4) was estimated for each visit. The accumulated incident (not lame at the first visit but lame on the second visit), chronic (lame on both visits) and recovered (lame on the first visit but sound on the second visit) cases of lameness were estimated using data from cows that were present at both visits to each farm. The number of observations differed in each model (Table 1) due to either missing cow identification numbers or the absence of records from some farms.

Multilevel analysis

Both herd- and cow-level analyses were fitted using the lme4 package of R (BATES et al., 2015) and P-values were obtained by Type II Wald Chi-squared tests. The effect of cow-level predictors on lameness was assessed using a multilevel binary (Bernoulli) logistic regression (KORNER-NIEVERGELT et al., 2015). To account for auto-correlated structure of lameness variation in herds, farm was included as random effect. We used 12 points for adaptive quadrature estimation in order to improve the approximation of the regressions. Three multivariable models were built: for incident, chronic, and recovered cases of lameness. The final models took the form:

 $\begin{array}{l} Pr(y \; j[i] = 1) = logit \; \text{-1} \; (\alpha + Xi \; \beta + \epsilon + \alpha j[i]), \; \text{for} \; i = 1, ..., n, \\ \text{Second level:} \\ \alpha j \sim N(0, \; \sigma \; 2\alpha), \; \text{for} \; j = 1, ..., n, \end{array}$

"Pr" is the logit transformation of the probability of lameness presence (incident or chronic) or absence (recovered) on the second visit; α is the regression intercept. X is the matrix of cow-level predictors, where "i" is the fixed effect of the "i th" level for each cowlevel predictor (Table 1); β the coefficient for predictor X; "j[i]" indexes the farm where each cow "i" is clustered; ε is the residual error; α j is a random effect to reflect residual variation between farms, which was normally distributed with mean of 0 and variance $\sigma 2\alpha$.

The effect of herd characteristics associated with herd-level lameness was assessed using a linear regression with a multilevel structure fit by restricted maximum likelihood estimation (KORNER- NIEVERGELT et al., 2015). Municipality was included as random effect. The final model took the form:

Yj[i] = $(\alpha + Xi \beta + \epsilon i + \alpha j[i])$ for i = 1,...,n, Second level: $\alpha j \sim N(0, \sigma 2\alpha)$, for j = 1,...,12,

Y is the cumulative incidence of lameness between the two visits; X is the matrix of herd-level predictors: where "i" is the fixed effect of the "i th" level for each herd-level predictor (Table 1); β the coefficient for predictor X; "j[i]" indexes the municipality where each herd "i" is clustered; ϵ is the residual error; α j is a random effect to reflect residual variation between municipalities, which was normally distributed with mean 0 and variance $\sigma 2\alpha$. All models were reduced using manual stepwise backward elimination using a P-value < 0.05 as the threshold for keeping the predictors in the model. Distributions of standard residuals were plotted to check the fit of the models. Posterior predictive simulation of the herd-level model to estimate the mean cumulative incidence of lameness was tested (BATES et al., 2015); the herd-level model was a good estimator of the mean lameness incidence and all the models fitted to the data.

3.3 RESULTS

3.3.1 Descriptive statistics

Cows were managed similarly across farms, briefly, they were milked twice per day sand provided access to pasture for a minimum of 16 h per day under rotational grazing systems. The cows were moved daily to or from the milking parlor by the farmers that walked behind or in front of the herd without using dogs or vehicles. At the second visit the herds (n = 37) covered, on average, a distance of 319 meters (SD = 335). During the hottest days of summer, some herds were also provided access to shaded tree areas or to the feeding areas to mitigate effects of thermal stress. Detailed information about the farms and visits is summarized in Table 2.

Table 1. Predictors and outcomes used in the multivariable models for cow- and herd-level analyses of risk factors for lameness in lactating cows assessed twice 4 months apart on 44 small-scale grazing dairy farms located in the south of Brazil.

Variable	Scale	Scores/level
Lameness occurrence		
Cumulative incidence ¹ Incident cases Chronic cases Recovered cases	Cow-level: 0: Non lame or 1: Lame 0: Non lame or 1: Chronic 0: Chronic or 1: Recovered Herd-level (%)	0: 565 1: 226 0: 565 1: 196 0: 196 1: 112 41 herds; 1,110 cows
Prevalence First visit Second visit	Cow-level 0: Non lame or 1: Lame Herd-level (%)	0: 1,133 1: 500 0: 1,206 1: 630 44 herds
Cow-level predictors (incidence)		
Breed	0: Jersey; 1: crossbreed; 2: Holstein	0: 197 1: 78 2: 516
Parity	0: first; 1: second-third; 2: > third	0: 133 1: 273 2: 100
Hoof abnormalities on second visit	0: absence or 1: presence	0:693 1:86
Body condition score on first visit	0: > 3; 1: 2-2.75; 2: 3	0:240 1:308 2:241
Total herds in the final model	-	40
Herd-level predictors (incidence)		
Cleanliness of holding area	0: clean or 1: dirt	0: 29 1: 14 herds
State of path to access the holding area	0: covered or 1: uncovered	0: 35 1: 9 herds
Stocking density on summer perennial pasture (cows/ha)	0: 1.3 to 5.5 or 1: > 5.5	0: 22 1: 22 herds
Distance covered by the herd when moved to or from milking (hundreds of meters)	Continuous variable	36 herds
Average speed of herd movement to or from milking (km/h)	Continuous variable	35 herds
Main breed of cows	0: Crossbreed	1 herd
	0: Jersey	4 herds
	0: Mixed	21 herds
	1: Holstein	18 herds
Municipality	Categorical variable	12
Total herds in the final model		35

¹ Categories for some variables in the models of recovered and chronic cases were different and are shown in Tables 4 and 5.

Item	Visit	
	First	Second
Date start – Date end (month-year)	01-2015 to 06-2015	07-2015 to 10-2015
Mean interval first-second visit (d)	129.4 ± 33.9 (48-212) ¹	
Mean area of farms (ha)	22.3 ± 11.5 (5-50)	
Mean area of perennial pasture (ha)	8.1 ± 5.9 (1.5-30)	
Mean area of annual pasture in summer (ha)	2.9 ± 4.1 (0-23)	
Mean area of annual pasture in winter (ha)	14.7 ± 7.2 (2-40)	
Mean area of corn planted in summer (ha)	11.7 ± 6.5 (4-40)	
Cows scored (n)	1,633	1,836
Mean number of cows in milk	37.6 ± 9.2 (24-67)	41.9 ± 11.2 (28-74)
Mean herd milk yield (L/month)	20,306 ± 6,162 (10,000-36,500)	
Estimated daily milk yield (L/cow/day) ²	18.8 ± 3.6 (11-25)	
Mean days in milk (n)	164.8 ± 108.2 (1-439) n = 1,065	153.2 ± 103.6 (1-438) n = 1,367
Desiter	2.7 + 1.7(1.11) = 960	$2.8 \pm 1.7 (1-10) \text{ n} = 1158$
Parity	2.7 ± 1.7 (1-11) n = 869	. ,
Mean body condition score (1-5)	3.0 ± 0.4 (1.75-4.5) n = 1,568	3.2 ± 0.5 (2-4.8) n =
		1,703
Feeding practices ³		
Silage (kg as fed /cow/day)	18 ± 7.5 (4-40)	
Concentrate (kg as fed /cow/day)	5.2 ± 1.6 (1.5-9)	

Table 2. Description of small-scale grazing dairy farms (n = 44) located in the south of Brazil that were visited twice.

¹ Values are shown as follows: mean ± standard deviation (range).

² The value was reported by farmers and records of milk production by herd were checked when available.

 $^{\scriptscriptstyle 3}$ The amount of silage and concentrate supplied to cows was estimated by farmers.

Lameness prevalence (locomotion score \geq 3) across the 44 farms was 31% (range: 10-70) and 35% (5-76) on the first and second visits, respectively. Severe lameness (locomotion score \geq 4) prevalence was 14.4% (0-57) and 4.8% (0-22.5) on the first and second visit, respectively. The cumulative incidence of lameness between the two visits across 41 farms was 29.6% (0-80) and the distribution of chronic, recovered and unaffected cases was 26.3%, 41.3% and 50.9%, respectively. The ratio of new cases to recovered cases was 2 (226/112); the ratio of unaffected cases to incident cases was 2.5 (565/226), and the

ratio of unaffected cases to chronic cases was 2.9 (565/196). This sample contained three kinds of breeds: Holstein, Jersey and crossbreed cows (Holstein x Jersey). The overall population across all herds changed between the first and second visits: of the 1,633 cows assessed during the first visit, 1,110 were present during the second visit, and 726 new individuals were introduced sometime between the two visits. The mean prevalence of hoof pathologies (n = 43 herds) was 24.2% (0-56.8) on the first visit and 11% (0-32.3) on the second visit. No farm had a regular preventive hoof trimming protocol, foot hygiene protocol, or records of lameness events.

3.3.2 Cow-level factors associated with lameness

Greater odds of being an incident case was observed in Holstein cows (compared to Jersey) in all herds (Table 3).

Variables	Category	Odds ratio	Predicted 95% CI	P-value
Breed	Jersey	Referent		
	Crossbreed	1.8	0.7-4.6	0.26
	Holstein	4.0	2.1-7.6	< 0.01
Parity	First	Referent		
	Second-Third	2.5	1.4-4.4	< 0.01
	> Third	6.6	3.3-13.1	< 0.01
Observed hoof abnormalities	Absence	Referent		
	Presence	2.5	1.3-4.8	< 0.01
Body condition score at	> 3	Referent		
the first visit (1-5 scale)	≤ 2.75	2.1	1.2-3.7	< 0.01
	3	2.0	1.1-3.6	< 0.05
Random effect	Variance	Standard deviation	ICC (%) ²	
Farm	0.15	0.4	4.5	

Table 3. Cow-level risk factors for accumulated incident cases of lameness (locomotion score \geq 3) in lactating cows (n = 498) observed twice 4 months apart on small-scale grazing dairy farms (n = 40) located in the south of Brazil.

¹ Incident cases: cows not lame at the first visit but lame on the second.

² Intraclass correlation was computed using the latent variable approach.

Compared to Jersey cows, Holstein and crossbreed cows had a higher probability of having a chronic case of lameness (Table 4). The odds of incident and chronic cases of lameness were greater in animals that were parity 2-3 or >3 (compared to parity 1) and in cows with visible hoof abnormalities (Tables 3 and 4). Incident cases were more common in cows with BCS \leq 3 at the first visit (Table 3), and chronic cases were more common in cows with BCS \leq 2.75 at the first visit (Table 4). Higher probability of recovery was observed in Jersey-crossbreed, and animals in parity 1 or 2 (Table 5).

Variables	Category	Odds ratio	Predicted 95% CI	P-value
Breed	Jersey	Referent		
	Crossbreed	7.5	1.8-30.5	< 0.01
	Holstein	10.1	3.2-31.5	< 0.01
Parity	First	Referent		
	Second-Third	8.6	3.0-24.6	< 0.01
	> Third	51.0	1.6-159	< 0.01
Observed hoof abnormalities	Absence	Referent		
	Presence	2.8	1.2-6.4	< 0.05
Body condition score at	> 2.75	Referent		
the first visit (1-5 scale)	≤ 2.75	2.1	1.1-3.8	< 0.05
Random effect	Variance	Standard deviation	ICC (%) ²	
Farm	1.24	1.1	27.4	

Table 4. Cow-level risk factors for chronic cases of lameness (locomotion score \geq 3) in lactating cows (n = 468) observed twice 4 months apart on small-scale grazing dairy farms (n = 40) located in the south of Brazil.

¹ Chronic cases: cows detected lame at both visits. CI = confidence interval. ² Intraclass correlation was computed using the latent variable approach.

3.3.3 Herd-level factors associated with lameness

Holstein herds had, on average, 13.5 percentage points greater cumulative incidence of lameness than herds made up by other breeds (Table 6). For every 1 km/h increase in average speed while moving the herd for milking, cumulative lameness incidence was 5 percentage points greater (Table 6). The minimum, maximum and mean speed of cows while being moved was 0.3, 4.6, and 1.9 km/h (SD = 0.9), respectively. The herds where the farmers pushed the cows walked at a greater average speed than the herds where the cows were allowed to

determine the walking speed (2.2 km/h 95% CI: 1.0-1.7 vs. 1.4 km/h 95% CI: 1.8-2.7, t = -3.1, n = 34, P-value = 0.03).

Table 5. Cow-level risk factors for recovered cases of lameness (locomotion score \geq 3) in lactating cows (n = 174) observed twice 4 months apart on small-scale grazing dairy farms (n = 37) located in the south of Brazil.

Variables	Category	Odds ratio	Predicted 95% confidence interval	P-value
Breed	Holstein	Referent		
	Jersey and crossbreed	3.2	1.3-8.1	< 0.05
Parity	\geq Third	Referent		
	First and Second	3.6	1.6-8.4	< 0.01
Random effect	Variance	Standard deviation	ICC (%) ²	
Farm	0.6	0.8	15.9	

¹ Recovered cases: cows lame at the first visit and not lame in the second. ² Intraclass correlation was computed using the latent variable approach.

Table 6. Herd-level risk factors for cumulative incidence of lameness (locomotion score \geq 3) in 35 small-scale grazing dairy farms visited twice 4 months apart in the south of Brazil.

Variables	Estimated	Predicted 95% confidence interval	P-value
Intercept	15.4	4.7-26.1	-
Main breed of herd: Holstein ¹	13.5	4.3-22.8	< 0.01
Average speed of herd movement to or from milking (km/h)	5.0	0.1-10.0	< 0.05
Random effect	Variance	Standard deviation	ICC (%) ²
Municipality	16.6	4.1	8.7

¹ Compared with Jersey, mixed and crossbreed herds.

² Intraclass correlation coefficient.

3.4 DISCUSSION

The prevalence and cumulative incidence of lameness were high in this population of small-scale grazing dairy herds. At the herd level, a high incidence of lameness was associated with the Holstein breed and with cows being forced to move at greater speeds to or from the milking parlor. At the cow level, Holstein cows were more likely to become lame, and being either Holstein or crossbreed increased the odds of cows being chronically lame. Low BCS, greater parity, and the presence of lesions on hooves were all associated with greater odds of a cow developing a new case of lameness or being identified as being chronically lame. Recovery from lameness was more frequent in cows in the first and second lactation and in Jersey and crossbreed cows. Overall, this study shows that lameness is a challenge in small-scale grazing herds in this region of Brazil, and highlights some areas of management that can be improved to reduce lameness.

Breed was associated with lameness at both the herd and cow levels. At the cow level, Holsteins had a higher incidence of lameness than Jersey cows. This pattern was also observed in the mixed breed herd analysis, which suggests that individual features of each breed may partly explain the association. Lameness has been mentioned as a major problem in Holstein herds (BARKER et al., 2010) with lower occurrence observed in grazing Jersey and crossbreed cows (DIAZ-LIRA et al., 2009). Differences in how different breeds are able to adapt to the environment have been previously suggested as a factor influencing the occurrence of lameness in pasture-based herds in the same region (COSTA et al., 2013).

However, breed may also be confounded with other risk factors for lameness. For example, attributes such as higher milk yield in early lactation and concomitant loss of BCS are known risk factors for lameness (NAVARRO; GREEN; TADICH, 2013; OIKONOMOU; COOK; BICALHO, 2013) that may be more common in Holstein cows. Genetic selection may influence susceptibility of cows to loss of BCS (KOUGIOUMTZIS et al., 2014); therefore, breeds selected for high milk yield, such as Holstein, may have higher risk of becoming lame if nutrition and management are inadequate.

Overall management practices and the design of facilities in smallholdings such as those participating in this study tend to be designed for the average cow in the herd, rather than for the individual. This may expose larger cows (i.e., Holstein) to uncomfortable or unfavorable conditions in mixed breed-herds. Thus, individual cows that vary in size may be affected differently by the design of the facilities and the amount of feed bunk space provided per cow. For instance, feed barriers built initially for smaller Jerseys or crossbreed may hinder the ability of the larger Holsteins to stand and feed comfortably. Overall, the effect of breed on lameness identified in this study appears to be explained, at least in part, by interactions between genetic and phenotypic characteristics of each breed, and environmental conditions under which these cows are cared for.

Moving the cows to or from the milking parlor at a higher speed increased the incidence of lameness, possibly by increasing the risk of injury to the hooves as cows attempted to navigate the paths. This factor may in fact be a surrogate measure for the cows' care and handling skill of the farmers. A study on grazing dairy herds showed that two surrogate measures for the farmers forcing the cows to walk faster were associated with greater lameness prevalence (CHESTERTON et al., 1989). Additionally, inappropriate handling of the cows on the path (e.g., causing sideways pushing among cows) was associated with greater prevalence of lameness in grazing dairy herds (RANJBAR et al., 2016). Also, cows forced to walk fast might be subjected to mechanical stresses on the foot similar to cows exposed to overstocking or restriction to free movement, conditions known to increase lameness (BARKER et al., 2010; RANJBAR et al., 2016).

Lameness incidence increased with higher parity, and cows with more than three parities had greater probability of being identified as a new or chronic case of lameness. It is not surprising that older cows are at increased risk of lameness; first, because they have increased exposure to potential risk factors and second, because aging is known to cause chronic degeneration of body structures responsible for the body posture. balance and locomotion, e.g., joints, ligaments, bones, digital cushion (RÄBER et al., 2004; KOUGIOUMTZIS et al., 2014; FODITSCH et al., 2016). Older cows also had higher odds of having previous lameness events and previous claw horn disruption lesions (CHDL), which might increase the occurrence of a subsequent lameness event (KOUGIOUMTZIS et al., 2014; FODITSCH et al., 2016). Age, previous events of lameness, and CHDL are also associated with the development of exostosis on the caudal aspect of the distal phalanx, a lesion that compromises the locomotion of cows (NEWSOME et al., 2016). The fat present in the digital cushion is progressively replaced by connective tissue (RÄBER et al., 2004) after the third parity, which might reduce the digital cushion padding capacity and promote the occurrence of CHDL (RÄBER et al., 2004; BICALHO; MACHADO; CAIXETA, 2009).

Cows with low BCS at the first visit had increased incidence of lameness. The identification of this association suggests that noninfectious causes of lameness (mainly CHDL) may be important in grazing dairy cows. Cows with low BCS are at higher risk of being lame (LIM et al., 2015). Some authors argue that digital cushion thickness is reduced in cows with low BCS, making these cows prone to suffer CHDL due to lower damping function of the hoof (BICALHO; MACHADO; CAIXETA, 2009; GREEN et al., 2014). However, thickness of the digital cushion also seems to be influenced by factors other than BCS, such as calving and integrity of suspensory apparatus (NEWSOME et al., 2017a), and the effects of BCS and digital cushion thickness on lameness may be independent (NEWSOME et al., 2017b). Thus, low BCS may be an intervening variable, or a factor that influences lameness through other mechanisms. Cows that were chronically lame were also identified as having a low BCS. Since low BCS is at once a consequence and a cause of lameness, chronically lame cows are clearly vulnerable and interventions should be directed to identifying and preventing the occurrence of both new (i.e., preventive measures) and chronic (i.e., lowering the tolerance of farmers to lameness, specific and prompt treatments, culling) cases of lameness.

Cows with visible hoof abnormalities (i.e., wall damage and chronic lesions affecting the skin around the hoof) had a higher probability of being a new or chronic case of lameness. We did not lift feet to fully assess the presence of foot lesions, so we detected only some chronic, obvious lesions on parts of the feet. Thus, although the presence of a lesion may not reflect the main cause of lameness, it may be indicative of farmers' higher tolerance to the abnormality, or minimal supervision of hoof status. We strongly encourage future work on the attitudes of farmers on this specific topic.

The average within-herd prevalence described in this study is higher than previous reports of lameness prevalence in grazing systems, e.g., 18.9% in (RANJBAR et al., 2016), 8.3% in (FABIAN; LAVEN; WHAY, 2014); however, the lameness prevalence was similar to prevalence reported in intensive systems in North America (e.g., 27.9%, 30.8% in (VON KEYSERLINGK et al., 2012)), Europe (e.g., 36.8% in (BARKER et al., 2010)), China (e.g., 31% in (CHAPINAL et al., 2014)), or in herds with access to pasture in Chile (e.g., 33.2%, 28.7% in (TADICH; FLOR; GREEN, 2010)).

Recent studies performed in intensive systems in North America have reported lower prevalence of lameness: 13.2% in (COOK et al., 2016), 21% in (SOLANO et al., 2015), 7.2% in (ADAMS et al., 2017), 15% in (WESTIN et al., 2016b), 14% in (FODITSCH et al., 2016); this is probably result of the implementation of preventive measures for lameness on those farms. Thus, regardless of the housing system, lameness is an important issue for dairy cows both in intensive and pasture-based systems, and there is a great opportunity to improve the management of the problem in these small-scale dairies. Additionally, specific herd management practices, as well as the application of preventive measures for lameness, may explain the differences of prevalence observed on the above-mentioned results and this study.

Lameness occurrence was highly variable between the herds. This variability among herds from the same region using a similar production system confirms that management and the specific design of the facilities strongly affect lameness, as suggested by (BICALHO; OIKONOMOU, 2013). The high prevalence of chronic lameness, particularly in the case of the older cows, added to our findings regarding the chronic hoof abnormalities, confirms that there were few attempts to reduce lameness in these herds. A high proportion of cows detected as lame on the first visit recovered, probably without intervention, whereas the ratio of new cases to recovered cases, was 2 indicating a progression of the issue. Because we found no adoption of basic prevention measures for lameness, part of the occurrence of lameness might have been driven by the continuing natural progression of the problem.

Due to the cross-sectional nature of the present study it is not possible to establish a causal relationship between significant explanatory variables and lameness. However, we recommend maximizing measures to control lameness in high-risk herds and cows (e.g., Holstein, older cows), establishing basic preventive measures for enhancing hoof health (i.e., hoof trimming and hoof care protocols), promoting appropriate nutritional management of cows to avoid low BCS, as well as gentle handling of cows during movement for milking in order to reduce lameness in grazing dairy herds.

3.5 CONCLUSION

Lameness was highly prevalent in this group of small-scale grazing dairy herds. Breed, parity, presence of hoof abnormalities and low BCS were cow-level risk factors for accumulated incident and chronic cases of lameness. The main breed of herd and the average speed when moving the cows were associated with greater herd lameness occurrence.

4 LAMENESS ON BRAZILIAN PASTURE BASED DAIRIES: FARMERS' AWARENESS AND ACTIONS

4.1 INTRODUCTION

Recently a considerable amount of scientific information on lameness control and prevention has become available, however, not all farmers succeed in achieving low rates of lameness. Thus, the adoption of preventative or therapeutic practices intended to improve the dairy cows' health are also mediated by factors other than the availability of information. An important factor that affects the adoption of good practices in some regions is the farmers' understanding of the health issues and therapeutic procedures. For instance, farmers might not adopt effective lameness control measures because they fail to recognize lame cows or view a lame cow as normal, or simply they do not accept that lameness is a problem on their farm (HUXLEY et al., 2012; LEACH et al., 2013; FABIAN; LAVEN; WHAY, 2014). Further issues, such as lack of time, labour or skilled labour, unpopularity or difficulty of tasks connected with lameness control, financial costs, deficit of information, and conflicting advice (HORSEMAN et al., 2013; LEACH et al., 2013) have all been mentioned as potential barriers preventing the implementation of measures to control lameness on farms. Clearly, understanding the farmers' awareness of the problem, the level of knowledge concerning available interventions targeted at reducing lameness and their desire to improve are needed for effective control of lameness on dairy farms. Therefore, the aims of this study were to verify farmers' awareness and knowledge about lameness in small scale grazing dairy herds and to analyze farmers' perspectives and actions regarding lameness management and prevention.

4.2 MATERIALS AND METHODS

The procedures of this cross-sectional survey were carried out in accordance with the guidelines of the Ethics Committees on Research on Human (Protocol # PP1237779) and Animals (Protocol # PP00949) of the Federal University of Santa Catarina, Brazil, and the University of British Columbia Animal Care committee (Protocol # A15-0082), Canada. The objectives, methods and specific procedures of the study were explained to all the participant farmers and informed consent was obtained.

4.2.1 Visited farms

Small scale grazing dairy farms (n = 44) distributed amongst 12 municipalities located in the Santa Catarina State of Brazil were visited twice in 2015. The sample of farms was chosen by convenience and potential participants identified by individuals working in the local dairy industry. This research was part of a larger study on lameness (BRAN et al., 2018b; COSTA et al., 2018), transition period diseases (DAROS et al., 2017) and stakeholder views of dairy cow health in grazing dairy herds (OLMOS et al., 2018).

4.2.2 Cows' visual locomotion score assessment

All lactating cows in the farms were locomotion scored during two farm visits (January – June and July – October) using a five point visual score (FLOWER; WEARY, 2006). Cows scored as 1 or 2 were considered non-lame; cows with score \geq 3, were considered clinically lame, and cows with score \geq 4 as being severely lame. The lead author, a veterinarian trained in locomotion scoring, did all of the locomotion scoring. Cows were observed while leaving the milking parlour as they walked along a flat hard surface.

4.2.3 Interview conducted with the farmers

The farmers were interviewed during the first visit using a predefined questionnaire presented using the Kobotoolbox software (PHAM et al., 2014). Farmers' knowledge on lameness and lameness management at the farms (Table 7), data characterizing the farms (farm area, average milk yield, breed of cows), and demographic variables of the families were also collected. During the completion of the questionnaire the presence of the farm manager or equivalent individual who had intimate knowledge of the farm was mandatory; in most cases, all persons working directly with the cows were present during the questionnaire and some answers reflect a consensus based on a discussion among all participants.

Table 7. Questionnaire conducted with farmers in 44 small scale grazing dairy farms located in the south of Brazil.

Questions relating to demographics

How many persons work regularly on your farm?

Working either full time or part time that either work directly with the animals or milk production

What age is the farm manager or equivalent person in charge of making decisions?

What is the highest educational level of all individuals identified in question 1?

How long has each family worked in the dairy industry?

Questions focused on farmers' knowledge and actions related to lameness control and prevention

What are the three main health problems of milking cows in your farm? (please rank by order of importance)

What are the three most common reasons for culling cows on your farm? (including voluntary and involuntary causes; please rank by order of importance)

Have you ever treated lame cows on your farm?

If yes, then, please describe how you managed and treated these cows¹

Do you usually practice preventative hoof trimming on cows?

Do you use routine preventative footbaths?

What are the main causes of lameness on your farm?²

Have you participated in any continuing education events related to animal health on the last two years? If yes, please describe the topic of the course.

Do you have veterinary support? If yes, then:

a) how frequent does the veterinarian visit the farm?

b) what are the main health issues that the veterinarian deals with when visiting your farm?

How many lactating lame cows are there on our farm today? ³

¹ The use of treatment records, when available, was used to verify responses and the specific name of the medications or protocols mentioned by the farmer.

² When farmers reported that lameness was uncommon at the farm, the question was phrased as: what are the main causes of lameness in dairy cows on herds similar to your farm productive conditions?

⁴ This question was phrased each time the veterinarian assessed locomotion of the cows and was used to estimate a farmers' reported prevalence.

The farmers' answers were categorized and data analyzed descriptively. Average measure-two-way mixed-effects model intraclass correlations coefficients (ICC 3,1) (SHROUT; FLEISS, 1979) were fitted to assess the consistency between the mean lameness prevalence estimated by the veterinarian and the mean prevalence reported by the farmers at the time of each visit.

The prevalence of clinical lameness estimated by the veterinarian in the first and second visit was compared among farms that provided different answers (yes/no) regarding the importance of lameness as a health problem in the farm and the impact of lameness on culling cows. Four univariable mixed-effects linear models were fitted (one model for each category of question regarding lameness importance as a health problem and impacts on culling cows at the farms). The response variable was the average prevalence estimated by the veterinarian at the moment of the visits and the predictor for each model was the farmer response category (No = intercept, Yes = slope). Municipality and farm, nested within municipality, were included as random effects to account for the repeated measurements of prevalence in the farms and also to account for the hierarchical structure of data distribution. The linear models were fit by restricted maximum likelihood estimation, using the lme4 package (BATES et al., 2015) and P-values were obtained by type II Wald chi-square test (KORNER-NIEVERGELT et al., 2015). Significance was set at P < 0.05.

The goodness of fit of the regressions was assessed through residual plot analysis and random effects' normality was checked graphically. The associations between lameness prevalence and farmers' answers (lameness estimated prevalence, answers regarding lameness relevance and impacts on culling) were used as a surrogate measure for farmers' awareness of lameness occurrence on their farms. All the statistical analyses were performed using R (R CORE TEAM, 2018).

4.3 RESULTS

4.3.1 Characterization of the families

Four persons on average (range 2-9) – consisting almost always of family members – worked regularly on the farm. Only 5 out of 44 farms employed non-family workers. The mean age of the farm manager

(or equivalent) was 41 years (range 23-60). Most of the farm managers were male (42 out of 44).

On average about half the managers reported making decisions regarding milk production collectively with the others working on the farm (24 out of 44 farms) with the management decision on the other farms made by the owner or manager (20 out of 44 farms). The level of education of the family member with the highest level varied across the farms, and was not always the farm manager, with 11 family members having elementary schooling, 22 stated that they had some secondary schooling, and 11 had post-secondary education (mostly animal science, agriculture or business administration). On average the families reported having been engaged in dairy production for approximately 20 years (range 5-35 years).

4.3.2 Farmers' estimated lameness prevalence

On average, the farmers estimated a lower prevalence of lameness on their farms than the veterinarian (Table 8 and Figure 1). Overall there was no agreement between the farmers' and veterinarian estimates of lameness prevalence. The only exception was in the case of severe lameness prevalence, which was similar (ICC 0.8, 95% CI: 0.6-0.9) for the estimates provided by both the trained veterinarian and the farmer on the second visit (Table 8 and Figure 1).

Table 8. Associations between severe lameness prevalence estimates provided by a trained veterinarian and those provided by farmers when asked the percentage of lame cows on their farms. Farms (n = 44) were small scale grazing dairies visited twice in 2015 in the south of Brazil.

Visit	Mean (SD) herd lameness estimated prevalence (%)		ICC ¹	95% co interva		P-value ⁴
	Veterinarian ²	Farmers	-	Lower	Upper	-
First	Severe: 14.4 (13.0)	6.5 (5.3)	0.2	-0.5	0.6	0.23
Second ³	Severe: 4.8 (5.0)	3.8 (3.7)	0.8	0.6	0.9	< 0.01

¹ Average measure-two-way mixed-effects model intraclass correlation coefficient (ICC) for assessing the consistency between the mean lameness prevalence estimated by all the farmers and the veterinarian.

² Severe lameness: locomotion score \geq 4.

 3 n = 43 (one farmer response was missing).

⁴ Significance was set at P<0.05; P-value testing if the correlation between the farmer and veterinarian estimate was different from zero.

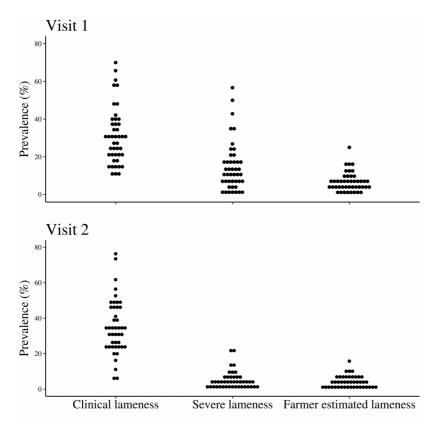


Figure 1. Distribution of veterinarian (clinical = locomotion score \geq 3; severe = locomotion score \geq 4) and farmer estimated lameness prevalence in 44 small-scale grazing dairy farms located in the south of Brazil visited twice in 2015. Farmer estimated prevalence in the second visit was obtained from 43 farms.

4.3.3 Farmers' suggested causes of lameness in dairy cows

The farmers mentioned different factors as causes of lameness that were grouped into five categories (Table 9). Trauma and conditions that exert excessive stress on the hoof were the most common factors identified as causes of lameness by farmers. This was followed by inadequate feeding practices with farmers frequently mentioning that ruminal acidosis (or giving excessive grain, or silage that promotes acidosis) was the main cause of lameness.

Farmers' suggested cause of lameness	n (%) ¹
Trauma	31 (70.5)
Rocky/stony ground	27 (61.4)
Mobility issues ²	7 (15.9)
Hard floors	1 (2.3)
Inadequate feeding practices	26 (59.1)
Ruminal acidosis	11 (25)
Excessive feeding of grain to cows	8 (18.2)
Excessive feeding of silage to cows	6 (13.6)
Low supply of minerals to cows	3 (6.8)
Environmental causes ³	23 (52.3)
Individual features of cows	7 (15.9)
Age (older cows)	4 (9.1)
Overweight	1 (2.3)
Inappropriate body conformation	1 (2.3)
Low body condition score	1 (2.3)
Hoof infection	1 (2.3)
Other causes	4 (9.1)

Table 9. Farmers' suggested causes of lameness in 44 small scale grazing dairy farms located in the south of Brazil and visited in 2015.

¹ Number of farmers who reported and percentage: categories are summarized per number of respondents and subcategories are expressed as number of responses (some farmers mentioned multiple subcategories).

² e.g., inappropriate paths, rush when chasing the cows to milking, cows walking long distances.

³Excessive moisture on paths and floors.

4.3.4 Farmers' sources of information

All the farmers reported having no specific lameness training, including no continuing education courses related to lameness prevention or management. Twenty farmers (45.5%) reported having attended some course or conference during the previous two years where health issues, but not lameness, in dairy cattle were discussed.

4.3.5 Lameness as a health issue or a reason for culling cows

Few farmers ranked lameness as the main health problem of cows on their farms; however, the majority did include it as one of the three most common health issues affecting cows on their farms (Table 10). Two other common health problems identified by the farmers were reproductive failure and mastitis. Lameness was frequently reported as one of the three main reasons for culling cows at their farms (Table 10).

Table 10. Distribution of estimated clinical lameness (locomotion score \geq 3) prevalence by gate scoring by a trained veterinarian between answers of farmers to questions about lameness relevance in the farm in 44 small scale grazing dairy farms visited twice in the south of Brazil in 2015.

Farmers' report regarding lameness in their farm		Lameness prevalence by visit ¹		
	answers	First	Second	
Lameness is the main health problem of milking cows	No (n = 37)	29.71 ± 15.33	33.76 ± 16.26	
	Yes (n = 7)	37.90 ± 12.69	41.67 ± 7.50	
Lameness is one of the 3 most common health problems affecting milking cows	No (n = 21)	26.37 ± 11.96	28.98 ± 12.18	
	Yes (n = 23)	35.25 ± 16.65	40.54 ± 16.23	
Lameness is the main reason for culling cows	No (n = 40)	30.42 ± 14.97	34.69 ± 15.82	
	Yes (n = 4)	36.99 ± 17.53	38.31 ± 11.83	
Lameness is one of the three reasons for culling cows	No (n = 29)	26.43 ± 12.08	30.94 ± 13.09	
	Yes (n = 15)	39.88 ± 16.78	42.92 ± 16.93	

¹ Mean (± standard deviation) of within-herd lameness prevalence (%) by farmer response category.

The farms where farmers identified lameness as either one of the three main health issues, or one of the common reasons for culling, had higher average lameness prevalence compared to farms where farmers did not list lameness as one of the main health issues or causes of culling (Table 11).

4.3.6 Veterinary assistance, prevention measures and treatments applied for lameness in the farms

With the exception of one farm, all farmers reported having treated lame cows on their farms. However, no farm kept records of specific treatments nor did they make use of veterinary or professional support to control lameness. Although 24 farmers (55%) reported having a veterinarian visit once a month, 16 farmers (36%) reported only calling a veterinarian for specific health issues, and 4 (9%) reported that they only had a veterinarian visit every 2-3 months. The most common veterinary assistance provided to these farms was in relation to reproduction (17 farms receiving periodical visits, either monthly, or every 2-3 months). Only

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two farmers mentioned having called a veterinarian to deal with lameness or for hooves' abnormalities in cows.

Table 11. Associations between farmers' answers to questions on farm lameness relevance and prevalence (locomotion score \geq 3) by gate scoring by a trained veterinarian on the first and second visit, in 44 small scale grazing dairy farms in the south of Brazil in 2015.

Farmers' report regarding		Estimate	95% CI ¹	P-value	Rando	m effect (SD)
lameness in their farm	answers				Farm	Municipality
Lameness is the main health problem of milking cows	Intercept Yes	31.74 8.04	24.93–34.54 -3.0–19.08	0.15	11.91	0
Lameness is one of the 3 most common health problems affecting milking cows	1	29.78 10.46	23.27–36.30 3.34–17.58	< 0.01	9.64	6.59
Lameness is the main reason for culling cows	Intercept Yes	33.48 4.83	28.41–38.55 -9.29–18.95	0.50	11.62	4.14
Lameness is one of the three reasons for culling cows	Intercept Yes	29.15 12.55	24.29–34.01 4.78–20.33	< 0.01	10.38	2.47

¹ CI = confidence interval. Coefficients were obtained from four univariable mixed-effects linear models (one model was fitted per each category report).

The main approaches to treatments of lameness described by the respondents are detailed in Table 12. The use of antibiotics to treat lame cows was frequently mentioned. Within this category the use of local and parenteral antibiotics was mentioned by 6 and 24 respondents, respectively, with 3 farmers reporting using both kind of antibiotics at the same time.

Table 12. Farmers' reported lameness treatment in dairy cows in 44 small scale grazing farms in the south of Brazil.

Question	Yes n (%) ¹
Treatment of lame cows	43 (97.7)
Use of antibiotics	27 (61.4)
Application of topic products on hooves ²	18 (40.9)
Use of anti-inflammatories/analgesics	12 (27.3)
Application of hoof trimming for treatment	11 (25)
Diet modifications for treatment	6 (13.6)
Application of foot-baths	4 (9.1)
Use of other measure/medication for treatment	4 (9.1)

¹ Number (n) and percentage of farmers.

² Ointments, spray, antiseptics, disinfectants.

When asked for the commercial or active principles of antibiotic used, nine respondents mentioned the use of cephalosporins and one reported the use of gentamicin. Only three farmers mentioned having practiced preventative hoof trimming in the past and one reported the use of hoof baths for some cows, but in both cases neither of these practices were routine.

4.4 DISCUSSION

Farmers estimated a lower prevalence of lameness compared to the trained veterinarian, with the greatest discrepancy noted on the first visit. The improvement in farmers' estimates (in comparison to the veterinarians) at the second visit may have been due to an increased sensitivity to the issue of lameness, particularly the severe cases which do not require the same degree of training to identify as clinical cases (FABIAN; LAVEN; WHAY, 2014). Farmers' underestimation of lameness occurrence is a barrier to be overcome if lameness is to be addressed. However, increasing the detection of lame cows is insufficient to fully address the problem, as increased farmer sensitivity to the problem per se is also necessary (LEACH et al., 2013).

Differences in estimated lameness prevalence between farmers and the trained veterinarian may be simply a matter of the farmers' underestimation of this malady, but it may also be explained, at least to some degree, to differences in what defines a lame cow (HORSEMAN et al., 2014). In cases where failure to recognize lameness is the main problem, the ability to identify lame cows has been reported to increase if the farmers have had previous contact with information, training and some technical orientation about the problem (LEACH et al., 2013). The responses to the questionnaire indicated little access to current information about lameness, which may have contributed to the farmers' failure to identify the majority of the lame cows. The large gap between farmers' and veterinarians' estimation of lameness prevalence suggests that efforts focusing on training farmers to identify lame cows (particularly mild lameness) may be a first step to reduce lameness in small scale grazing herds in this region.

Lameness in dairy cows is a clinical sign frequently associated with pain arising by foot infectious diseases or claw horn disruption lesions (CHDL) (HUXLEY et al., 2012). Some farmers mentioned factors that have been previously associated with higher rates of foot lameness, or conditions that result in greater stress on the cows' feet, such as hoof trauma and excessive moisture on walking surfaces (COOK; NORDLUND, 2009; NEWSOME et al., 2017b). However the farmers did not mention diseases associated with lameness, nor did they bring up any of the common CHDL when asked about lameness causes. This provides further evidence that there is low familiarity with technical information regarding lameness in cattle among this group of farmers. Since farmers likely make decisions based on their circumstances and agricultural context (RITTER et al., 2017), farmers' knowledge about factors affecting lameness on their own conditions might be used as a basis for advisers to discuss and motivate the enforcement of actions aimed to control risk factors for lameness.

Most farmers believed that acidosis arising from poor nutrition was the cause of lameness. The idea that a causal relationship exists between rumen acidosis and laminitis in dairy cows and thus is the major cause of lameness has been largely disregarded in the modern literature (DANSCHER; TOELBOELL; WATTLE, 2010; BICALHO; OIKONOMOU, 2013; NEWSOME et al., 2017b). An alternative hypothesis suggesting that the causes of most CHDL and diseases leading to foot lameness are multifactorial is gaining acceptance (NEWSOME et al., 2017b, 2017a). However, it is possible that sources of information, including those brought forward by extension agents and other dairy advisors working in the area, may be historic in nature and thus not represent current knowledge. Equally important to having the correct information available for education is the farmers' desire to incorporate the newest knowledge into the dairy production system. The fact that many of the farmers' exclusively linked acidosis to lameness indicates that, at least to some degree, there are some knowledge gaps either within the advisor or the farmer community, or both. In summary, it seems that the farmers did not recognize lameness as a sign of pain or discomfort caused by multiple conditions; however, they identified common factors that might be associated with higher occurrence of lameness in herds or cows, but that are not necessarily intervening or causal factors.

The frequent mention of antibiotic use for treating lame cows may suggest a possible suspicion of infectious causes of lameness by the farmers. Foot root and digital dermatitis are infectious diseases that may be common in dairy cows at the region. Previously we described the presence of digital dermatitis on this region (BRAN et al., 2018b). However, as none of the farmers mentioned any of these diseases on their responses, we can not link the treatments reported by farmers with any specific foot infectious disease.

A lack of understanding of the role of antibiotics in treating disease may also be associated with the frequent mention of these medications to treat lame cows. Conflicting advice or information given to farmers by different stakeholders such as their veterinarian and feed-store vendors has been mentioned as factors contributing to the misuse of antibiotics in small-scale dairy farms (REDDING et al., 2014). Also, given that input vendors are a frequent source of advice for farmers, they may exert a proportionally larger influence on farmers' management decisions compared to others. Thus, the widespread reported use of antibiotics – despite the fact that farmers did not mention infectious causes of lameness as an important issue – may reflect an untargeted approach to treating lameness as a result of misinformation and advising.

The low reported use of analgesics may reflect farmers' unawareness regarding inflammation and pain associated with lameness and the great importance of pain control to preserve the health and welfare of lame cows (TADICH et al., 2013; COETZEE et al., 2017). Another study surveying the opinions of dairy farmers in the same region to dehorning practices reported that farmers recognize and are concerned by pain in their animals, but this unfortunately rarely reflected in adoption of measures to control it (CARDOSO; VON KEYSERLINGK; HÖTZEL, 2016). The recommendations of dairy advisers may also have influenced the decisions of farmers to only make infrequent use of analgesics to control pain associated with lameness. It has been shown that, with regard to other painful situations, extension workers in the region did not recommend medication for pain control (HÖTZEL; SNEDDON, 2013). Advisers may also have different criteria to assess the pain severity in cows (REMNANT et al., 2017) and this may affect the recommendations given to the farmers regarding analgesic use. Improving the understanding of the role(s) of advisers may be important, as they may be influential when attempting to advocate for pain mitigation in lame cows on the current farms. This, plus the identification of other barriers preventing effective solutions that control pain (and ultimately lameness), is important as there is need to continue to look for practical, motivational or subjective reasons behind the non-adoption of this basic measure.

Almost all farmers reported treating lame cows in the recent past, although they appeared to be more aware of severe than moderate cases

of lameness, which may imply that they prioritize the treatment of severe lame cows. This approach might be insufficient to control lameness in the herds since severe lame cows have lower probability of after treatment (MIGUEL-PACHECO recoverv et al., 2017). notwithstanding the welfare impairment on these cows. Additionally, a surprisingly large number of the farmers failed to routinely request veterinary assistance when dealing with lameness cases which may indicate that the treatments for lame cows on these farms may be partially driven by advisers others than veterinarians, which might influence the application of untargeted measures to treat and prevent lameness in cows. Enforcement of preventive measures may be required in order to reduce the impacts of lameness on health, productivity and welfare of the cows. Thus, not just giving adequate treatment to the affected individuals, but reducing the causes of health issues should be a priority when taking a preventive health approach. A structured program aimed to control lameness in the assessed population should contemplate measures directed at identifying and treating both mild and severe lame cows, as well as preventing and controlling lameness at the population level.

Most farmers seemed to be aware that lameness is a common issue affecting the health and the culling rate of their cows, a finding that seems coherent with the higher occurrence of lameness observed on farms that reported that lameness was a challenge. However, they did not seem aware of the scope of the problem, given that they underestimated the number of lame cows present on their farms. Thus, the farmers may consider lameness as a secondary problem, thereby placing a priority on other health issues (i.e., reproductive failure and mastitis). Therefore, many of the farmers' efforts are presumably targeted towards the issues that they prioritize, resulting in fewer efforts and resources available to control lameness. A first step needed when addressing this malady is that farmers must acknowledge the existence of the problem and accept the responsibility of taking actions (RITTER et al., 2017). Hence, ignoring the existence of the problem may result in farmers avoiding the adoption of actions intended to reduce the negative impacts of lameness in the herds.

Farmers' adoption of recommended practices to prevent and control health issues in dairy cows is a complex process mediated by the comprehension of the problem by farmers, but also influenced by multiple factors such as socio-economic conditions or the influence of social referents like advisors, veterinarians, or other farmers (RITTER et al., 2017). Thus, it seems that there are no simple protocols to deal with this issue; nevertheless, some general recommendations to overcome barriers associated with low adoption of practices intended to prevent lameness have been proposed previously. Providing information to farmers about lameness occurrence (CHAPINAL et al., 2014), risk factors, and the main measures needed to prevent lameness is important to motivate changes directed to control the problem (WHAY et al., 2012). Also, training the farmers to recognize and timely treat the mildly lame cows has been suggested as a main measure for controlling lameness (GREEN et al., 2010). It is also crucial to engage the farmers in the process of planning preventive measures for lameness to ensure their application and success in reducing lameness. Interventions that promote engagement of farmers with assistance of facilitators seem to be effective in the generation of control strategies for lameness (WHAY et al., 2012; CHAPINAL et al., 2014). Thus, providing the farmers access to proper technical information on lameness in dairy cows and promoting the adoption of programs and strategies focusing on reducing lameness, as well as ensuring that correct advice is given are important measures that should help reduce the impact of lameness in this region.

4.5 CONCLUSION

The farmers in the assessed population seemed unaware of the actual occurrence of lameness on their farms and the relevance of the problem in relation to other health related issues (e.g., mastitis or reproductive failure). The knowledge regarding lameness aetiology was minimal and restricted to farmers' empirical observations, with an almost complete absence of any technical training or advising from specialists on the issue, despite apparent routine contact with veterinarians and other advisors. Possibly as a consequence, the strategies of lameness management were mainly focused on treating individual cases, whereas measures directed to prevent or control this disease at the population level were neglected.

5 ASSOCIATIONS BETWEEN COW- AND HERD-LEVEL RISK FACTORS AND LAMENESS PREVALENCE IN FREESTALL AND COMPOST-BEDDED PACK DAIRY BARNS IN SOUTHERN BRAZIL

5.1 INTRODUCTION

Some aspects of housing systems have been mentioned as factors influencing lameness in dairy cows. Cows in compost-bedded packs are believed to have lower lameness occurrence than cows housed in freestall barns, due to the improved lying surfaces and reduced risk of foot contusions that may be associated with freestall housing. However, some studies have failed to show differences in lameness distribution between different types of housing systems (BURGSTALLER et al., 2016; ECKELKAMP et al., 2016). Few epidemiological studies have explored risk factors associated with lameness in dairy cows in Brazil, and also most observational studies are based in sampling of cows within farms or did not contemplate the population structure and spatial distribution within the barns. In the present study we assessed the entire population of cows on the participating farms and used mixed-effects models in order to investigate the proportion of lameness variation that may be explained by the heterogeneous fragmentation of herds in different pens within the barns. The aim of the present study was to investigate cow- and herd-level risk factors associated with lameness in lactating dairy cows housed in compost bedded-pack and freestall barns located in southern Brazil.

5.2 MATERIALS AND METHODS

All the procedures applied in this study were approved by the Ethics Committees on Research in Animals (Protocol PP00949) and Humans (Protocol PP1237779) of the Federal University of Santa Catarina and the University of British Columbia Animal Care Committee (Protocol A15–0082). The procedures and study methods were explained to all the farmers and informed consent was obtained. The study report was conducted in compliance with the STROBE Veterinary Statement (SARGEANT et al., 2016). This cross-sectional study was conducted in 2016. Data analyses were based on information collected from a convenience sample of 50 dairies visited once (Table 13) located in four cities situated in Paraná state, Brazil. The recruitment

of volunteers was based on information provided by people working in the dairy industry at the region.

5.2.1 Animal-based evaluations

All lactating cows present at the moment of the visit were individually identified and examined by the same trained researcher during or immediately following milking. Body condition score (BCS) was assessed using a five points categorical scale with 0.25 unit increments (EDMONSON et al., 1989). The presence and severity of superficial injuries compromising the skin or soft tissues in the frontal carpal (carpal injuries) and lateral tarsal (hock injuries) joint region was registered, scoring one limb per cow. For this purpose we applied a (modified) three points score developed by Cornell University (https://ahdc.vet.cornell.edu/programs/NYSCHAP/docs/HockScoringCh art-NYSCHAP-4-04.pdf; Accessed: 2017; Hock Assessment Chart for Cattle, Cornell Cooperative Extension). Briefly, cows without any injury were classified as score 1, cows with mild swelling and/or balding were classified as score 2, and cows with mild swelling and/or open wound were classified as score 3. The skin around the udder was inspected for cleanliness using a three point hygiene score (Lombard et al., 2010): 0 = Absence of any dirt or manure; 1 = presence of small amount of manure or dirt; 2 = presence of large amounts of manure or dirt).

All lactating cows present at the time of each visit were locomotion scored using a five points scale visual score (FLOWER; WEARY, 2006) immediately after milking when they walked along a flat hard surface. Cows with locomotion score 1 and 3 were considered sound; \geq 3 were considered clinically lame; and cows with score \geq 4 were considered severely lame.

5.2.2 Interview on herd management

A face to face interview was conducted with every farm manager and/or farm owner (hereafter called "farmer") at the beginning of each visit to obtain information on routine herd management. The questionnaire consisted of both open and closed ended questions, using a predefined form (PHAM et al., 2014). The questionnaire set out: a) to characterize farm features, i.e., herd size, barn type and building years, milk yield, milking routine, number of pens per farm, provision of pasture access to cows and heifers, manure alley management, stall base, bedding type and bed management, use of health and production records; and b) to characterize feeding management of cows and c) to verify the use of on farm routine preventative measures for lameness, i.e., record of lameness events, veterinary assistance, structured protocols for early treatment of lame cows, routine hoof trimming and use of foot-baths in the herds. Farmers' responses were recorded using a notebook.

5.2.3 Farm inspection

We inspected the milking parlor, holding area, cow feed and cow traffic alleys, pens, alleys, feed bunk area and lying stalls to evaluate potential environmental risk factors for lameness. A check list was used to verify flooring surfaces and condition in each barn section and along the tracks, the feed bunk space available per cow, the bedding base and type of lying stalls, the barn manure cleaning system, and the ventilation systems. Additionally, we assessed the slipperiness of the barn flooring (cow-feed and cow traffic alleys floor) through a subjective score (0 = non-slippery surface, 1 = slightly slippery, 2 = very slippery) and a question was directly phrased to farmers during the interview in order to triangulate this information (Did the cows often fall when being moved in the barn? Response: no, often, rare, sometimes).

5.2.4 Data analyses

Data management and unconditional associations assessment

Data were screened for errors or inconsistent measurements and incorrect entries were excluded of the dataset. All the variables considered in the models were categorized based both on biological meaning or data distribution in the population (Tables 14–18). Data were described, associations between pairs of explanatory variables checked, and unconditional associations between explanatory variables and the occurrence of clinical lameness (locomotion score \geq 3) in cows and herds tested. We checked for potential confounders or intervening variables between all the assessed predictors through detailed description and logical inference, with reference to causal diagrams. Then, outcome variables associated with lameness (P-value < 0.2) identified through univariable tests were assessed in multivariable models. All statistical analyses were performed using R (R Core Team, 2018).

Item	Values
Date start – Date end (month-year)	March to October 2016
Barn type (n)	Freestall: 38, compost-bedded pack: 12
Barn ventilation (n)	Natural: 14, fan: 23, fan and misting system: 7 fan and sprinkler: 6
Milking/day (herds)	Two: 28, Three: 21, Four: 1
Access to pasture for cows and heifers (herds)	Dry cows and heifers: 31, lactating, dry cow and heifers: 13, heifers: 2, lactating cows only 1, no access: 3
Number of lactating cows in farm per worker	26.90 ± 10.34 (26.08, 10.75–70.17)
Average herd size (cows)	308.6 ± 226.21 (233.5, 49.0–1000)
Average lactating herd size ¹	274.1 ± 201.68 (204.5, 41–901)
a) Compost-bedded pack barns	104.3 ± 62.94 (86, 41–239)
b) Freestall barns	327.7 ± 200.97 (324, 71–901)
Average years since barn was built	11.52 ± 10.72 (5, <1–35)
a) Compost-bedded pack barns	1.58 ± 0.90 (1.5, <1-3)
b) Freestall barns	14.66 ± 10.48 (15.0, 1–35)
Average number of pens per farm	4.68 ± 2.44 (5, 1–12)
Average number of cows housed per pen within farms	58.57 ± 39.77 (53.5, 2–227)
Adopted feeding practices (farms)	TMR: 47, Top-dressing: 3
Number of feeds provided per day (farms)	One: 5, Two: 27, Three: 13, Four: 4, Six: 1
Annual frequency of preventive hoof trimming (farms)	Zero: 8, Once: 9, Twice: 25, More than twice: 8
Routinely foot bath use (farms)	Yes: 44, No: 6
Cows scored (n)	13706
Breed (n, cows)	Holstein: 13147, Other: 559
Average body condition score (1-5)	2.94 ± 0.26 (3.0, 1.5–5.0)
Average daily milk yield (K/day)	
Primiparous cows (n = 2084)	33.65 ± 8.54 (34.1, 4.20–56.80)
Second lactation cows (n = 1482)	36.57 ± 11.19 (37.0, 4.0–69.90)
Multiparous cows (n = 1963)	37.25 ± 13.07 (37.30, 3.5–84.20)
Average days in milk (n = 5360 cows)	207.1 ± 135.48 (6–782)
Parity (n = 5529 cows)	2.28 ± 1.40 (1-10)

Table 13. Main features and management practices of 50 confined dairy farms located in Paraná state – south of Brazil that were visited once in 2016.

¹ Descriptive statistics for quantitative variables are shown as follows: mean ± standard deviation (median, min–max).

Multivariable analyses

Associations between cow-level predictors and lameness were determined using mixed-effects binary logistic regressions (Bernoulli distribution) (KORNER-NIEVERGELT et al., 2015), using the Laplace approximation method, fitted using the lme4 package of R (BATES et al., 2015). P-values were obtained using Type II Wald Chi-squared test. Since most farms housed cows in groups within pens, we used group as a random effect nested within each farm in order to account for both the hierarchical structure of herds' division and the auto correlated variation of measurements collected from clustered animals. A linear regression, using the response variable lameness prevalence of clinical lameness was built to assess the herd-level associations with lameness.

Multivariable models were reduced by manual stepwise backward elimination where predictors with a P-value < 0.05 were retained in the models. If removing a specific variable from the multivariable model was associated with 30% or more of the coefficients for other significant predictors, that variable was considered a confounder. Plausible biological interactions were tested between significant explanatory variables in all of the multivariable models. Goodness of fit of the regressions was assessed through residual plot analyses and likelihood-ratio tests comparing the final models with null models; posterior predictive simulation was conducted to estimate if the herd-level model was a good predictor of the average lameness prevalence. The inclusion of random effects improved the cow-level models fit and all models retained fitted to the data.

Model 1. Cow-level associations with lameness in the overall population of inspected cows:

We initially tested factors that were predicted to be associated with lameness based on previous research and included BCS, presence and severity of superficial skin injuries in the hock and carpal region and udder cleanliness score. We also assessed how much of the proportional unknown variation in lameness was accounted by the random-effects (groups of cows within farms and farms, hereafter, "grouped factors").

The final model included the following three levels:

Y ijk ~ Bernoulli (probability = π ijk), Logit (π 1ijk / π 0ijk) = α + Xi β + vk + vjk, for i = 1,..., n, vk ~ N (0, $\sigma 2\alpha$), $\upsilon jk \sim N (0, \sigma 2\alpha),$

where Y ijk is the occurrence of lameness on a cow "i" housed in the "jth" pen within the "kth" farm; π ijk is the probability of lameness occurrence; α is the regression intercept. X is the matrix of cow-level predictors and the subscript "i" is the fixed effect of the "i th" level for each cow-level predictor considered in the model; β represents the coefficient for predictors X; vk and vjk are random effects to reflect residual variation between groups of cows nested in farms and clustered in pens within each farm, respectively. Random effects followed a normal distribution with mean of 0 and variance $_{\sigma 2\alpha}$.

Model 2. Cow-level associations of BCS, leg injuries, udder cleanliness score and lameness accounted for by controlling for the effects of milk yield, days in milk and parity:

The second model was similar in nature to the first model with the exception that we assessed cow-level associations with lameness in a sub-sample of 16 farms using individual cow data on milk yield, days in milk (DIM) and parity obtained from a regional dairy herd improvement association database. Three models were constructed: a) for parity=1 (hereafter, primiparous cows), b) parity = 2, and c) parity > 2 (hereafter, multiparous cows). In all three models we considered the same predictors offered to the first model, and the explanatory variables DIM and individual milk yield (Tables 16 and 17); in the model for multiparous cows, parity was also included as a dichotomous predictor (cows in the third or greater parity).

Model 3. Herd-level associations with lameness accounting for the effects herd-size and barn system:

A linear regression was built to investigate the herd-level associations with the predicted lameness prevalence using the following model:

Yi = α + Xi β + ϵ i, for i = 1,..., n,

Yi is the within herd lameness prevalence; α represents the regression intercept and ϵ i the residual error, that was independent, normally distributed with mean 0 and standard deviation σ . X is the matrix of herd-level predictors and the subscript "i" is the fixed effect of

the "i th" level for each herd-level predictor considered in the model (Tables 14 and 18); β represents the coefficients for predictors X.

5.3 RESULTS

5.3.1 Data description

Overall, 13706 lactating cows were locomotion scored across the 50 herds. The population of herds was variable regarding barn system and lactating herd size (Table 13), and the distribution of lameness prevalence in this population follows a pattern associated with these variables (Table 14). The prevalence of lameness was variable across farms and, overall it was superior in freestall, compared with compostbedded pack barns, yet, lower lameness prevalence was observed in both small freestall and compost-bedded farms (40 to 160 lactating cows), compared with larger farms (Table 14). The data distribution of herd size between farm type was not balanced, with compost-bedded pack barns on average being smaller and thus under represented in the larger farm category (herd size >first quartile) (Table 14). Most farms housed the cows according to productive needs (e.g., DIM, milk yield, parity) of the farms, or to the health status of cows (e.g., special care cows, lame cows, cows with elevated somatic cell count) which resulted in more than one pen within each farm, (Table 13). The number of cows housed per pen and the number of pens per farm was highly variable across farms (Table 13). Overall, larger farms had more groups than smaller farms. The lameness prevalence was highly variable between the groups of cows that were housed in different pens within each farm (Table 14).

5.3.2 Cow-level factors associated with lameness

Associations of BCS, leg injuries and udder cleanliness score with lameness in the overall population of lactating cows:

The odds of lameness increased in cows with skin leg injuries, low BCS and dirt udders (Table 19). The cows grouped in pens within each farm had greater similarity (27%) regarding within-pen unknown lameness proportion variation.

	Overall pop	ulation				Freesta	ll barns				Compost-bedded pack barns		
Category	Lameness ¹	Cows	Herds	Mean (SD)	Min-Max	Cows	Herds	Mean (SD)	Min-Max	Cows	Herds	Mean (SD)	Min-Max
Within-herd	Clinical	13706	50	41.14 (11.30)	13.76-64.46	12454	38	43.96 (10.39)	13.76-64.46	1252	12	32.22 (9.61)	16.67–51.16
	Severe			20.91 (9.10)	2.82-37.82			22.99 (8.56)	2.82-37.82			14.33 (7.69)	4.73–30.23
Within-pens ²	Clinical			45.83 (24.13)	0–100			47.55 (24.41)	0–100			32.02 (16.40)	0–62.5
	Severe			24.61 (19.09)	0–91.67			25.92 (19.43)	0–91.67			14.11 (11.84)	0–50
Herd size													
40-140	Clinical	1096	13	33.03 (12.11)	13.76–51.16	560	5	32.82 (15.53)	13.76–50.82	536	8	33.17 (10.66)	16.67–51.16
	Severe			15.72 (9.96)	2.82-32.79			16.18 (12.66)	2.82-32.79			15.43 (8.84)	6.0-30.23
141-204	Clinical	1995	12	42.90 (12.49)	18.24-64.46	1518	9	47.34 (10.08)	35.36-64.46	477	3	29.58 (9.82)	18.24–35.67
	Severe			21.95 (11.08)	4.73–37.82			25.36 (10.34)	9.94–37.82			11.71 (6.04)	21.3–36.0
205–359	Clinical	3575	12	43.40 (7.84)	30.63-59.06	3333	11	44.42 (10.08)	35.36-64.46	242	1	32.23	
	Severe			23.04 (6.07)	13.22-31.89			23.93 (7.35)	30.63-59.06			13.22	
360–901	Clinical	7050	13	45.52 (8.73)	30.86	7050	13	45.52 (8.73)	30.86-59.28				
	Severe			23.22 (7.14)	12.56			23.22 (7.14)	12.56-33.85				

Table 14. Distribution of lameness prevalence in herds, pens within herds and by lactating herd size in compost-bedded pack
 and freestall dairy barns visited once in 2016 and located in the south of Brazil.

¹ Lameness prevalence was classified as clinical (locomotion score \geq 3) or severe (locomotion score \geq 4). ² Lameness prevalence within groups of cows grouped by each herd.

Table 15. Distribution of lameness cases by categories of predictors considered in multivariable models for assessment of cow-level factors associated with lameness prevalence in lactating dairy cows housed in compost-bedded pack (n = 12 herds) and freestall barns (n = 38 herds) visited once in 2016 and located in the south of Brazil.

		Overall population of cows					Sub sample of cows (n = 16 herds)				
Variable	Category	Non la	me	Lame	•	Total	Non la	ne	Lame	•	Total
		n	%	n	%		n			%	
Lameness prevalence	Clinical lame cows (locomotion score \geq 3)	7760	56.62	5946	43.38	13706	3372	60.99	2157	39.01	5529
	Severe lame cows (locomotion score \geq 4)	10656	77.75	3050	22.25	13706	4475	80.94	1054	19.06	5529
Barn type	Compost-bedded	849	67.81	403	32.19	1252	32	82.05	7	17.95	39
	Freestall	6911	55.49	5543	44.5	12454	3340	60.84	2150	39.16	5490
Body condition score (1–5)	≤ 2.75	2866	47.95	3111	52.05	5977	1232	49.88	1238	50.12	2470
	> 2.75	4894	63.32	2835	36.68	7729	2140	69.96	919	30.04	3059
Hock injuries	Absence	5740	61.57	3583	38.43	9323	2693	63.11	1574	36.89	4267
	Mild swelling and/or balding	1695	48.73	1783	51.26	3478	571	55.87	451	44.13	1022
	Swelling and/or open wound	325	35.91	580	64.09	905	108	45.0	132	55.0	240
Carpal injuries	Absence	5089	64.01	2861	36.0	7950	2231	67.48	1075	32.52	3306
	Mild swelling and/or balding	2321	49.76	2343	50.23	4664	985	53.91	842	46.09	1827
	Swelling and/or open wound	350	32.05	742	67.95	1092	156	39.39	240	60.61	396
Udder cleanliness score	Clean	5971	58.49	4232	41.5	10207	2456	63.53	1410	36.47	3866
	Presence of small amount of manure or dirt	1164	52.38	1058	47.61	2222	488	55.96	384	44.04	872
	Presence of large amounts of manure or dirt	625	48.94	652	51.06	1277	428	54.11	363	45.89	791

Table 16. Distribution of lameness cases by categories of predictors considered in multivariable models for assessment of cow-level factors associated with lameness prevalence in lactating cows housed in compost-bedded pack (n = 39 cows in 1 herd) and freestall barns (n = 5490 cows in 15 herds) visited once in 2016 and located in the south of Brazil.

Variable	Category	Non lam	e cows	Lame	cows	Total
		n	%	n	%	
Parity	First	1760	84.45	324	15.55	2084
	Second	941	63.50	541	36.50	1482
	Third	435	44.30	547	55.70	982
	Greater than third	236	24.06	745	75.94	981
Days in milk ¹	≤ 120	1082	63.65	618	36.35	1700
	121–175	469	58.48	333	41.52	802
	176–230	513	65.77	267	34.23	780
	231–280	417	64.05	234	35.94	651
	281–335	339	63.72	193	36.28	532
	336–782	451	50.39	444	49.61	895
	Missing data	101	59.76	68	40.24	169

¹ Cows with values superior to 800 days in milk (n = 169) were ruled out of this variable before running the models since most of those data seemed inconsistent for that predictor in the descriptive analyses (e.g., greater milk yield than similar categories of days in milk, inconsistencies in accumulated milk yield). Data on cows with missing measurements for this predictor were excluded from multivariable regression analyses.

		Primi	Primiparous cows			Second-lactation cows				Multiparous cows (> 2 nd lactation)						
Variable	Category	Non l	ame	Lam	ie	Total	Non	lame	Lan	e	Total	Non	lame	Lame	•	Total
		n	%	n	%		n	%	n	%		n	%	n	%	
Lameness proportion	Overall population	1760	84.45	324	15.55	2084	941	63.49	541	36.50	1482	671	34.18	1292	65.82	1963
Days in milk ¹	≤ 120	533	91.11	52	8.89	585	332	67.75	158	32.25	490	217	34.72	408	65.28	625
	121–175	226	81.0	53	19.0	279	133	60.45	87	39.55	220	110	36.30	193	63.70	303
	176–230	272	88.03	37	11.97	309	137	67.82	65	32.18	202	104	38.66	165	61.34	269
	231-280	260	87.84	36	12.16	296	96	60.38	63	39.62	159	61	31.12	135	68.88	196
	281–335	174	80.55	42	19.45	216	94	70.15	40	29.85	134	71	39.01	111	60.99	182
	336–782	236	74.45	81	25.55	317	124	53.22	109	46.78	233	91	26.38	254	73.62	345
	Total	1701	84.96	301	15.03	2002	916	63.70	522	36.30	1438	654	34.06	1266	65.94	1920
	Missing data	59	71.95	23	28.05	169	25	56.82	19	43.18	44	17	39.53	26	60.47	43
Body condition score (1–5)	> 2.75	1135	88.12	153	11.88	1288	571	72.55	216	27.45	787	434	44.11	550	55.89	984
	2–2.75	625	78.52	171	21.48	796	370	53.24	325	46.76	695	237	24.21	742	75.79	979
Milk yield (k/cow) ²	First quartile	410	78.54	112	21.46	522	221	59.09	153	40.91	374	145	29.41	348	70.59	493
	Median	448	85.17	78	14.83	526	222	60.16	147	39.84	369	159	32.51	330	67.49	489
	Third quartile	457	87.72	64	12.28	521	251	67.84	119	32.16	370	173	35.16	319	64.84	492
	Max	445	86.41	70	13.59	515	247	66.94	122	33.06	369	194	39.67	295	60.33	489

Table 17. Distribution of lameness cases by parity and days in milk in lactating dairy cows housed in compost-bedded pack (n = 39 cows in 1 herd) and freestall barns (n = 5490 cows in 15 herds) located in the south of Brazil.

¹ Cows with values superior to 800 days in milk (n = 169) were ruled out of this variable before running the models since most of those data seemed inconsistent for that predictor in the descriptive analyses (e.g., greater milk yield than similar categories

of days in milk, inconsistencies in accumulated milk yield). Data on cows with missing measurements for this predictor were excluded from multivariable regression analyses.

² Quartiles in primiparous cows: 4–28, 28.1–34.1, 34.2–39.7, 39.8–56.8; quartiles in second lactation cows: 4–28.5, 28.6–37.0, 37.1–44.5, 44.6–69.9; quartiles in multiparous cows: 3.5–27.9, 28.0–37.3, 37.4–46.9, 47.0–84.2.

Table 18. Distribution of lameness prevalence (locomotion score \geq 3) by categories of predictors considered in multivariable models for assessment of herd-level factors associated with lameness in lactating cows housed in compost-bedded pack (n = 12 herds) and freestall barns (n = 38 herds) visited once in 2016 and located in the south of Brazil.

		Overall population			Freestall barns				Compost-bedded pack barns							
Variable	Category	Herds	Mean	SD	Min	Max	Herds	Mean	SD	Min	Max	Herds	Mean	SD	Min	Max
Did the cows usually fall at the barn? ¹	No	14	34.42	12.2	16.67	56.48	7	40.86	13.1	22.53	56.48	7	27.98	7.4	16.67	35.53
	Yes	36	43.75	9.9	13.76	64.46	31	44.66	9.8	13.76	64.46	5	38.08	9.9	25.0	51.16
Alley manure cleaner system	Scrapper	17	44.42	11.7	18.24	59.05	13	48.39	7.8	31.16	59.05	4	31.53	14.2	18.24	51.16
	Tractor	33	39.44	10.9	13.76	64.46	25	41.66	10.9	13.76	64.46	8	32.52	7.6	16.67	43.75
Stall cleaning frequency (day)	Once-twice	23	41.33	12.3	13.76	64.46	19	43.44	12.4	13.76	64.46	4	31.33	4.9	25.0	35.53
	Three	19	44.48	8.3	30.63	59.27	19	44.48	8.3	30.63	59.27	0				
	> three	8	32.62	11.6	16.67	51.16	0					8	32.62	11.6	16.67	51.16
Stall bedding ²	Compost	12	32.19	9.6	16.67	51.16	0					12	32.19	9.6	16.67	51.16
	Deep-bedding	11	39.33	11.2	13.76	52.89	11	39.33	11.3	13.76	52.89	0				
	Mattress	16	47.92	10.7	22.53	64.46	16	47.92	10.7	22.53	64.46	0				
	Other	11	42.83	7.3	30.86	59.27	11	42.83	7.3	30.86	59.27	0				
Roof covering the holding area ³	Covered	40	39.85	11.2	13.76	59.27	30	43.42	10.1	13.76	59.27	10	29.13	6.9	16.67	35.67
	Uncovered	10	46.27	10.6	22.50	64.46	8	45.97	11.9	22.53	64.46	2	47.46	5.2	43.75	51.16
Cow-feed alley floor	non-slippery	12	33.65	11.4	13.76	53.74	9	35.72	11.9	13.76	53.74	3	27.39	7.9	18.24	32.23
slipperiness score	Slightly	20	41.41	11.6	16.67	64.46	16	45.05	9.4	30.63	64.46	4	26.83	8.1	16.67	35.67
	Very	15	47.91	7.1	34.81	59.27	12	49.76	5.5	40.91	59.27	4	40.50	9.2	34.81	51.16
	Missing data	3	35.48	7.2	31.16	43.75	1	31.16				2	37.64	8.6	31.52	43.75

Dry period length ⁴	Sixty days	32	39.09	10.3	13.76	58.40	26	41.09	10.0	13.76	58.40	6	30.41	6.3	18.24	35.67
	Less than sixty days	18	44.77	12.4	16.67	64.46	12	50.18	8.4	35.40	64.46	6	33.97	12.5	16.67	51.16

¹ The question was directly posed to farmers and answers were categorized (no = cows never fall, yes = cows fall often, sometimes or rare times, n = 4, 17 and 15 respectively). We used this question in order to triangulate information of slipperiness score of floors at the cow alley in each barn, but not as a main predictor in the multivariable model.

² We tested the associations of lameness with stall base categories used in freestall barns, compared with compost-bedded pack barns. Compost bedded-pack barns used shaving wood as bedding; deep-bedding material commonly used was sand and stalls with mattresses used different bedding types; "others" represent a category for farms that did not use the same bedding for all lactating cows, instead the farms used a) mattress and compost-bedded barns (n = 4), b) mattress and deep-bedding (n = 4) or c) mattress, deep-bedding and compost-bedded barns (n = 2).

³ Covered = partially (50% or more) or totally covered holding area.

⁴ Dry period length criteria for farms with less than sixty days = 45, 50, 55 and 57 days (n = 6, 5, 6, and 1 farm respectively).

Cow-level associations with lameness accounting for the effects of milk yield and DIM in primiparous, second-lactation and multiparous cows:

The lameness prevalence increased with DIM and parity (Table 17); cows in their first parity had lower lameness prevalence in their first 120 lactation days (9%) whereas older cows in their last third of lactation had greatest prevalence (73.6%) (Table 17).

Table 19. Cow-level factors associated with lameness prevalence (locomotion score \geq 3) in lactating dairy cows (n = 13706 cows in 50 herds) housed in freestall (n = 12454 cows in 38 herds) and compost-bedded pack barns (n = 1252 in 12 herds) visited once in 2016 and located in the south of Brazil.

Variables	Category	Estimate	Odds ratio	Predicted 95% CI	P-value
Body condition score (1–5)	> 3	Referent			
	1.5–2.75	0.80	2.23	2.1–2.5	< 0.001
Hock injuries	Absence	Referent			
	Mild swelling and/or balding	0.34	1.41	1.27-1.56	< 0.001
	Swelling and/or open wound	0.66	1.94	1.63–2.32	< 0.001
Carpal injuries	Absence	Referent			
	Mild swelling and/or balding	0.41	1.51	1.38–1.65	< 0.001
	Swelling and/or open wound	1.08	2.94	2.50-3.46	< 0.001
Udder cleanliness	Clean	Referent			
score	Presence of small amount of manure/dirt	0.27	1.31	1.15–1.48	< 0.001
	Presence of large amounts of manure/dirt	0.46	1.59	1.31–1.92	< 0.001
Random effect	Observations (n)	Variance	Standard deviation	ICC % ²	
Groups of cows within farms	234	1.24	1.11	27.43	
Farms	50	0.02	0.15	0.66	

 1 CI = confidence interval

 $^{\rm 2}$ Intraclass correlation coefficient (ICC %) was computed using the latent variable approach.

After accounting for individual milk yield, DIM and parity effect, the cow-level associations and the proportion of unknown variation in lameness associated with the grouping factors were resulted in three

final models for each parity category. Milk yield and poor udder cleanliness were not associated with lameness and therefore will not be mentioned again. Leg injuries had a distribution associated with each parity category (Tables 20-22) and low BCS was associated with greater odds of lameness in cows in all the parities, with similar coefficients (Tables 20-22).

Table 20. Cow-level factors associated with lameness prevalence (locomotion
score \geq 3) in primiparous lactating dairy cows (n = 2002 cows in 16 herds)
housed in freestall ($n = 15$ herds) and compost-bedded pack barns ($n = 1$ herd)
visited once in 2016 and located in the south of Brazil.

Variables	Category	Estimate	Odds ratio	Predicted 95% CI ¹	P-value
Body condition score (1–5)	> 2.75	Referent			
	2.0–2.75	0.88	2.41	1.82–3.19	< 0.001
Days in milk	≤ 120	Referent			
	121–175	1.08	2.95	1.89–4.59	< 0.001
	176–230	0.46	1.58	0.98–2.55	0.06
	231–280	0.57	1.76	1.07–2.89	0.02
	281–335	1.10	2.99	1.83-4.90	< 0.001
	336–782	1.49	4.44	2.81-7.03	< 0.001
Carpal injuries	Absence	Referent			
	Mild swelling and/or balding	0.33	1.40	1.03–1.88	< 0.03
	Swelling and/or open wound	1.31	3.72	2.23-6.19	< 0.001
Random effect	Observations (n)	Variance	Standard deviation	ICC % ²	
Groups of cows within farms	79	0.32	0.57	8.98	
Farms	16	0.07	0.26	2.07	

 1 CI = confidence interval.

 $^{\rm 2}$ Intraclass correlation coefficient (ICC %) was computed using the latent variable approach.

Primiparous cows within 121-175 and > 281 DIM had between 1.8 to 7 times greater odds of lameness compared with cows that were within 120 DIM (Table 20). The odds of lameness increased in primiparous cows with the severity of carpal injuries (Table 20). After accounting for the associations of DIM, BCS and carpal injuries in

primiparous cows, the cows grouped in pens within each farm had greater similarity (9%) regarding within-pen unknown lameness proportion variation, if compared with the similarity of cows housed within each farm (2%). Compared with second lactation cows in the first 120 DIM, second lactation cows with greater DIM (121-175 DIM, 231-280 DIM and >336 DIM) had 1.1 to 4 times greater odds of lameness (Table 21).

Variables	Category	Estimate	Odds ratio	Predicted 95% CI ¹	P-value
Body condition score (1–5)	> 2.75	Referent			
	2.0-2.75	0.97	2.65	2.07-3.38	< 0.001
Days in milk	≤ 120	Referent			
	121–175	0.51	1.66	1.15–2.38	< 0.01
	176–230	0.18	1.20	0.81-1.76	0.36
	231–280	0.49	1.63	1.09–2.44	0.02
	281–335	0.19	1.21	0.77–1.90	0.41
	336–782	1.02	2.76	1.91–3.99	< 0.001
Hock injuries	Absence	Referent			
	Mild swelling and/or balding	0.31	1.36	0.99–1.87	0.05
	Swelling and/or open wound	0.93	2.54	1.43–4.48	< 0.01
Random effect	Observations (n)	Variance	Standard deviation	ICC % ²	
Groups of cows within farms	87	0.05	0.23	1.57	
Farms	16	0.18	0.42	5.15	

Table 21. Cow-level factors associated with lameness prevalence (locomotion score \geq 3) in second-lactation dairy cows (n = 1438 cows in 16 herds) housed in freestall (n = 15 herds) and compost-bedded pack barns (n = 1 herd) visited once in 2016 and located in the south of Brazil.

 1 CI = confidence interval.

 $^{\rm 2}$ Intraclass correlation coefficient (ICC %) was computed using the latent variable approach.

Second parity cows with severe hock injuries had greater odds of lameness than cows without injuries (Table 21). After accounting for the associations of lameness with DIM, BCS and hock injuries in second lactation cows, the cows grouped in pens within each farm had lower similarity (1.6%) regarding within-pen unknown lameness proportion variation, if compared with the similarity of cows housed within each farm (5%) (Table 21).

The prevalence of lameness was greatest for cows with three or more parities, compared with three-lactation cows, and in multiparous cows at the end of lactation (\geq 336 DIM) than in cows in the first 120 DIM. (Table 22).

The odds of lameness also increased in multiparous cows with mild and severe carpal injuries (Table 22). After accounting for the associations of lameness with DIM, BCS and carpal injuries in multiparous cows, the cows grouped in pens within each farm had slight great similarity (6.5%) regarding within-pen unknown lameness proportion variation, if compared with the similarity of cows housed within each farm (5%) (Table 22).

Table 22. Cow-level factors associated with lameness prevalence (locomotion score \geq 3) in multiparous (> second parity) dairy cows (n = 1920 cows in 16 herds) housed in freestall (n = 15 herds) and compost-bedded pack barns (n = 1 herd) visited once in 2016 and located in the south of Brazil.

Variables	Category	Estimate	Odds ratio	Predicted 95%CI ¹	P-value
Body condition score (1–5)	> 2.75	Referent			
	2.0–2.75	1.04	2.83	2.29–3.59	< 0.001
Days in milk	≤ 120	Referent			
	121–175	-0.03	0.97	0.70–1.35	0.86
	176–230	-0.08	0.92	0.65–1.30	0.64
	231–280	0.32	1.37	0.93–2.04	0.11
	281–335	-0.03	0.97	0.65–1.44	0.89
	336–782	0.58	1.79	1.25–2.56	< 0.01
Parity	Third	Referent			
	Greater than third	0.95	2.59	2.08-3.22	< 0.001
Carpal injuries	Absence	Referent			
	Mild swelling and/or balding	0.32	1.38	1.09–1.75	< 0.01
	Swelling and/or open wound	0.69	2.0	1.34–3.00	< 0.001
Random effect	Observations (n)	Variance	Standard deviation	ICC % ²	
Groups of cows within farms	77	0.23	0.48	6.48	
Farms	16	0.18		5.31	

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¹ CI = confidence interval.

 $^{\rm 2}$ Intraclass correlation coefficient (ICC %) was computed using the latent variable approach.

5.3.3 Herd-level factors associated with lameness

Farms using mattresses as stall base had a greater predicted lameness prevalence than compost-bedded farms, but were similar to freestall farms using other stall bases (Table 23).

Table 23. Herd-level factors associated with lameness prevalence (locomotion score \geq 3) in lactating dairy cows housed in freestall (n = 37 herds) and compost-bedded pack barns (n = 10 herds) visited once in 2016 and located in the south of Brazil.

Variables	Category	Estimate	Predicted 95% CI	P-value
Intercept	-	17.32	10.45–24.19	
Stall bedding type ¹	Compost	Referent		
	Deep-bedding	5.552	-1.74–12.79	0.13
	Mattress	14.20	7.35–21.05	< 0.001
	Other	4.45	-4.04–12.93	0.29
Cow-feed alley floor slipperiness score	Non-slippery	Referent		
	Slightly slippery	6.56	0.82-12.30	< 0.05
	Very slippery	12.42	6.53–18.29	< 0.001
Dry period length ²	Sixty days	Referent		
	Less than sixty days	6.83	2.01-11.66	< 0.05
Lactating herd size ³	40–140	Referent		
	141–204	10.54	3.45-17.62	< 0.01
	205–359	8.53	0.90–16.17	< 0.05
	360–901	11.63	3.48–19.77	< 0.01

¹ Data correspondent to the results of a simple linear regression analyses (P-value < 0.001, adjusted R squared: 0.59). The coefficients and confidence intervals (CI) are shown for each and category. The category for "other" bedding type comprise barns that used diverse stall base and bedding material for different pens within the farm.

² Dry period length criteria for farms with less than sixty days = 45, 50, 55 and 57 days (n = 6, 5, 6, and 1 farm respectively).

³ Lactating herd size was categorized by quartiles.

The predicted lameness prevalence increased in farms with the slipperiness of floors at the cow-feed alley (Table 23), When the data was filtered according to farm lameness prevalence, flooring slipperiness score and farmers' responses to the question regarding how often cows fall, the distribution of prevalence [mean \pm SE (range)] was lower on farms where farmers stated that the cows never fell compared to farms where farmers reported that cows fell [27.12 \pm 2.84 (18.24–32.23) vs 38.29 \pm 4.62 (13.76–53.74)], and on farms with non-slippery compared to farms with and slippery cow-alley floors [39.35 \pm 4.83 (16.67–56.48) vs 45.63 \pm 1.73 (25.0–64.46) in barns, respectively].

Predicted lameness prevalence was closer to the actual lameness prevalence in larger than in small herds (40 to 140 lactating cows (herd size first quartile)) (Table 23). Additionally, farms managing to a dry period less than 60 days had a greater predicted lameness prevalence than farms targeting a 60 day dry off period (Table 23). The significant herd level variables accounted for approximately 60% (adjusted R squared) of the predicted average lameness prevalence in the linear model.

5.4 DISCUSSION

This study describes the results of a cross-sectional study reporting associations between cow and herd level factors with lameness in cows housed in freestall and compost-bedded dairy farms in southern Brazil. The odds of lameness was greater in cows with increasing parity category, the presence of leg injuries and the low BCS (≤ 2.75). In freestall farms, cow-alley floor slipperiness, the use of mattresses, greater lactating herd size, and farms with dry period length < 60 days in the herds were all associated with increases in predicted herd-level lameness prevalence. We are not aware of previous epidemiological studies describing these associations on farms with similar conditions in Brazil.

The associations of lameness in cows with leg injuries varied by each parity category: first lactation and cows with more than 2 lactations had greater odds of lameness associated with carpal injuries, while second lactation cows had greater odds of lameness associated with severe hock injuries. This pattern may suggest a differential effect of facilities' design in cows of differing parity but to our knowledge there has been no work specifically addressing this issue. It is not surprising that there is an association between carpal and hock injuries and lameness; a result that is likely driven by certain facility characteristic affecting both of these maladies. For instance previous work has shown that the use of deep bedding in stalls (BARRIENTOS et al., 2013; CHAPINAL et al., 2014) are risk factors for both hock injuries and lameness. Similarly, the softness and length of the lying surface and the height of free space within the confines of the cubicle space (BRENNINKMEYER et al., 2013) have also been shown to be associated with both injuries and lameness.

After accounting for DIM, BCS and leg injuries we were only able to explain some proportion of the variation in lameness leaving a large proportion of the variance explained by the grouping of cows in pens within farms (cow-level). The cow-level analyses provides to our knowledge the first evidence of the heterogeneity of factors related with pen structure and management within a farm that might influence the prevalence of lameness, thus future work should explore specific risk factors for within-pen prevalence of lameness.

Our study has identified that first lactation cows are at increased risk of being lame when they are in their first 120 DIM. Other parity categories, however, showed greater odds of lameness with increased DIM and increased parity. Of interest is that in both first and secondlactation cows, specific times during the lactation were associated with greater occurrence of lameness. In contrast cows in their third or greater lactation cows were at even odds regardless of their DIM, which may represent the cumulative effect of chronic cases which may mask the possible patterns of new cases' occurrence.

In one prospective UK study involving freestall farms, lameness occurred throughout the 305-day lactation (GREEN et al., 2014). A longitudinal study showed that cows between greater than 91 DIM were more likely to become lame that cows in the first days in lactation (< 90 DIM) (LIM et al., 2015). Other authors have also reported that the cumulative incidence of lameness (compared with 0–56 days) increased gradually from 119–168 DIM until the end of lactation (RANDALL et al., 2016). This latter study reports a similar pattern to our current study, likely reflecting a specific period of greater susceptibility of cows to became lame in the first and second-lactation cows. However, others authors have found an inverse pattern of lameness distribution by DIM: cows at 60–120 and 121–180 DIM had lower odds of being lame than cows in the first 60 DIM (MORABITO et al., 2017). This latter cross-sectional study, however, describes the lameness distribution by DIM

for cows in all parity categories (likely reflecting the cumulative proportion of new and chronic cases for all parities). Caution is warranted when comparing this study to our own given differences in sampling; the higher proportion of cows sampled in the Morabito et al., (2017) that were 120 DIM making comparison with the present study difficult.

The fact that lameness prevalence increased in primiparous cows at around lactation peak (i.e., 120 DIM) is of particular interest. It appears that the susceptibility to lameness changes as cows proceed through the lactation curve. Whether this increased risk is associated with claw horn disruption diseases is not known but there is some evidence that claw horn disruption may be related with high energy demands for milk production and the metabolic status of the cows (WILHELM; WILHELM; FÜRLL, 2017). In contrast, our finding that the second point of convergence was associated with the lactation curves of primiparous and adult cows in later lactation (i.e., 255–295 DIM) may be a consequence of accumulated chronic cases.

Cows alter their time budget throughout the lactation period and with increases in parity. Specifically early lactation cows have lower lying times than cows in late lactation (BAK; HERSKIN; JENSEN, 2016; WESTIN et al., 2016a), and multiparous cows have longer lying times than primiparous cows (SOLANO et al., 2016; WESTIN et al., 2016a; MORABITO et al., 2017). These differences in lying patterns may predispose early lactation and primiparous cows increased risk of hoof disorders, due in large part to increased standing times. The first lactation cows are of special interest from a preventive perspective, since some causes of lameness may induce chronic changes in the foot and previous lameness cases are risk factors with great impacts in subsequent lameness events (RANDALL et al., 2016, 2018). Preventing the occurrence of the first lameness episode in a cow may have a significant impact in the reduction in lameness in dairy herds; but work is needed to verify this statement.

The association between low BCS and lameness has been reported in a number of studies (RANDALL et al., 2015; NEWSOME et al., 2017a). However, what remains unclear is if low BCS is directly associated with diseases causing lameness, or if it is an intervening factor in a causal pathway (LIM et al., 2015; RANDALL et al., 2015). In the present study we are not able to indicate the direction of this association, a well noted shortcoming of cross-sectional studies, given that the outcome and the factors are measured at the same time. In the case of BCS it is also further complicated by the fact that it is confounded with changes in milk yield: high producing cows with low BCS may became lame, and after being lame, both milk yield and BCS decreases.

The increases in lameness noted in cows that were after lactation peak maybe explained by the complex metabolic conditions that high producing cows must cope with at the beginning of lactation (ZACHUT et al., 2013). Since the majority of lameness is the consequence of previous exposures acting on claw-diseases, we encourage more work on investigating the physiological aspects that may be used as early predictors of a lesion (and lameness). Cows experience a series of metabolic changes during the transition period, including decline in dry matter intake, and alterations in insulin actions which prioritize energy mobilization for milk yield over the provision of energy to other tissues, which results in lipolysis and gluconeogenesis (ZACHUT et al., 2013). Those changes may affect the health status of cows independently of the nutritional management and housing provided to the cows while in the transition period. Occasionally, impaired energetic metabolism and inadequate management practices may result in hyperglycemia and serious health alterations such as ketosis.

The effects of negative energetic balance in transition cows on claw-health is an under explored area; likely because the energy metabolism in the transition period may be affected by milk yield, low BCS, and the presence of claw-horn diseases. Work in the human literature provides some interesting discussion that may help disentangle these various factors; most diabetic patients are at increased risk of foot ulcers due to the downstream effects of abnormal glucose metabolism. Inadequate glycaemic control (hyperglycemia) may predispose them to peripheral neuropathy, oxidative stress and ischaemia resulting in foot deformity and foot ulcers (AHMAD, 2016). Future studies on lameness should also investigate the specific effects of glycemia or energetic metabolism in transition cows on the development of the first event of claw-horn disruption diseases as a first step to disentangle the complex associations between DIM, BCS, milk yield and lameness.

Smaller farms (40 to 140 lactating cows) had similar lameness prevalence regardless of housing type but this must be viewed with caution given that we had a lack of orthogonality of the data in our sample (we had few large farm that used compost bedding). Thus, the average within-herd prevalence reported in this study was strongly influenced by the measurements taken in the freestall barns and most of the population at risk was concentrated in this barn system.

The issue of farm size and lameness risk has been of great interest to many working within the dairy industry (ROBBINS et al., 2016). We noted a greater lameness prevalence in larger farms. This finding that may be attributed at least in part to the greater cow numbers which may have resulted in increased probability that a particular cow could become exposed to risk factors for claw-diseases or infectious agents causing lameness, since the dynamics of transmission of infectious agents depends on the number of possible contacts between individuals which would be much greater compared to smaller herds.

The complexity of interactions on a farm also increases with increases in herd size. For instance, the number of social interactions between an individual cow increases with increasing group size, the diversity of environments at each lactation phase, and the differences in facilities and management within each pen or group of cows housed on the farm.

The larger farms also likely have greater walking distances to and from the milking parlor and trackways features that may differ between individual groups of cows. Unfortunately we did not measure this but do recognize that the observed greater lameness prevalence in cows that were greater than 120 DIM (in first and second lactation cows) may have also been confounded by the fact that these cows may have had to walk the furthest. Previous work by von Keyserlingk et al. (2012) reported that distance to and from the milking parlor varied by 0 to more than 1 km in freestall housed cows in US. Thus, the distance traveled by the cows, and the nature of the walking track may contribute to lameness prevalence.

Additionally, the inspection of individual cows might be more difficult in large herds. Clearly, the use of automatic devices (VAN DE GUCHT et al., 2017) to detect lame cows in larger farms may be particularly useful given that individual inspection of cows may be extremely time consuming and difficult given that the cow to employ ratio also increases with increasing farm size (BEWLEY et al., 2010).

Increasing herd-size has been previously associated with both greater (SJÖSTRÖM et al., 2018) or lower lameness prevalence in dairy herds (CHAPINAL et al., 2013, 2014; SOLANO et al., 2015). However, comparisons between the associations found by those studies and the present should be assessed carefully, since each study used a different variable to represent herd-size and, in contrast with the present study,

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the prevalence was estimated in a sample of cows that were almost always < 120 DIM. These studies explored a linear relationship between lameness and herd size, while in the present study the association was demonstrated between categorical variables: small, compared with each of three categories of larger farms that had similar predicted lameness prevalence and showed no evidence of linear increases thorough the levels.

We think that herd-size is an intermediate variable that represents diverse management practices adopted by the farmers and differences in facilities' design of the barns. In addition, this association is not causal, which explains why different studies, or even the same study (SJÖSTRÖM et al., 2018) have found associations with different directions. Also, when accounting for other herd-level variables associated with lameness (e.g., time of lameness treatment, active observation of locomotion in cows by the farmers, or features of lying surfaces and flooring), the herd-size no longer was associated with lameness (BARKER et al., 2010). The high variation in within-pen lameness prevalence in the present study supports our hypothesis that other intervening variables may explain the herd-size association: larger farms with cows housed in multiple pens had different levels of lameness prevalence, and when controlled for relevant cow-level factors the variation in lameness prevalence remained high (when compared with the variation of lameness prevalence between farms). Clearly, the effect of within farm variation in lameness must be further explored.

Using mattresses as the stall base was associated with greater prevalence of lameness compared to compost-bedded pack barns but this association was not observed in freestall barns that used deepbedding type or diverse stall bases and bedding types. The greater prevalence of lameness in herds using mattress has been previously shown in US (CHAPINAL et al., 2013) and China (CHAPINAL et al., 2014) freestall dairy farms; dairy cows housed on deep bedded stalls have prolonged lying times than other bedding types such as rubber mats (BAK; HERSKIN; JENSEN, 2016).

In a recent US study, the lameness prevalence was found to be similar in freestall barns (40.80%, n = 8 herds and average of 84 cows housed per herd) and compost bedded barns (39.24%, n = 7 herds and average 178 cows housed per herd) (ECKELKAMP et al., 2016). An Austrian study reported similar prevalence of lameness when comparing 5 freestall (14.9%) and 5 compost-bedded dairy barns (18.7%) where the Flekvieh herd sizes ranging from 20 to 41 cows (BURGSTALLER

et al., 2016). Although we noted that the prevalence of lameness was lower in compost-bedded barns compared with most of the freestall farms, however, the within-herd lameness prevalence was high on those herds, if compared with others study; in addition, bedding in compost barns may be a protective factor for lameness, however if other risk factors for lameness are not controlled (e.g., flooring, BCS, cows' management), lameness will be a prevalent issue.

The presence of slippery floors at the cow-feed alley was also associated with greater predicted lameness prevalence, possibly explained by the non abrasive surfaces resulting in lower stability while walking which may have caused the cows to compensate and engage in the adoption of more abnormal postures or movements. Floor slipperiness has been previously associated with greater lameness prevalence (SOLANO et al., 2015). Slippery floors may also potentially induce abnormal hoof wearing and also musculoskeletal damage caused by slips or falls.

The prevalence of lameness was higher in farms that targeted a dry off period that was less than 60 days, compared with farms that targeted 60 days. We speculate that this may be used as a proxy for how the management of dry cows can impact the occurrence of lameness in the herd, however we are unable to provide any causal links on how management practices of dry cows may affect lameness. This is an important theme for future studies on lameness.

5.5 CONCLUSION

We found greater odds of lameness in cows with presence of leg injuries, with low BCS, and with advanced parity and within certain times of the lactation curve for cows in first and second parity. The use of mattresses as stall base and the presence of slippery floors at the cowfeed alley were the herd-level predictors with stronger associations with lameness, suggesting that interventions aimed at modifying these conditions should be explored. Although we noted associations with lactating herd size and the dry period length due to the cross-sectional nature of the present study, we cannot make specific inferences regarding the direction of the associations, or the possible causal effects of the predictor on the lameness prevalence. These variables are important factors to be explored in future research.

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6 GENERAL CONCLUSIONS

We described associations with lameness both at the cow- and herd-level in different housing systems in southern Brazil. Some cowlevel factors, for instance, BCS seems similar between the two studies (grazing systems and intensive managed freestall and compost-bedded pack barns). Other predictors at the cow level, seems to be particular of each productive system: leg injuries, for instance are nonexistent in grazing cows and very common in confined cows. The herd-level predictors also seems to be highly associated with the type of farm. In freestall and compost-bedded pack barns the effects of flooring and stall characteristics are of main importance to control lameness. In smallscale grazing herds, the adaptation of the breeds, and one factor associated with cows' mobility and human-animal relationship (high speed when moving the cows) were strongly associated with increases in lameness. The farmers' perspectives and actions seems to be of great importance to understand the high lameness occurrence observed in grazing herds, but also, the exploration of these perspectives and actions may help to think in appropriate intervention measures directed do control the problem.

Overall, lameness is a significant health and welfare issue highly prevalent in the assessed populations of cows. What is seen on this theses as numbers, represents a great proportion of animals that may be in pain and suffering. Thus, actions might be promoted from official and private dairy stakeholders to reduce the impacts of lameness in Brazilian dairies. Due to the great variation in lameness occurrence in the visited herds, there is a great opportunity to improve the health and welfare of these animals. Any reduction in lameness should represent greater economical benefits for the farmers, but also is a very important condition to improve the regional and national dairy industry.

Future research efforts should be directed to better understand the dynamics of lameness in other dairy herds in Brazil and also to understand how professionals and farmers understand this problem and how much they are aware and interested in improving the hoof health and the welfare of lame cows at their herds. Future research on this issue should focus in knowing about the specific diseases causing lameness and their dynamics. Identifying the main risk factors for those diseases is a main objective. Longitudinal studies, designed to know the causes of the first lameness event in dairy cows and also focusing in disentangling any possible causal association between the metabolic

status of transition cows and claw-horn abnormalities may be interesting in order to better comprehend this issue and to enforce effective preventive measures.

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