Université de Montréal

Traitement antibiotique sélectif au tarissement des vaches laitières

Par

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Cette thèse intitulée

Traitement antibiotique sélectif au tarissement des vaches laitières

Présentée par

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A été évaluée par un jury composé des personnes suivantes

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Résumé

Le traitement sélectif (TS) des vaches laitières au tarissement (où seuls les quartiers ou les vaches infectées sont traités avec des antimicrobiens) constitue une alternative potentielle au traitement universel (TU, où tous les quartiers de toutes les vaches reçoivent des antimicrobiens, quel que soit leur statut infectieux), pour une utilisation plus judicieuse des antimicrobiens. L'objectif de cette thèse était d'apporter plus de lumière sur les décisions de traitement antimicrobien ciblant les quartiers ou vaches infecté(e)s au tarissement. Différents devis et méthodologies ont été utilisés pour répondre à cet objectif.

Un essai contrôlé randomisé a été conçu et 569 vaches (2,251 quartiers) provenant de 9 troupeaux laitiers du Québec avec un comptage de cellules somatiques (CCS) du réservoir <250 000 cellules/mL ont été systématiquement enrôlées et réparties au hasard dans 4 groupes : 1) traitement antimicrobien seul pour tous les quartiers ; 2) traitement antimicrobien combiné avec un scellant interne à trayon pour tous les quartiers ; 3) traitement antimicrobien sélectif seul basé sur les résultats de la culture bactériologique du lait sur Petrifilm® ; et 4) traitement antimicrobien sélectif combiné avec un scellant interne à trayon basé sur les résultats de la culture du lait sur Petrifilm®. Dans les groupes de TS, les quartiers non infectés n'ont reçu qu'un scellant interne à trayon. Aucune différence significative n'a été détectée entre le TS par quartier et le TU des vaches laitières au tarissement, en termes d'élimination des infections intramammaires (IIM) et de prévention de nouvelles IIM pendant la période de tarissement, de risque d'un premier cas de mammite clinique (MC), de production laitière moyenne quotidienne et de CCS au cours des 120 premiers jours de la lactation suivante. Un TS reposant sur les résultats d'une culture de lait de quartier sur Petrifilm® au tarissement a permis de réduire l'utilisation d'antimicrobiens de 52% (IC à 95%: 39 – 64) par rapport à un TU.

En plus de cet essai contrôlé randomisé, la culture du lait par quartier à l'aide de Petrifilm® a été comparée à l'historique du CCS par une estimation bayésienne de leur précision pour identifier les quartiers ou les vaches qui devraient être traités avec des antimicrobiens dans des protocoles de TS au tarissement. Compte tenu de la disponibilité des données de CCS, de la facilité

d'utilisation du dernier test de CCS pré-tarissement et de la valeur prédictive négative élevée qui pourrait être obtenue, les producteurs pourraient envisager d'utiliser uniquement le dernier test de CCS pré-tarissement comme outil potentiel pour identifier les vaches qui devraient être traitées avec des antimicrobiens au tarissement. Le dernier test de CCS pré-tarissement peut être utilisé seul ou en combinaison avec la culture de lait par quartier sur Petrifilm® sur les vaches avec un CCS élevé pour identifier encore plus spécifiquement les quartiers qui doivent être traités. L'ajout d'une culture de lait par quartier à la ferme sur Petrifilm® pour les vaches identifiées comme infectées à l'aide des données du CCS améliorerait la précision du test (principalement la valeur prédictive positive) et réduirait davantage l'utilisation d'antimicrobiens.

Également, une revue systématique et une série de méta-analyses ont été menées pour étudier l'efficacité du TS par rapport au TU, afin de guider les décideurs et les utilisateurs qui s'engagent dans une utilisation plus efficace et judicieuse des antimicrobiens au moment du tarissement. Treize articles représentant 12 essais contrôlés, randomisés ou non, étaient disponibles pour les analyses. Le TS a permis de réduire de 66% (IC à 95%: 49 – 80) l'utilisation d'antimicrobiens au moment du tarissement. Les résultats appuient fortement l'idée que le TS réduirait l'utilisation d'antimicrobiens au moment du tarissement, sans effet négatif sur la santé du pis ou la production laitière au cours des premiers mois de la lactation subséquente, si, et seulement si, les scellant internes à trayons sont utilisés pour les quartiers non traités avec des antimicrobiens.

Enfin, le suivi de l'utilisation d'un scellant interne à trayon a été effectué pour déterminer la proportion de quartiers qui ont conservé le bouchon de scellant jusqu'à la première traite après le vêlage et la persistance de résidus de scellant dans le lait après le vêlage. Un bouchon de scellant était présent jusqu'à la première traite pour 83% des quartiers, et nous pourrions émettre l'hypothèse que la perte du bouchon s'est produite près du vêlage secondaire à la tétée ou pour une autre raison (ex., la pression hydrostatique du lait), étant donné que les associations observées entre la présence ou non d'un bouchon de scellant observable et les chances de nouvelles IIM étaient relativement faibles. Les résidus de scellant pouvaient être observés dans le lait jusqu'à 12 jours après le vêlage, quoique 75% des quartiers n'excrétaient plus de scellant au bout de 5 jours en lait.

Mots-clés : Vaches laitières, tarissement, infection intramammaire, Petrifilm®, comptage de cellules somatiques, précision diagnostique, traitement antimicrobien sélectif, scellant interne à trayons, utilisation d'antimicrobiens.

Abstract

Selective dry cow therapy (SDCT, in which only infected quarters or cows are treated with antimicrobials) represents an alternative to blanket dry cow therapy (BDCT, in which all quarters of all cows at dry off are treated with antimicrobials, regardless of their infection status), for a more judicious use of antimicrobials. The objective of this thesis was to shed more light on targeted antimicrobial treatment decisions of infected quarters or cows at dry-off. Different study designs and methodologies were used to meet this objective.

A randomized controlled trial was designed and a total of 569 cows (2,251 quarters) from 9 dairy herds in Québec with bulk tank somatic cell count (SCC) <250,000 cells/mL were systematically enrolled and randomly allocated to 4 groups: 1) antimicrobial treatment alone of all quarters; 2) antimicrobial treatment combined with an internal teat sealant (ITS) of all quarters; 3) selective antimicrobial treatment alone based on milk bacteriological culture results on Petrifilm®; and 4) selective antimicrobial treatment combined with an ITS based on milk culture results on Petrifilm®. In the selective antimicrobial treatment groups, uninfected quarters received only an ITS. No significant differences were detected between quarter-based selective and blanket dry cow therapies, in terms of elimination of intramammary infections (IMI) and prevention of new IMI during the dry period, risk of a first case of clinical mastitis (CM), daily average milk yield and somatic cell count in the first 120 days of the subsequent lactation. A selective antimicrobial treatment relying on results of quarter milk culture using Petrifilm® at dry off enabled a reduction in antimicrobial use of 52% (95% CI: 39 – 64) as compared to blanket dry cow treatment.

In addition to this randomized controlled trial, quarter milk culture using Petrifilm® was compared with SCC history through a Bayesian estimation of diagnostic accuracy to identify quarters or cows that should be treated with antimicrobials in selective treatment protocols at dry off. Considering the availability of SCC data, the easiness of using just the last Dairy Herd Improvement (DHI) test before dry off, and the high negative predictive value that could be achieved, producers may consider using just the last DHI test before dry off results as a potential tool to identify cows that should be treated with antimicrobials at dry off. The last SCC test before

dry off may be used alone or in combination with quarter-level on-farm Petrifilm® milk culture on high SCC cows to more specifically identify quarters that need to be treated. Adding quarter-level milk culture using Petrifilm® to cows identified as unhealthy using cow-level SCC data could improve the test accuracy (mainly the positive predictive value) and further reduced the use of antimicrobials.

Also, a systematic review and a series of meta-analyses were conducted to investigate the efficacy of SDCT compared with BDCT, to guide decision-makers and users to engage in a more effective and judicious use of antimicrobials at dry-off. Thirteen articles representing 12 controlled trials, randomized or not, were available for analyses. SDCT reduced the use of antimicrobials at dry off by 66% (95% CI: 49 – 80). Evidences strongly support that SDCT would reduce the use of antimicrobials at dry off, without any detrimental effect on udder health or milk production during the first months of the subsequent lactation, if, and only if, ITS are used for healthy quarters untreated with antimicrobials.

Finally, a follow up on the use of ITS was performed to determine the proportion of quarters that had retained the sealant plug until the first milking after calving and the persistence of ITS residues in milk after calving. A sealant plug was present at first milking after calving for 83% of the quarters, and we could hypothesize that the loss of the plug occurred closely around calving due to suckling or for another reason (e.g., milk hydrostatic pressure), since the observed associations between the presence or not of an observable sealant plug and the odds of new IMI were relatively small. The sealant residues could be observed in milk up to 12 days in milk, although 75% of the quarters had expelled the last ITS residues by 5 days in milk.

Keywords: Dairy cows, dry off, intramammary infection, Petrifilm®, somatic cell counts, Diagnostic accuracy, selective antimicrobial treatment, internal teat sealant, antimicrobial use.

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AABP: American Association of Bovine Practitioners

BDCT: blanket dry cow therapy

BTSCC: bulk tank somatic cell count

CBMRN: Canadian Bovine Mastitis Research Network

CCS: comptage de cellules somatiques

CFU: colony-forming units

CI : confidence interval

CM: clinical mastitis

CMT: California mastitis test

CNS: coagulase negative staphylococci

Covn: negative covariance

Covp: positive covariance

CT: controlled Trial

d: day

DCT: dry cow therapy

DHI: Dairy Herd Improvement

DIM: days in milk

GRADE: Grading of Recommendations Assessment, Development and Evaluation

IC: intervalle de confiance

IIM : infection intramammaire

IMI : intramammary infection

IMIenv: IMI by environmental bacteria

IMM: intramammary

ITS: internal teat sealant

Kg : kilogramme

LCM: latent class model

lnSCC: natural logarithm of the somatic cell count

MALDI-TOF MS: matrix assisted laser desorption/ionization time-of-flight mass spectrometry

MC: mammite clinique

MD : mean difference

mL: millilitre

NAS: non-aureus staphylococci

NIMI: new intramammary infection

NIMIenv: new intramammary infection by environmental bacteria

NMA: network meta-analysis

NMC: National Mastitis Council

NPV: negative predictive value

NR: not reported

NRSI: nonrandomized studies of interventions

PCR: polymerase chain reaction

PPV: positive predictive value

PRISMA: Preferred Reporting Items for Systematic Review and Meta-Analysis

PRISMA-P: Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols

QSDCT: quarter-based selective dry cow therapy

RCT: randomized controlled trial

REFLECT: Reporting Guidelines for Randomized Controlled Trials for Livestock and Food Safety

REML: restricted maximum likelihood

RoB: risk of bias tool

ROBINS-I: Risk Of Bias In Non-randomized Studies – of Interventions

RR: relative risk (risque relatif)

SCC: somatic cell counts

SCS: somatic cell score

SDCT: selective dry cow therapy

Se: sensitivity

SE: standard error

Sp: specificity

STROBE-Vet: Strengthening the Reporting of Observational Studies in Epidemiology – In veterinary medicine

TS : traitement sélectif

TU : traitement universel

wk : week

Dédicace

« Une main toute seule ne peut pas applaudir! » Proverbe africain

À tous ceux qui, de près ou de loin, m'ont guidé, soutenu et accompagné.

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Chapitre 1 – Introduction générale

Le traitement universel (TU) au tarissement des vaches laitières a été préconisé il y a de nombreuses années (Neave et al., 1969) et est largement utilisée par les producteurs laitiers. Selon cette pratique, les quatre quartiers de la vache au tarissement reçoivent un traitement antimicrobien avec deux objectifs : (1) l'élimination des infections intramammaires (IIM) existantes au tarissement et (2) la prévention des nouvelles IIM (NIIM) durant la période de tarissement. Cependant, l'utilisation judicieuse des antimicrobiens dans l'industrie laitière exige une réévaluation du TU, qui comprend l'utilisation d'antimicrobiens à titre préventif dans les quartiers ou vaches non infecté(e)s au tarissement.

Le traitement sélectif (TS) des vaches ou quartiers infecté(e)s pourrait constituer une solution intéressante comme alternative potentielle pour réduire la quantité d'antimicrobiens utilisée en production laitière, sans compromettre la santé du pis ou la production de lait au cours de la lactation subséquente (Cameron et al., 2014, Rowe et al., 2020a, Vasquez et al., 2018). Ainsi, seuls les quartiers ou vaches diagnostiqués infectés par une IIM seront traitées avec des antimicrobiens au tarissement. Cependant, les quartiers ou vaches saines au tarissement et, par conséquent, qui ne reçoivent pas d'antimicrobiens pourraient être à haut risque de nouvelles IIM pendant la période de tarissement.

Le scellant interne à trayon pourrait constituer une solution pour les quartiers qui ne reçoivent pas d'antimicrobiens lors du programme de TS au tarissement. En effet, dans les quartiers où il n'existe pas d'infection au moment du tarissement, l'administration intracisternale d'un scellant interne à trayon a montré son efficacité dans la prévention des NIIM durant la période de tarissement (Dufour et al., 2019, Rabiee and Lean, 2013, Woolford et al., 1998).

La réussite du programme de TS au tarissement dépend de la disponibilité d'une méthode simple, rapide, économique et précise de détection des quartiers ou vaches infecté(e)s au moment du tarissement (Sanford et al., 2006b). Des méthodes rapides ont été proposées comme outils pour identifier les quartiers ou vaches infecté(e)s. Il s'agit, par exemple, du test de mammite de Californie (CMT) (Bhutto et al., 2012, Sanford et al., 2006b), de la culture bactériologique de

lait, simplement nommé culture de lait tout au long de la présente thèse, à l'aide de Petrifilm® (Cameron et al., 2013, McCarron et al., 2009) ou du Minnesota Easy Culture System (McCarron et al., 2009, Rowe et al., 2020a), ou des données de comptage de cellules somatiques du contrôle laitier (Scherpenzeel et al., 2016a).

En considérant les données de comptage de cellules somatiques (CCS) et les résultats de culture à la ferme sur Petrifilm® pour différencier les vaches infectées et saines, un protocole de TS + scellant interne à trayon a permis une réduction de 22% de l'utilisation des antimicrobiens au tarissement (Cameron et al., 2013) sans effet négatif sur la santé du pis ou la production de lait durant la lactation suivante (Cameron et al., 2015, Cameron et al., 2014). Cependant, étant donné que de nombreuses vaches pourraient n'avoir qu'un seul quartier infecté (83% de quartiers sains ont été rapportés par Cameron et al. (2013)), une sélection au niveau quartier pourrait améliorer davantage la réduction de l'utilisation d'antimicrobiens associée à une approche de TS.

Ainsi, les décisions de TS au tarissement pourraient être prises soit au niveau quartier, soit au niveau vache. Certaines études ont rapporté l'interdépendance des quartiers de la vache, vis-à-vis des IIM (Berry et al., 2003, Robert et al., 2006a), ce qui supporterait la prise de décision au niveau vache. Cependant, il est possible que cette interdépendance des quartiers d'une vache soit réduite durant la période de tarissement, en présence d'un scellant interne à trayon, étant donné qu'il crée une barrière physique à l'entrée des bactéries dans le trayon.

Objectifs de recherche

L'objectif général de cette thèse était d'apporter plus de lumière sur le TS au tarissement des vaches laitières. Les objectifs spécifiques étaient de:

(1) Synthétiser la littérature comparant le TS et le TU chez la vache laitière au tarissement en terme de méthodes de sélection des vaches à traiter, de réduction atteinte de l'utilisation d'antimicrobiens, de l'impact des protocoles de TS et TU sur des indices de santé de la glande mammaire (élimination des IIM existantes et prévention de nouvelles IIM durant la période de tarissement, incidence de MC et CCS durant la lactation suivante) et sur la production de lait dans la lactation suivante;

(2) Quantifier la réduction d'utilisation des antimicrobiens et déterminer si le TS par quartier au tarissement basé sur la culture de lait à l'aide de Petrifilm® affecte le risque de guérison des infections et l'incidence de nouvelles IIM durant le tarissement, et, durant les 120 premiers jours de la lactation suivante, le risque d'un premier cas de MC, le CCS et la production de lait ;

(3) Évaluer la précision des approches diagnostiques basées sur des données du CCS et/ou d'un système de culture bactériologique du lait à l'aide des Petrifilm®, pour différencier les quartiers sains et infectés au tarissement, en comparaison avec la bactériologie standard du lait (test de référence);

Enfin, dans le but d'évaluer certains aspects rapportés comme problématiques par certains producteurs laitiers tels que la présence de résidus de scellant dans le lait après le vêlage et la présence de bouchon de scellant dans le trayon tout le long de la période de tarissement, un objectif secondaire a été ajouté :

(4) Évaluer la persistance des résidus de scellant interne à trayon dans le lait après le vêlage et les facteurs de risque pouvant l'affecter.

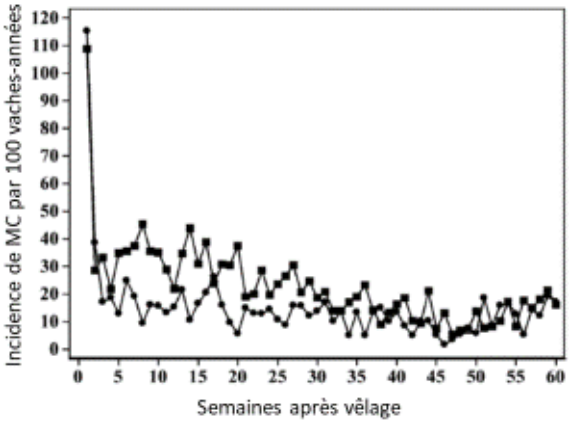
Cette thèse inclut sept chapitres, en plus des chapitres d'introduction générale et celui de la revue de la littérature. Le chapitre sur la revue de la littérature est présenté, en plus de deux chapitres sur la revue systématique et méta-analyse, pour définir certains concepts importants pour une bonne compréhension du contenu de cette thèse. **Le troisième chapitre** concerne le protocole d'une revue systématique et méta-analyse pour comparer différents éléments liés au traitement au tarissement, dont une comparaison des approches de TU et TS au tarissement des vaches laitières. **Le quatrième** porte sur les résultats de la revue systématique et des méta-analyses pour comparer les approches de TU et TS au tarissement des vaches laitières. **Le cinquième** porte sur un essai clinique contrôlé randomisé qui compare le TS et le TU, pour la proportion de réduction d'antimicrobiens, la santé du pis et la production de lait durant la lactation subséquente. **Le sixième** porte sur l'évaluation des tests diagnostiques, pour déterminer les vaches ou même les quartiers qui devraient être traités avec des antimicrobiens au tarissement. **Le septième** chapitre porte sur l'évaluation de la persistance des résidus de scellant interne à trayon dans le lait après le vêlage et ses facteurs de risque. Les deux derniers chapitres, **huitième** et **neuvième**, sont consacrés à la discussion générale et la conclusion générale, respectivement.

Chapitre 2 – Revue de la littérature

La mammite

Principalement causée par des IIM, la mammite est une inflammation de la glande mammaire. Ces IIM sont généralement dues à l'envahissement du canal du trayon par des microorganismes (bactéries principalement, levures, algues). La mammite s'accompagne de changements visibles dans le lait avec ou sans signes d'inflammation du pis comme la chaleur, la douleur, la rougeur et le gonflement (MC) ou ne présente aucun signe clinique (mammite subclinique), selon le degré d'inflammation. Un test diagnostic (culture de lait) est nécessaire pour détecter la présence d'une IIM. Le CCS effectué par le contrôle laitier ainsi que la mesure indirecte de la concentration des cellules somatiques par le CMT permettent de mettre en évidence une augmentation de l'inflammation de la glande mammaire.

L'incidence de mammite clinique varie d'une ferme à l'autre et en fonction des jours en lait et de l'âge de la vache (Figure 1 ; (Olde Riekerink et al., 2008)). L'incidence est très élevée durant les deux premières semaines suivant le vêlage, puis se stabilise durant le reste de la lactation.



*vaches primipares (●) ; *vaches pluripares (■)

Figure 1. – Distribution de l'incidence de mammites cliniques (MC) par semaine après le vêlage pour les vaches primipares et les vaches pluripares ; Adapté de Olde Riekerink et al. (2008).

La gravité de la mammite clinique est décrite selon le degré de sévérité (légère ou grade 1, modérée ou grade 2 et sévère ou grade 3). Pour la MC de grade 1, il y a seulement changement d'apparence du lait. Pour le grade 2, le quartier change aussi d'apparence. En cas de MC sévère ou de grade 3, des signes systémiques tels que la fièvre, anorexie, tachycardie, tachypnée et déshydratation sont présents. La sévérité de la MC dépend de l'agent pathogène en cause (Schabauer et al., 2018), bien que d'autres facteurs liés à l'environnement et la vache peuvent intervenir. Comme la figure 2 le montre, dans certains cas d'inflammation de la glande mammaire, il peut y avoir un retard dans l'apparition des signes cliniques, avec une période de mammite subclinique avant l'apparition des signes cliniques (National Mastitis Council, 2016).

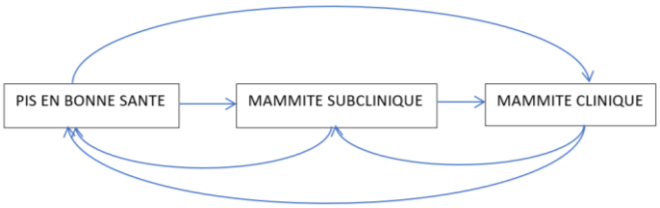


Figure 2. – Relation entre les mammites subcliniques et cliniques, Adapté de National Mastitis Council (2016).

La mammite est une maladie très courante et coûteuse dans l'industrie laitière (Aghamohammadi et al., 2018, Halasa et al., 2009a). Elle affecte non seulement l'état de santé des animaux, mais aussi la rentabilité de la ferme et le bien-être des animaux. Les conséquences économiques de la mammite concernent, par exemple, la baisse de la production laitière, les médicaments et les frais de diagnostic, les rejets de lait, les services vétérinaires, la main-d'œuvre, la qualité du lait et la réforme précoce ou même la mortalité des vaches laitières (National Mastitis Council, 2016).

Agents pathogènes de la mammite bovine

Les microorganismes qui causent le plus fréquemment la mammite bovine peuvent être divisés en deux catégories (National Mastitis Council, 2016): les agents pathogènes contagieux qui peuvent se propager d'une vache à l'autre principalement durant la traite par l'équipement de traite et les mains des trayeurs; et les agents pathogènes environnementaux qui n'infectent normalement pas la glande mammaire mais qui peuvent le faire lorsque l'environnement de la vache, les trayons et le pis, les équipements de traite ou les mains trayeurs sont contaminés par

ces organismes environnementaux et qu'ils accèdent au canal du trayon (National Mastitis Council, 2016).

Les agents pathogènes contagieux courants comprennent, par exemples, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma bovis*, *Mycoplasma* spp et *Corynebacterium bovis* (National Mastitis Council, 2016). Les exemples des agents pathogènes environnementaux sont *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Escherichia coli*, *Klebsiella* spp, *Enterobacter* spp, *Citrobacter* spp, *Enterococcus faecalis*, *Enterococcus faecium* et d'autres bactéries à gram-négatif, les staphylocoques non-aureus (NAS) comme *Staphylococcus chromogenes*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus simulans* et *Staphylococcus xylosus* (National Mastitis Council, 2016). D'autres agents pathogènes peuvent provoquer des mammites, mais ces infections sont peu fréquentes. Cependant, elles peuvent provoquer des cas individuels graves dans les troupeaux laitiers. Les exemples de ces agents pathogènes sont *Serratia* spp, *Pseudomonas* spp, les levures ou les champignons, *Prototheca*, *Trueperella pyogenes* et *Nocardia* spp (National Mastitis Council, 2016).

Bien que la distinction entre les agents pathogènes contagieux et environnementaux soit fréquemment utilisée, des études ont démontré que cette distinction n'est pas toujours claire. Certaines espèces bactériennes responsables de la mammite présentent à la fois des propriétés "contagieuses" (c'est-à-dire la capacité de se propager d'une vache infectée à une autre) et "environnementales" (c'est-à-dire la capacité de survivre dans l'environnement) (Bradley et al., 2012). Par exemple, *Streptococcus uberis* présente à la fois des propriétés "contagieuses" et "environnementales" (Zadoks et al., 2003). Cependant, cette distinction revêt une importance pratique puisque des stratégies différentes sont recommandées et utilisées pour lutter contre les agents pathogènes contagieux et environnementaux (National Mastitis Council, 2016). Également, les NAS sont un groupe de bactéries causant des mammites comprenant des espèces environnementales et contagieuses (Piessens et al., 2012).

Parfois, les agents pathogènes de la mammite bovine sont catégorisés en agents pathogènes majeurs comme *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae, Mycoplasma* spp. et les coliformes et en agents pathogènes mineurs comme les *Corynebacterium*

spp. et les NAS. Les agents pathogènes majeurs provoquent souvent des épisodes de MC, entraînant des dommages à la glande mammaire, ainsi qu'une inflammation importante, détectable par une augmentation du CCS. À l'inverse, les agents pathogènes mineurs de la mammite provoquent généralement des inflammations moins sévères.

Importance de la période de tarissement

En production laitière, la période de tarissement est une période entre deux lactations successives et durant laquelle la vache ne produit pas de lait. Il s'agit d'une période de repos qui dure généralement de 6 à 8 semaines. Ce repos permet de bien se préparer à la prochaine lactation. La durée de la période de tarissement a une influence sur la fertilité et l'incidence des maladies métaboliques ou autres maladies post-partum comme la mammite, la métrite ou le déplacement de la caillette durant la lactation suivante.

Les IIM peuvent être acquises aussi bien pendant la période de tarissement que pendant la période de lactation chez les vaches laitières (Barkema et al., 1998). Cependant, le risque d'apparition de nouvelles infections (NIIM) augmente durant la période de tarissement (Figure 3), principalement au début et vers la fin de la période de tarissement (Bradley and Green, 2000, Bradley and Green, 2004, Smith et al., 1985b). L'augmentation de l'incidence des IIM au début de la période de tarissement est en partie due à l'arrêt de la traite, aux changements physiologiques liés à l'involution des glandes mammaires et au délai avant la formation d'un bouchon de kératine dans le trayon.

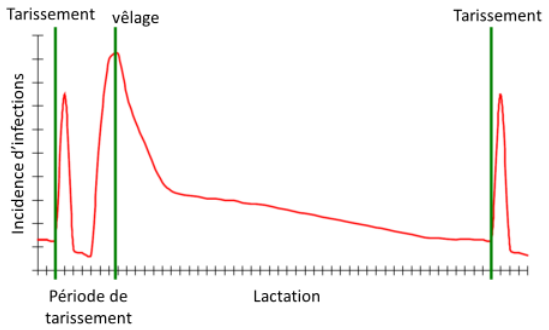


Figure 3. – Illustration schématique de l'incidence d'infections intramammaires au cours du cycle de lactation; Adapté de Bradley and Green (2004).

En absence d'un traitement approprié, les IIM qui persistent de la lactation précédente, ou de NIIM qui se développent durant la période de tarissement, peuvent contribuer à la prévalence des IIM au vêlage subséquent et à l'incidence de MC au cours de la lactation suivante (Berry and Hillerton, 2002b, Green et al., 2002). Par conséquent, la prévention des NIIM pendant la période de tarissement est importante pour la santé du pis pendant la période de tarissement et les premiers mois de la lactation suivante. Ainsi, il importe de bien traiter les IIM existantes au moment du tarissement et prévenir les NIIM durant la période de tarissement. Aussi, il faut noter qu'en ce qui concerne le contrôle des IIM, la période de tarissement est considérée comme une période idéale car des taux de guérison élevés peuvent être atteints pour les IIM existantes (Dingwell et al., 2003, Halasa et al., 2009a, Smith et al., 1985a). Également, il n'y a pas de problème avec les temps de retrait pour le lait parce que la vache n'est pas en lactation.

Scellant interne à trayon

Le scellant interne à trayon constitue une alternative au traitement antimicrobien pour la prévention des NIIM pendant la période de tarissement. Également, le scellant interne à trayon en combinaison avec un antimicrobien réduit significativement le risque de NIIM durant la période de tarissement, en comparaison avec un antimicrobien seul (Bradley et al., 2010, Godden et al., 2003, Rabiee and Lean, 2013).

L'ingrédient principal retrouvé dans la majorité des produits homologués comme scellant interne à trayon est le subnitrate de bismuth (65% p/p). Le scellant interne à trayon est un produit à infusion intracisternale, stérile, non-antibiotique sous la forme d'une pâte visqueuse. Il forme une barrière physique à l'entrée des bactéries dans le trayon et persiste dans le trayon jusqu'au vêlage (Woolford et al., 1998). En fait, il mime la fonction du bouchon de kératine naturelle de la vache en fermant instantanément les trayons de façon étanche et prévient ainsi l'envahissement du canal du trayon par les bactéries. Il reste dans le canal du trayon pendant toute la période de tarissement jusqu'à ce qu'il soit retiré manuellement lors de la première traite ou par la tétée du veau. Toutefois, un scellant interne à trayon seul ne peut être utilisé que pour les quartiers non infectés (Godden et al., 2003).

Peu de données sont actuellement disponibles sur la persistance des résidus de scellant dans le lait après le vêlage et les facteurs qui peuvent affecter l'excrétion de scellant après le vêlage. Certains auteurs ont signalé la présence de résidus de scellant dans le lait jusqu'à 3 semaines après le vêlage (Berry and Hillerton, 2002a). Bhutto et al. (2011) ont rapporté que la plupart du produit était éliminé lors de la première traite, mais que certains résidus pouvaient être observés au cours des traites suivantes. Cependant, ces auteurs n'ont pas étudié la durée moyenne d'excrétion des résidus.

Des préoccupations ont été soulevées concernant la persistance des résidus de scellant après le vêlage et l'accumulation possible de ses particules dans l'équipement de traite. En fait, il est rapporté de manière anecdotique que les résidus de scellant après le vêlage peuvent s'accumuler dans les systèmes de traite si des procédures de nettoyage optimales ne sont pas utilisées. Encore plus, des défauts de taches noires ont été observés dans certains fromages et ont été potentiellement associés à la présence de résidus de scellant dans le lait (Lay et al., 2007).

Traitement antimicrobien au tarissement

Dans la pratique courante, tous les quartiers de toutes les vaches au tarissement reçoivent des antimicrobiens (Aghamohammadi et al., 2018). Cette pratique est qualifiée de TU et constitue la première raison d'utilisation d'antimicrobiens sur les fermes laitières au Canada (Lardé et al., 2020, Lardé et al., 2021, Saini et al., 2012). Cependant, l'opinion public commence à se faire entendre par rapport à l'impact de l'utilisation d'antimicrobiens en production animale ainsi que sur le problème grandissant de résistance des bactéries aux antimicrobiens (Call et al., 2008, Oliver et al., 2011). En effet, l'utilisation d'antimicrobiens est considérée comme un facteur important favorisant le développement de la résistance par les bactéries (Call et al., 2008). Ainsi, toutes les possibilités alternatives, dans le but de limiter leur usage, seraient les bienvenues.

Les progrès réalisés en épidémiologie de la mammite expliquent pourquoi le TU de toutes les vaches au tarissement n'est plus primordial (Rajala-Schultz et al., 2011, Robert et al., 2006b). En effet, la santé de la glande mammaire s'est grandement améliorée sur les fermes laitières depuis les années 70. Ce qui fait que, maintenant, beaucoup de vaches arrivent au tarissement en santé, alors que ce n'était pas le cas auparavant. À la suite des préoccupations croissantes sur

l'antibiorésistance, et ayant constaté que de nombreuses vaches sont non infectées au tarissement, en plus de la disponibilité du scellant interne à trayon, la recommandation d'un TU au tarissement devrait être revue (Berry and Hillerton, 2002a). Ce qui renforce l'intérêt à adopter l'approche de TS épargnant l'utilisation d'antimicrobiens en prévention. En effet, il est très important que les antimicrobiens soient utilisés de façon judicieuse. Le TS pourrait être utilisé pour allouer les traitements antimicrobiens aux quartiers ou vaches infecté(e)s, et ainsi mieux cibler et réduire l'utilisation des antimicrobiens au moment du tarissement.

Cependant, les préoccupations concernant les effets négatifs potentiels sur la santé du pis ou la production laitière durant la lactation suivante pourrait freiner les producteurs à adopter le TS au tarissement. L'échec du TS peut être dû à l'utilisation d'un test diagnostique ayant une faible sensibilité et spécificité pour détecter les quartiers ou vaches infecté(e)s (Poutrel and Rainard, 1981). Également, cet échec peut s'expliquer par le fait de ne pas utiliser un scellant interne à trayons pour prévenir les nouvelles IIM pendant la période de tarissement pour les quartiers non infectés et qui n'ont pas reçu, par conséquent, d'antimicrobiens (Kabera et al., 2021a, Winder et al., 2019).

Critères de sélection des quartiers ou vaches éligibles au traitement antimicrobien au tarissement

Il importe donc de savoir comment sélectionner les quartiers ou vaches éligibles au traitement antimicrobien au tarissement et quel serait l'impact de cette sélection sur la santé du pis durant la période de tarissement ainsi que la lactation suivante. En effet, le succès dépend de la capacité de déterminer avec précision le statut d'un quartier ou d'une vache au tarissement afin que le traitement approprié soit appliqué (Huxley et al., 2002, Robert et al., 2008, Torres et al., 2008). Le TS et les critères de sélection ont potentiellement un effet sur la prévalence d'infection au vêlage, l'incidence de la MC et la qualité du lait durant les premiers mois de la lactation suivante, la réduction d'utilisation d'antimicrobiens, le bien-être animal et la faisabilité pratique et économique. Depuis quelques années, plusieurs méthodes ont été décrites pour sélectionner les quartiers ou vaches infecté(e)s.

La considération des données de CCS, associées ou non avec l'historique de MC, est la méthode la plus utilisée (McDougall, 2010, Rajala-Schultz et al., 2011, Torres et al., 2008). Bien que cette méthode ne soit pas parfaite, elle est très pratique et peu coûteuse pour évaluer l'état de santé du pis (Schukken et al., 2003). Le fait que les données de CCS soient facilement disponibles dans de nombreux troupeaux rend cette méthode plus facilement réalisable que les autres. Une sensibilité de 70% et une spécificité de 63% ont été rapportées pour identifier les vaches infectées au moment du tarissement, en considérant la moyenne des trois derniers tests DHI à un seuil de 200,000 cellules/mL et l'historique de MC durant la lactation (Torres et al., 2008).

Chapitre 3 – Antimicrobial-based dry cow therapy approaches for cure and prevention of intramammary infections: a protocol for a systematic review and meta-analysis

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Abstract

In dairy herds, application of antimicrobials at drying-off is a common mastitis control measure. This article describes a protocol for systematic review and meta-analysis to address three crucial points regarding antimicrobial usage at drying-off: (1) comparative efficacy of antimicrobials used for preventing new and eliminating existing intramammary infections (IMI); (2) comparison of selective and blanket dry cow therapy approaches in preventing new and eliminating existing IMI; and (3) assessment of the extra prevention against new IMI that can be gained from using antimicrobial-teat sealant combinations versus antimicrobials alone. Five PICO (Population, Intervention, Comparator, Outcome) questions were formulated to cover the three objectives of the review. Medline, CAB Abstracts, Web of Science, and conference proceedings will be searched along with iterative screening of references. Articles will be eligible if: (1) published after 1966; (2) written in English or French; and (3) reporting field clinical trials and observational studies, conducted on dairy cows at drying-off, with at least one antimicrobial-treated group and one IMI-related outcome. Authors will independently assess the relevance of titles and abstracts, extract data, and assess bias and the overall quality of evidence. Results will be synthesized and analyzed using pairwise and network meta-analysis. The proposed study will significantly update previously conducted reviews.

Introduction

Intramammary infections (IMI) are a perpetual threat to the productivity and, consequently, the profitability of the dairy industry worldwide (Halasa et al., 2007). Dry cow therapy (DCT; i.e. treatment of all or some cows with antimicrobials at drying-off) is a cornerstone of mastitis control. DCT is recommended for both treatment of existing IMI and for prevention of new IMI acquisition during the dry period, and various drugs have been specifically designed for such use. Despite the controversy surrounding prophylactic use of antimicrobials in production animals, the National Mastitis Council's Recommended Mastitis Control Program still suggests treatment of all

cows (i.e. blanket DCT) at drying-off (National Mastitis Council, 2006). Recently, however, identification of infected cows at drying-off (using diagnostic tests) and treatment of infected cows only, also known as selective DCT, has been the object of research (Berry and Hillerton, 2002b, Cameron et al., 2013). It has also been shown that a teat sealant (ITS) can be used in conjunction with blanket or selective DCT to prevent IMI acquisition during the dry period (Cameron et al., 2015, Cameron et al., 2014, Sanford et al., 2006a). Therefore, on modern dairy farms, managers have to make decisions regarding: (1) the type of antimicrobials to be used at drying-off; (2) whether all (blanket DCT) or some (selective DCT) cows will be treated at drying-off; and (3) whether an ITS will be used in conjunction with the antimicrobial treatment. The objective of this protocol is to describe the methodology for a systematic review and meta-analysis of the various antimicrobial-based DCT strategies that can be used at drying-off to cure or prevent IMI. This review will complement an ongoing review on non-antimicrobial drying-off strategies (Francoz et al., 2016).

Objectives

The general objective of this review is to identify and compare the different antimicrobial-based strategies that can be used at drying-off to treat and prevent IMI in dairy cows. The specific objectives are described in the following five PICO (Population, Intervention, Comparator, Outcome) questions.

Choice of antimicrobial at drying-off

(1) In dairy cows (i.e. the population), which antimicrobial treatment (i.e. the comparators) when administered at dry-off (i.e. the intervention) is the most efficient for preventing new IMI (i.e. the outcome)?

(2) In infected dairy cows (i.e. the population), which antimicrobial treatment (i.e. the comparators) when administered at dry-off (i.e. the intervention) is the most efficient for eliminating existing IMI (i.e. the outcome)?

Blanket versus selective dry-cow treatment

(3) In dairy cows (i.e. the population), is selective DCT (i.e. the intervention) as efficient as blanket DCT (i.e. the comparator) in preventing new IMI (i.e. the outcome)?

(4) In infected dairy cows (i.e. the population), is selective DCT (i.e. the intervention) as efficient as blanket DCT (i.e. the comparator) in eliminating existing IMI (i.e. the outcome)?

Complementing an antimicrobial treatment with an ITS

(5) In dairy cows (i.e. the population), how does the efficacy of an antimicrobial–ITS combination administered at dry-off (i.e. the intervention) compared with an antimicrobial alone (i.e. the comparator) for preventing new IMI (i.e. the outcome)?

Materials and methods

This protocol is written in accordance with the PRISMA-P (Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols) statement (Moher et al., 2015). The systematic review and network meta-analysis (NMA) will be reported following the PRISMA-NMA extension statement to structure the contents of the final report (Hutton et al., 2015).

Eligibility criteria

Study design

Controlled trials, randomized or not (i.e. cows/quarters allocated to interventions by non-randomization methods), will be included in our systematic review. In addition, studies in which cows were naturally or experimentally infected with any type of mastitis-causing pathogen will be retained. Both split udder and split herd designs will be included. Based on the experience of Francoz et al. (2017), the number of observational studies (case–control and cohort) answering our PICO questions is expected to be nil or very low. These study designs, however, will not be excluded a priori. Sometimes, the distinction between non-randomization trials and cohort studies is not quite clear, so they will be included, along with case–control studies, as 'nonrandomized studies of interventions' (NRSI) (Di Girolamo et al., 2017, O'Connor and Sargeant,

2014). Other study designs, including cross-sectional studies and descriptive studies such as case-series, case-reports, or expert opinions will be excluded.

Review articles and meta-analyses will not be included per se; however, every single study involved in those reviews will be evaluated for inclusion.

Population

The population of interest will be lactating dairy cows at drying off; tropical and exotic breeds will be excluded. For evaluating IMI elimination, infected quarters (or cows) at drying-off will be our target population. Because infected quarters at drying-off can still acquire new IMI by a different pathogen over the dry period, both non-infected and infected quarters (or cows) at drying-off will be included when assessing the prevention of new IMI.

Interventions and comparators

For the first two PICO questions, the interventions are all antimicrobials that can be administered by all routes with any dose; the corresponding comparators are placebo or no treatment, or active controls if other antimicrobials (with the same treatment regimen regarding blanket vs. selective and use of ITSs) were used. For the third and fourth PICO questions, the interventions are selective DCT regimens involving any antimicrobials as described above; the comparators are blanket DCT regimens for the same antimicrobials. For the fifth PICO question, the interventions are antimicrobial–ITS combinations involving any antimicrobials as described above; the comparators are the same antimicrobials used without the ITS. Studies investigating the efficacy of ITS alone will be excluded, since this topic is already under investigation in an ongoing review (Francoz et al., 2016).

Outcomes

The primary outcomes under investigation will be IMI incidence risk for the first, third, and fifth PICO questions and IMI cure risk for the remaining PICO questions. Since some studies may only report on IMI prevalence post-calving, this outcome will also be considered as a primary outcome (a proxy for IMI incidence and cure risk). For determination of the quarter (or cow) IMI pre-dry and post-calving statuses, only studies using the following diagnostic tests will be retained: milk

somatic cell count (SCC), milk bacteriological culture (external laboratory or on farm), and polymerase chain reaction (PCR). In studies using milk bacteriological culture or PCR as a diagnostic test, milk samples will have to have been collected aseptically. Moreover, the post-calving IMI status will have to have been measured within 14 days of calving, to ensure that the infection or cure most likely occurred during the dry period. A quarter will be deemed to have experienced a new IMI when a specific pathogen species is isolated in the calving or post-calving samples from a quarter that was free of the pathogen species in the drying-off sample. Furthermore, a cure of IMI will have occurred if a specific pathogen species was present at drying-off and not found in the post-calving sample.

Report characteristics

To be included, articles will have to be published after 1966, because the oldest article retained in a previous review on this topic was published in 1967 (Halasa et al., 2009a, Halasa et al., 2009b). In addition, articles will have to be written in English or French. Finally, if two or more articles present results from the same trial (e.g. preliminary vs. final results), only the most complete article will be included.

Information sources

Three electronic sources of information will be used: Medline, CAB Abstracts, and Web of Science. These sources have shown to cover most of the veterinary literature (Grindlay et al., 2012). Conference proceedings from the National Mastitis Council and the American Association of Bovine Practitioners will also be searched. In addition, the list of references from each included paper will be searched to identify additional publications not initially obtained by the database search.

Search strategy

A search strategy was developed with search terms adapted from the Halasa et al. (2009b), Halasa et al. (2009a), Francoz et al. (2016) and Francoz et al. (2017) papers. The search terms were divided into four components describing: (1) the population of interest (i.e. dairy cows); (2) the outcome studied (i.e. mastitis); (3) the specific period of interest (i.e. the dry period); and (4) the

interventions and comparators (i.e. antimicrobials and/or ITS). The Boolean operator 'AND' was used to combine the four components, while the 'OR' operator was used to join the terms within each component. Search terms and keywords have been adapted to the specifications required for each database. Development of the search terms and elaboration of the search strategy were done in collaboration with a librarian (Rafael Rangel Braga), Faculté de Médecine Vétérinaire, Université de Montréal, as per Shamseer et al. (2015). The algorithm for searching each database is presented in Appendix 1.

Study records

Data management

All search result citations will be imported and managed in EndNote bibliographic software (version X8.2 for Windows, Thomson Reuters, New York, NY, USA), then duplicate records will be detected automatically, based on title, author(s) and publication year, and further screened out manually. After full retrieval of articles, a custom-built Access database (version 2016, Microsoft Corp., Redmond, WA, USA) will be used for data extraction.

Selection process

In order to identify potentially relevant studies, each title and abstract will be evaluated by two independent reviewers. Each abstract will be reviewed by one of the first two authors (MA and FK), and one of the other co-authors will be selected to act as the second reviewer. A screening checklist designed according to the predefined inclusion and exclusion criteria will be used to assess the relevance of the abstracts. Only abstracts with a positive or unclear response to all questions will be eligible to proceed to the next stage, and only when the reviewers agree. Any disagreement will be resolved by consensus among the research team. Reviewers will be blinded to author names, journal, and year of publication when reviewing the abstracts. The screening tool is included in the Appendix 2.

A second evaluation will be conducted to retain only citations where a full text is available in French or English, and where all answers to the checklist of Appendix are 'yes'. This evaluation

will be done by two independent reviewers, in the same fashion as described above. A PRISMA flow diagram will be used to document the flow of records (Moher et al., 2009).

Data collection process

A data extraction form will be developed for the current project based on the forms used in the previous systematic review projects (Dufour et al., 2011, Francoz et al., 2017, Francoz et al., 2016). Data extraction will be performed by three independent reviewers (MA and FK as well as one of the other co-authors). Any discrepancies in the extracted data will be resolved by consensus among the research team.

Authors of studies, for which some of the needed information is unclear or missing, will be contacted for clarification via email, and a follow-up email will be sent 2 weeks later if no feedback is received. Then, authors will be provided 2 more weeks to respond. If there is no response from authors and the missing information is crucial, the study will not proceed to the meta-analysis.

Data items

The following information will be extracted: (1) study characteristics: year of publication, type of publication (journal article vs. conference proceeding), country; (2) study methods: study design (RCT, NRSI or case–control), type of exposure (natural IMI vs. experimental challenge), the study's main objective (e.g. noninferiority trial, analysis of risk factors); (3) population-related information: number of herds, number of cows, number of quarters, inclusion criteria (age, breed, minimal or maximal planned dry period length, and other inclusion and exclusion criteria), and study unit (quarter, cow, or herd); (4) intervention and comparator-related information: antibiotic (trade name, active ingredient, dose, route and frequency of administration, and treatment duration if multiple administrations were needed), ITS (trade name, active ingredient, dose, route (systemic vs. intramammary infusion) and frequency of application, and treatment duration, if applied more than once), description of negative control (in particular, whether a placebo or no treatment was used), and for selective DCT the approach by which infected cows/quarters were selected for treatment at drying-off; (5) outcome-related information: unit of assessment (cow vs. quarter), diagnostic tests for the detection of IMI (SCC, bacteriological culture, or PCR), thresholds used for the definition of IMI incidence and cure risk, follow-up time,

results for targeted outcomes; and (6) quality-related information: whether intention-to-treat analysis was used, and whether an a priori sample size calculation was reported.

Outcome and prioritization

Primary outcomes are: IMI cure risk and IMI incidence risk over the dry period, and post-calving IMI prevalence. Secondary outcomes that will be extracted are: early lactation (i.e. 0–4 months), clinical mastitis incidence, subsequent lactation milk production, and SCC, and for studies investigating selective DCT, proportion of untreated cows.

Risk of bias in individual studies

Clarity, completeness, and accuracy of reporting are going to be assessed using a full or reduced (modified) checklist of items based on the REFLECT statement (O'Connor et al., 2010) for controlled trials and STROBE-VET statement (Sargeant et al., 2016) for observational studies.

Sources of bias will be assessed as part of the data extraction using the revised Cochrane risk of bias tool (RoB 2.0) for randomized trials (Higgins et al., 2016). Five domains will be used to assess the bias arising from the randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result. The risk of bias will be reported as 'low risk', 'high risk', or 'unclear'. An overall risk of bias judgment for the outcome is based on the collective domain-level judgments. Additional considerations will be made for different trial designs (simple parallel-group trials, cluster-randomized trials, and cross-over trials). For NRSI, the Cochrane ROBINS-I (Risk Of Bias In Non-randomized Studies – of Interventions; (Sterne et al., 2016)) tool will be used.

Data synthesis and meta-bias

Descriptive results of all selected studies will be computed. Incidence (risk) ratios (RR) will be computed for each comparison in each study. For both IMI incidence and cure, the ratio will be computed by dividing incidence (cure) of IMI in treated quarters/cows by incidence (cure) of IMI in control quarters/cows. The number needed to treat for either preventing or curing one case of IMI will be computed whenever data are available (Schunemann et al., 2017). Secondary outcome analyses will be determined by the number of articles reporting them.

Pairwise meta-analysis will be conducted to synthesize the results of studies addressing the last three PICO questions. For the first two questions, pairwise comparisons will be used for the studies with similar comparisons, either active to non-active control or active to active treatment arms. The RR from each study will be pooled using a random-effects model because of the anticipated variability between trials.

Meta-regression will be used to identify the underlying sources of heterogeneity. Potential explanatory variables include: publication year and type, study design, exposure type, diagnostic test, type of antimicrobial, type of ITS, dose, route, bias-domain variables, and baseline risk. If the underlying risk contributes both substantially and significantly to the between-study heterogeneity, a random slopes model will be implemented in either a Bayesian or a frequentist framework, as described by Dohoo et al. (2007). If the number of studies for a given comparison is sufficient, a multivariable model may be developed based on epidemiological and statistical considerations.

Sensitivity analyses will be performed by eliminating each study, one at the time, to investigate the impact of each individual study on the overall summary effect. Publication bias will be assessed graphically using funnel plots and if asymmetry is noted, a contour-enhanced funnel plot will be sketched to investigate the cause of asymmetry (Peters et al., 2008).

For the first two review questions, and as the data allow, a NMA will be used to combine and compare treatment effects of all antimicrobials, by integrating direct and indirect evidence (Caldwell et al., 2005, Dias et al., 2018a, Dias et al., 2018b, Jansen et al., 2008, Lu and Ades, 2004, White et al., 2012). Interventions that cannot be included in the NMA will be summarized and narratively described in the final review.

Confidence in cumulative evidence

The quality of evidence for all outcomes will be rated, by two review authors (MA and FK), independently, as 'high', 'moderate', 'low', or 'very low' following the Grading of Recommendations Assessment, Development and Evaluation (GRADE) working group methodology (Schünemann et al., 2013). Any discrepancies will be resolved by consensus within the research team. Judgments will be justified, documented, and incorporated into the reporting

of results for each outcome. For NMA, the quality of each direct and indirect effect estimate will be rated according to Brignardello-Petersen et al. (2018). A summary of findings table will be prepared using GRADE pro software (GRADEpro, 2015).

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Author contributions

The first two authors (MA and FK) equally contributed to the elaboration of the protocol and writing of the paper. All other authors were consulted for the elaboration of the protocol and will be involved for conducting the review. SD is the guarantor of the proposed review. All authors reviewed and provided feedback on the manuscript.

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Role of funder

As per the research agreement, aside from providing financial support, the funders have no role in the design and conduct of the studies, data collection and analysis, or interpretation of the data. Researchers maintain independence in conducting their studies, own their data, and report

the outcomes regardless of the results. The decision to publish the findings rests solely with the researchers.

References

Berry EA and Hillerton JE (2002) The effect of selective dry cow treatment on new intramammary infections. *J. Dairy Sci.* 85, 112–121.

Brignardello-Petersen R, Bonner A, Alexander PE, Siemieniuk RA, Furukawa TA, Rochwerg B, Hazlewood GS, Alhazzani W, Mustafa RA, Murad MH, Puhan MA, Schünemann HJ and Guyatt GH, GRADE Working Group (2018). Advances in the GRADE approach to rate the certainty in estimates from a network meta-analysis. *J Clin Epidemiol* 93, 36–44.

Caldwell DM, Ades AE and Higgins JPT (2005) Simultaneous comparison of multiple treatments: combining direct and indirect evidence. *BMJ* 331, 897–900.

Cameron M, Keefe GP, Roy JP, Dohoo IR, MacDonald KA and McKenna SL (2013) Evaluation of a 3M petrifilm on-farm culture system for the detection of intramammary infection at the end of lactation. *Prev. Vet. Med.* 111, 1–9.

Cameron M, McKenna SL, MacDonald KA, Dohoo IR, Roy JP and Keefe GP (2014) Evaluation of selective dry cow treatment following on-farm culture: risk of postcalving intramammary infection and clinical mastitis in the subsequent lactation. *J. Dairy Sci.* 97, 270–284.

Cameron M, Keefe GP, Roy J-P, Stryhn H, Dohoo IR and McKenna SL (2015) Evaluation of selective dry cow treatment following on-farm culture: milk yield and somatic cell count in the subsequent lactation. *J. Dairy Sci.* 98, 2427–2436.

Di Girolamo N, Giuffrida MA, Winter AL and Reynders RM (2017). In veterinary trials reporting and communication regarding randomization procedures is suboptimal. *Vet. Rec.* 181, 195–200.

Dias S, Ades AE, Welton NJ, Jansen JP and Sutton AJ (eds) (2018a). Generalised linear models. In Network Meta-Analysis for Decision Making. New Jersey: John Wiley & Sons, Ltd, pp. 93–153.

Dias S, Ades AE, Welton NJ, Jansen JP and Sutton AJ (eds) (2018b) Meta-regression for relative treatment effects. In Network Meta-Analysis for Decision Making. New Jersey: John Wiley & Sons, Ltd, pp. 227–271.

Dohoo I, Stryhn H and Sanchez J (2007) Evaluation of underlying risk as a source of heterogeneity in meta-analyses: a simulation study of Bayesian and frequentist implementations of three models. *Prev. Vet. Med.* 81, 38–55.

Dufour S, Fréchette A, Barkema HW, Mussell A and Scholl DT (2011) Invited review: effect of udder health management practices on herd somatic cell count. J. Dairy Sci. 94, 563–579.

Francoz D, Wellemans V, Roy J-P, Lacasse P, Ordonez-Iturriaga A, Labelle F and Dufour S (2016) Non-antibiotic approaches at drying-off for treating and preventing intramammary infections: a protocol for a systematic review and meta-analysis. *Anim. Health Res. Rev.* 17, 169–175.

Francoz D, Wellemans V, Dupré JP, Roy JP, Labelle F, Lacasse P and Dufour S (2017) Invited review: a systematic review and qualitative analysis of treatments other than conventional antimicrobials for clinical mastitis in dairy cows. *J. Dairy Sci.* 100, 7751–7770.

GRADEpro GDT: GRADEpro Guideline Development Tool [Software] (2015). Evidence Prime, Inc., McMaster University.

Grindlay DJ, Brennan ML and Dean RS (2012) Searching the veterinary literature: a comparison of the coverage of veterinary journals by nine bibliographic databases. *J Vet Med Educ.* 39, 404–412.

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

Halasa T, Nielen M, Whist AC and Østerås O (2009a) Meta-analysis of dry cow management for dairy cattle. Part 2. Cure of existing intramammary infections. *J. Dairy Sci.* 92, 3150–3157.

Halasa T, Østerås O, Hogeveen H, Werven T and van Nielen M (2009b) Meta-analysis of dry cow management for dairy cattle. Part 1. Protection against new intramammary infections. *J. Dairy Sci.* 92, 3134–3149.

Higgins JPT, Sterne JAC, Savović J, Hróbjartsson A, Boutron I, Reeves B and Eldridge S (2016). A revised tool for assessing risk of bias in randomized trials. In Chandler J, McKenzie J, Welch V (eds.) Cochrane Methods, Cochrane Database of Syst Rev.. Oxford: John Wiley & Sons, Ltd., pp. 29–31.

Hutton B, Salanti G, Caldwell DM, Chaimani A, Schmid CH, Cameron C, Ioannidis JPA, Straus S, Thorlund K, Jansen JP, Mulrow C, Catalá-López F, Gøtzsche PC, Dickersin K, Boutron I, Altman DG and Moher D (2015) The PRISMA extension statement for reporting of Syst Rev. incorporating network meta-analyses of health care interventions: checklist and explanations. *Ann. Intern. Med.* 162, 777–784.

Jansen JP, Crawford B, Bergman G and Stam W (2008) Bayesian meta-analysis of multiple treatment comparisons: an introduction to mixed treatment comparisons. *Value Health* 11, 956–964.

Lu G and Ades AE (2004) Combination of direct and indirect evidence in mixed treatment comparisons. *Stat Med.* 23, 3105–3124.

Moher D, Liberati A, Tetzlaff J and Altman DG, PRISMA Group, (2009). Preferred reporting items for Syst Rev. and meta-analyses: the PRISMA statement. *BMJ* 339, b2535.

Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P and Stewart LA, PRISMA-P Group (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev.* 4, 1.

NMC (National Mastitis Council) (2006). Recommended Mastitis Control Program. Available from http://www.nmconline.org/wp-content/uploads/2016/08/RECOMMENDED-MASTITIS-CONTROL-PROGRAM-International.pdf (accessed August 2017).

O'Connor AM and Sargeant JM (2014) Meta-analyses including data from observational studies. *Prev. Vet. Med.* 113, 313–322.

O'Connor AM, Sargeant JM, Gardner IA, Dickson JS, Torrence ME, Consensus Meeting Participants, Dewey CE, Dohoo IR, Evans RB, Gray JT, Greiner M, Keefe G, Lefebvre SL, Morley PS, Ramirez A, Sischo W, Smith DR, Snedeker K, Sofos J, Ward MP and Wills R (2010) The REFLECT statement: methods and processes of creating reporting guidelines for randomized controlled

trials for livestock and food safety by modifying the CONSORT statement. *Zoonoses Public Health* 57, 95–104.

Peters JL, Sutton AJ, Jones DR, Abrams KR and Rushton L (2008) Contour-enhanced meta-analysis funnel plots help distinguish publication bias from other causes of asymmetry. *J Clin Epidemiol* 61, 991–996.

Sanford CJ, Keefe GP, Dohoo IR, Leslie KE, Dingwell RT, DesCôteaux L and Barkema HW (2006) Efficacy of using an internal teat sealer to prevent new intramammary infections in nonlactating dairy cattle. *J. Am. Vet. Med. Assoc.* 228, 1565–1573.

Sargeant JM, O'Connor A, Dohoo IR, Erb HN, Cevallos M, Egger M, Ersbøll AK, Martin SW, Nielsen LR, Pearl DL, Pfeiffer DU, Sanchez J, Torrence ME, Vigre H, Waldner C and Ward MP (2016) Methods and processes of developing the strengthening the reporting of observational studies in epidemiology – veterinary (STROBE-Vet) statement. *Prev. Vet. Med.* 134, 188–196.

Schünemann HJ, Brożek J, Guyatt G and Oxman AD (eds) (2013) GRADE Handbook for Grading Quality of Evidence and Strength of Recommendations. Available from guidelinedevelopment.org/handbook (accessed January 2017).

Schunemann HJ, Oxman AD, Vist GE and Higgins JPT (2017) Chapter 12: interpreting results and drawing conclusions. In Higgins JPT, Chandler J and Cumpston MS (eds), Cochrane Handbook for Syst Rev. of Interventions Version 5.2.0. pp. 12:1–12:26. Available from www.training.cochrane.org/handbook (accessed September 2017).

Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P and Stewart LA (2015) Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 349, g7647.

Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, Henry D, Altman DG, Ansari MT, Boutron I, Carpenter JR, Chan A-W, Churchill R, Deeks JJ, Hróbjartsson A, Kirkham J, Jüni P, Loke YK, Pigott TD, Ramsay CR, Regidor D, Rothstein HR, Sandhu L, Santaguida PL, Schünemann HJ, Shea B, Shrier I, Tugwell P, Turner L, Valentine JC, Waddington H, Waters E, Wells

GA, Whiting PF and Higgins JP (2016) ROBINS-I: a tool for assessing risk of bias in nonrandomized studies of interventions. *BMJ* 355, i4919.

White IR, Barrett JK, Jackson D and Higgins JPT (2012) Consistency and inconsistency in network meta-analysis: model estimation using multivariate meta-regression. *Res. Synth. Methods* 3, 111–125.

Chapitre 4 – Comparing blanket versus selective dry-cow treatment approaches for cure and prevention of intramammary infections during the dry period: a systematic review and meta-analysis

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Abstract

A systematic review and a series of meta-analyses were conducted to investigate the efficacy of selective dry cow antimicrobial treatment (SDCT; in which only infected quarters/cows were treated with an antimicrobial) compared with blanket dry cow treatment (BDCT; all quarters/all cows received an antimicrobial, regardless to their infection status). A full detailed protocol was published before initiating this review. Studies reporting on: 1) proportion of untreated quarters or cows when using SDCT; 2) intramammary infections (IMI) incidence risk over the dry period; 3) IMI elimination risk; 4) post-calving IMI prevalence; 5) early lactation clinical mastitis incidence; or 6) subsequent lactation milk yield, and somatic cells counts were considered eligible. Thirteen articles representing 12 controlled trials, whether randomized or not, were available for analyses. SDCT reduced the use of antimicrobials at dry-off by 66% (95% CI: 49 – 80). There was no difference in the elimination of existing IMI at dry off, between SDCT and BDCT. Meta-regression showed that risk of acquisition of new IMI during the dry period, IMI risk at calving, early lactation clinical mastitis risk, and early lactation milk yield and somatic cells counts did not differ between SDCT and BDCT as long as an internal teat sealant (65% bismuth subnitrate) was administered to untreated healthy quarters/cows at dry off. For trials not using internal teat sealants, SDCT resulted in higher risk than BDCT of acquiring a new IMI during the dry period and of harboring an IMI at calving. Evidences strongly supports that SDCT would reduce the use of antimicrobials at dry off, without any detrimental effect on udder health or milk production during the first months of the subsequent lactation, if, and only if, internal teat sealants are used for healthy, untreated quarters/cows.

Key words: Dairy cows, dry period, selective antimicrobial treatment, intramammary infection, antimicrobial use.

Introduction

Blanket dry cow therapy (BDCT), where all quarters of all cows are treated with a long acting antimicrobial at dry-off, was introduced many years ago (Neave et al., 1969) and is widely used by dairy farmers. This practice permits to increase the elimination of existing intramammary infections (IMI) at dry-off and prevent the occurrence of new IMI during the dry period. In fact,

persistent and new IMI during the dry period can result in the development of clinical mastitis (CM) early in the next lactation (Bradley and Green, 2000, Bradley and Green, 2004, Green et al., 2002).

However, with changes in mastitis epidemiology and increasing public health concerns regarding the use of antimicrobials in food-producing animals, selective dry cow therapy (SDCT) is a potential alternative to BDCT to reduce antimicrobial usage in dairies (Cameron et al., 2014, Kabera et al., 2020, Rindsig et al., 1978). With a SDCT approach, antimicrobial treatment is reserved for cows or quarters suspected of having an IMI, while uninfected cows and quarters usually do not receive an antimicrobial treatment. In addition, internal teat sealants (ITS) have been shown to be a very effective non-antimicrobial alternative to prevent new IMI during the dry period (Dufour et al., 2019, Huxley et al., 2002, Sanford et al., 2006a). A teat sealant could be used to protect untreated cows or quarters when a selective antimicrobial treatment approach at dry off is applied. Thus, SDCT could prevent the use of antimicrobials for a prophylactic purpose, and that, possibly without detrimental changes to udder health parameters (Vanhoudt et al., 2018).

A systematic review comparing blanket and selective dry-cow therapy and describing the various advantages and potential negative impacts would be of great importance for decision-makers to engage in an effective and judicious use of antimicrobials at dry-off. Recently, a systematic review (Winder et al., 2019) reported on impact of selective vs. blanket dry cow therapy, but on only one outcome, prevalence of IMI at calving. In this latter study, reduction in use of antimicrobials at dry-off (the main reason for choosing SDCT) was not investigated, nor was risk of CM, milk yield, or SCC in the early next lactation. These outcomes are all very important for choosing the best dry-cow treatment protocol. Moreover, IMI dynamics during the dry period (i.e., acquisition and elimination of IMI during the dry period) was not investigated in the Winder et al. (2019) study. Nevertheless, studying IMI dynamics can provide better insights on the underlying biological processes, compared to studying prevalence at a single point in time (e.g., at calving).

Objective

The objective of the current study was to investigate the efficacy of SDCT, compared to the treatment of all quarters of all cows, for: 1) reducing use of antimicrobials at dry-off; 2) preventing acquisition of new IMI during the dry period; 3) eliminating existing IMI at dry off; 4) reducing the prevalence of IMI at calving; and 5) preventing early lactation CM. The objectives were also to investigate whether milk yield and SCC in the early lactation would be affected. Our hypothesis was that SDCT protocols could be implemented without negative health or production effects and would result in a substantially lower usage of antimicrobials at dry-off.

The PICO (population, intervention, comparator, outcome) questions answered by the current study was formulated as: In dairy cows (i.e. the population), is SDCT (i.e. the intervention) as efficient as BDCT (i.e. the comparator), (1) in preventing new IMI during the dry period; (2) in eliminating existing IMI at dry off; (3) in reducing IMI risk at calving; and (4) in preventing early lactation CM; (5) and to describe the impact of the dry cow treatment approach on milk yield and SCC in the early lactation (i.e. the outcomes)?

Methods

This current systematic review was reported using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines (Moher et al., 2009). The detailed protocol for this review was published elsewhere prior to initiating the review (Afifi et al., 2018). The complete protocol targeted three independent objectives: (1) choice of antimicrobial at drying-off; (2) comparison of blanket versus selective dry cow treatment; and (3) complementing an antimicrobial treatment with a teat sealant. However, the current manuscript reports only on the comparison of blanket and selective dry cow treatments. The other two objectives will be addressed in two future independent manuscripts.

The complete search strategy described in the published protocol was initially conducted May 1st, 2018, and updated June 16th, 2020, prior to finalizing the analyses and manuscript. The search strategies were all conducted on the same day for the three electronic sources of information (Medline, CAB Abstracts, and Web of Science) and for conference proceedings from the National Mastitis Council and the American Association of Bovine Practitioners. Modifications and

precisions to the published protocol with their justifications are described in the following sections.

Modifications and/or precisions to the published protocol

Eligibility criteria

In the published protocol, we planned to include studies where the post-calving IMI status was determined within 14 days in milk (DIM), to ensure that the new IMI or elimination most likely occurred during the dry period (vs. in the early lactation). However, in some articles, cows were sampled twice after calving (for instance, 3-4 DIM and 5-18 DIM) and a parallel interpretation of the two milk samples was used to define IMI status. Hence, some studies relied on testing within an interval that extended slightly beyond 14 DIM, but was mostly within 0-14 DIM. We decided to retain these studies (Cameron et al., 2014, Kabera et al., 2020, Ward and Schultz, 1974). In the published protocol, we indicated CM incidence during the first 0 – 4 months after calving as a studied outcome. More precisely, we did use the CM data from studies with a shorter follow-up period and otherwise extracted the data up to a maximum of 4 months in milk.

Risk of bias in individual studies

As it was planned in the protocol, we proposed to record different domains of risk of bias (ROB) by outcome's type. In fact, the ROB 2.0 makes it clear that the assessment is typically specific to a particular result and consequently, the assessments of risk of bias need to be outcome-specific (Higgins et al., 2016). However, all measured outcomes yielded the same evaluation within a given trial. Hence, for simplicity, we only reported risk for a domain for all outcomes of a trial at once. As all included studies were controlled trials (whether randomized or not), only the Cochrane Collaboration's tool for assessing risk of bias was used for assessing risk of bias in selected studies (Cochrane Handbook for Systematic Reviews of Interventions, version 5.1.0).

Summary measures

Daily mean milk production (kg/d) or mean natural logarithm of SCC (ln SCC) during the first four months were extracted directly from included trials or obtained from personal communications

with the authors. Thus, raw mean difference (MD) was used as the effect size, for those two outcomes.

Data synthesis and meta-analysis

Meta-analyses were conducted in R version 4.0.0 (R Foundation for Statistical Computing Platform: x86_64-w64-mingw32/x64 (64-bit)) using RStudio version 1.2.1335 (RStudio Inc., Boston, MA) using the 'meta' package version: 4.12-0 (2020-05-04). Studies were weighed using the inverse variance method based on the logit transformation. A random effects approach was used, as it was described in the published protocol (Afifi et al., 2018) and the between-study variability was estimated using the method of restricted maximum likelihood (REML) (Langan et al., 2019) and the Knapp-Hartung adjustment for random effects model (Knapp and Hartung, 2003). Heterogeneity was assessed by the I^2 statistic. Effects of trial level characteristics were tested using a meta-regression model with one covariate, and only if at least three trials were included in each category of the covariate.

Confidence in cumulative estimates

The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach involves rating, for each comparison made, the confidence in effect estimate based on an assessment of eight domains: number of trials, risk of bias, inconsistency, indirectness, imprecision, publication bias, number of individuals (in our case quarters or cows) followed, and a summary measure of association with its 95% CI. Then, an overall assessment is made regarding the level of confidence in the summary effect estimate observed. For rating the different domains of the GRADE, in the current review, we used the guidelines suggested by Dufour et al (Dufour et al., 2019).

Briefly, for the risk of bias domain, a trial was rated at low risk of bias, when at least four out of seven evaluated domains for an individual trial were rated at low risk with a maximum of one domain evaluated at high risk. When at least four domains were rated at low risk but with two domains evaluated at high risk, the trial was rated at moderate risk of bias. In other cases, the trial was rated at high risk of bias. For the inconsistency domain, we visually appraised, using forest plot, whether a uni-, bi-, or multi-modal distribution of point estimates was observed across

trials and rated these, respectively, as no serious, serious, and very serious limitations. Regarding the indirectness domain, we computed independently the proportion of trials for which the investigated population, intervention, and outcome matched those of interest, and an equal weight was given to these three sub-domains. Comparisons with a score ≥66%, between 65 and 33%, and of ≤33% for that domain were then rated as no serious, serious, and very serious limitations, respectively.

For the imprecision domain, the difference between the natural logarithm of the higher and lower bounds of the summary relative risk (RR) was computed. Comparisons with confidence interval bounds differences ≤1.1 on the logarithmic scale (equivalent to an RR interval of 1.0–3.0 points), between 1.1 and 1.6 (equivalent to an RR interval of 3.0–5.0 points), and ≥1.6 (equivalent to an RR interval of ≥5.0 points) were rated, respectively, as no serious, serious, and very serious limitations. For the imprecision domain for milk yield and SCC, in addition to the examination of upper and lower limits of the 95% confidence intervals, we considered the calculation of an optimal information size (Guyatt et al., 2011). When the optimal information size criterion was not met, the precision was rated as serious limitations. When the optimal information size criterion was met and the 95% CI length < 2 (i.e. a mean difference of -1.00 to +1.00) for milk yield and ln SCC, we rated precision as no serious limitations. When the optimal information size criterion was met, and the 95% CI length ≥ 2 and ≤ 4 for milk yield and ln SCC, the precision was rated as serious limitations. When the optimal information size criterion was met, and the 95% CI length ≥ 4 for milk yield and ln SCC, the precision was rated as very serious limitations.

Finally, for the publication bias domains, we considered whether number of trials allowed us to fully appraise funnel plot asymmetry. We also considered whether the outcomes studied would be associated with any commercial advantages.

Results

Study selection

Results of the different steps for searching and assessing eligibility of studies are presented in Figure 4. After removing duplicates and exclusion due to language restriction, a total of 991

records were identified from three databases: Cab Abstracts, Web of Science, and Medline. Of the 991 records, after reviewing the content of the abstracts and full texts, only 89 records met the inclusion criteria for at least one of the PICO questions on antimicrobial-based dry cow therapy approaches.

In addition, 43 records were identified through the search of NMC (National Mastitis Council) and AABP (American Association of Bovine Practitioners) conference proceedings. Finally, after excluding proceeding papers for which an equivalent full article was available (n=27), 105 records combining 89 full articles and 16 conference papers were included. The references cited in these 105 retained records and 78 non-primary studies were screened for any additional relevant study which was not initially retrieved through databases or conference proceedings search, but no additional eligible records were identified from this process for the comparison of SDCT and BDCT.

Of the 105 records retained, 13 articles representing 12 trials reported on the comparison of SDCT and BDCT and, therefore, were included in this part of the systematic review. Other retained records will be discussed in two other manuscripts reporting on the choice of antimicrobial at drying-off or on complementing an antimicrobial treatment with an ITS.

Included studies

Characteristics of the 13 included articles representing 12 trials are described in Table 1. Those twelve trials include 6 trials reported in 6 articles (Hassan et al., 1999, Patel et al., 2017, Rindsig et al., 1978, Roguinsky and Serieys, 1977, Ward and Schultz, 1974, Williamson et al., 1995), two trials where each trial was reported in two articles for different outcomes (Browning et al., 1990, Browning et al., 1994, Cameron et al., 2015, Cameron et al., 2014) two trials reported in two articles where each article reported on both trials (Rowe et al., 2020a, b) and two trials reported in one article (Kabera et al., 2020). Furthermore, the description of the SDCT group and of the reported outcomes are summarized in Table 2. Finally, the follow-up period after calving and definitions of IMI at dry off and calving, of new IMI, and of elimination of IMI during the dry period used in each study are provided in Table 3.

Briefly, six included trials were randomized controlled trials and six did not clearly report a randomization process and were, therefore, considered simply as controlled trials. Seven trials

reported using herd and/or cow level recruitment criteria, one trial did not set criteria to recruit cows and/or quarters, and four trials did not report on selection criteria. One trial set selection criteria at herd level only, while the other six trials set them both at cow and herd levels. The most common herd-level selection criteria was to have a bulk milk SCC below a predetermined threshold (ranging from 250,000 to 400,000 cells/mL). For cow-level criteria, having a standard expected dry period length was often used, as well as no recent treatment prior to dry off, and no CM at dry-off. Among the 6 trials where breed was reported, three were conducted in crossbred and purebred (Holstein and Holstein-Jersey or Friesian and Friesian-Jersey), while the other three were conducted solely in purebred cows (Holstein/Friesian). Of 12 trials, the selection approach was based at quarter level for eight trials and at cow level for the other four trials.

In all trials, measures of new IMI, of IMI elimination, and of prevalence of IMI were based on bacteriological culture of milk samples collected before drying-off and after calving. Pre-dry off samples were taken within one month before dry off and days in milk at post-calving sampling ranged from 0 to 4 weeks. Of the 12 included trials, acquisition of new IMI during the dry period was the most often reported outcome (n = 11), followed by elimination of IMI during the dry period (n=10) and prevalence of IMI at calving (n=9). Clinical mastitis in the subsequent lactation was reported in ten trials. However, two of the ten trials reporting on CM in the subsequent lactation were excluded from the meta-analysis, as the follow-up period was more than 4 months and it was not possible to have data specifically for the 0-120 DIM period.

Four trials reported daily mean milk production during the first 120 DIM of the subsequent lactation (Kabera et al., 2020, Rowe et al., 2020b), and one trial reported daily mean milk production during the first 180 DIM (Cameron et al., 2015). Six trials reported on SCC during the subsequent lactation. One trial reported on an arithmetic mean scale for the first week and between 28 and 56 days after calving (Rindsig et al., 1978). One trial reported test-day ln SCC 0-180 DIM (Cameron et al., 2015), two on mean milk somatic cell score for 0-120 DIM (Kabera et al., 2020) and two on SCC geometric mean for 0-120 DIM (Rowe et al., 2020b). After contacting authors, data could be obtained on a same scale (mean ln SCC) for five trials. Moreover, for the trial reporting on a period of 0-180 DIM, we were able to obtain data specifically for the 0-120

DIM period. This latter trial was, therefore, included in the meta-analysis comparing average milk yield and ln SCC between SDCT and BDCT.

Risk of bias within studies

The risk of bias for each individual study is reported in Figure 5. A summary of the risk of bias assessment for the 12 trials included in the meta-analysis is presented in Figure 6. All trials had at least one potential source of bias rated as high or unclear. The risk of bias was evaluated for 13 articles reporting on 12 trials and the components with the smallest proportion of low risk trials were blinding of participants and personnel (0/12), then allocation concealment (2/12), and, finally, random sequence generation (6/12). The method used to generate a random sequence was described for only six trials (Cameron et al., 2015, Cameron et al., 2014, Kabera et al., 2020, Patel et al., 2017, Rowe et al., 2020a, b). Two trials had cows allocated alternately to two treatment groups and were consequently assessed as 'high risk' (Rindsig et al., 1978, Ward and Schultz, 1974) and four other trials did not report on the randomization process in sufficient details for assessing potential bias (Browning et al., 1990, Browning et al., 1994, Hassan et al., 1999, Roguinsky and Serieys, 1977, Williamson et al., 1995). The allocation concealment was not described at all, in eight trials. Consequently, they were classified as having an unclear risk regarding potential source of bias. It was appraised as 'low risk' in two trials (Kabera et al., 2020) and as 'high risk' in two other trials where cows were allocated alternately (Rindsig et al., 1978, Ward and Schultz, 1974). Similarly, blinding of participants and personnel was not mentioned in seven trials. This latter component was evaluated at high risk in five other trials, as producers were not blinded to the treatment and, thus, we could not exclude an influence on the management of cows in different treatment groups. Bias due to blinding of outcome assessment (detection bias) was considered 'low risk' in all trials relying mainly on laboratory analyses, which was considered to be an objective measurement.

Meta-analyses comparing selective and blanket dry-cow therapies

A total of 12 trials reported on the effect of SDCT on IMI during the dry period and on udder health and milk production in the subsequent lactation, in comparison with BDCT. In addition to a positive control group (BDCT), one trial (Hassan et al., 1999) included a second control group

where cows did not receive any therapy at dry off. Data from this control group were not extracted, as our focus was the comparison between SDCT and BDCT.

The most important study characteristics suspected as potential source of heterogeneity and tested in meta-regression were: (1) method used to identify infected cows/quarters at dry off (milk culture vs. combination of SCC and/or history of clinical mastitis and/or California mastitis test and/or N-acetylf-D-glucosaminidase); (2) whether the selective treatment was applied at cow or quarter level; and (3) whether an ITS was applied for healthy cows/quarters. Meta-regression by the preceding variables was attempted if at least three trials were included in each category. For all the meta-analyses conducted, results by category of the covariate were presented rather than a general summary measure, whenever a variable tested in a meta-regression yielded a p-value < 0.05.

Reduction of antimicrobial use at dry off

Eleven trials reported on the reduction of antimicrobial use, however, only ten of them could be used to summarize reduction of usage of antimicrobial at dry-off. In fact, one of the trials (Cameron et al., 2015, Cameron et al., 2014) reported on the reduction in use of antimicrobials in cows preselected (individual SCC < 200,000 cells/mL and no CM on last three Dairy Herd Improvement (DHI) tests; Table 1) before the randomization into selective and blanket treatment groups. Thus, it was not comparable with other trials, regarding the reduction of antimicrobial use.

Three trial characteristics (diagnostic test used to identify infected cows/quarters at dry off; whether the selective antimicrobial treatment was applied at cow or quarter level and whether an ITS was applied for untreated (healthy) cows/quarters or not) were tested. None of them could explain the observed heterogeneity (I^2=97%). Figure 7 presents the proportion of antimicrobial use reduction for each trial and a summary measure.

Effects of selective dry cow therapy on IMI incidence over the dry period

In 11 trials, IMI incidence risk during the dry period was investigated and reported at quarter level. When comparing IMI incidence over the dry period in SDCT and BDCT, one trial characteristic

(whether an ITS was applied for untreated (healthy) cows/quarters) was significantly associated with the estimate effect size ($p<0.01$; tau2=0.00). An ITS consisting of 65% bismuth subnitrate was used in the six trials where it was applied for untreated (healthy) cows/quarters. Figure 8 presents the RR comparing risk of acquiring a new IMI over the dry period between selective and BDCT for each trial, as well as summaries of RR for trials using ITS or not.

For studies without ITS, the risk of new IMI during dry period was significantly higher for selectively compared to blanket dry treated cows/quarters (RR=2.00, 95% CI: 1.41 – 2.84). Conversely, for studies where an ITS was used to protect healthy cows/quarters, the risk of new IMI during the dry period was not different for selectively compared to blanket dry treated cows/quarters (RR=1.04, 95% CI: 1.00 – 1.07).

Effects of selective dry cow therapy on IMI elimination over the dry period

In 10 trials, elimination of IMI during the dry period was investigated. None of the variables evaluated in the meta-regressions were significantly associated with risk of IMI elimination. Figure 9 presents the RR comparing risk of IMI elimination over the dry period between selective and BDCT for each trial, as well as a summary measure for all trials together. There was no difference (RR=0.99, 95% CI: 0.96 – 1.03) between SDCT and BDCT, regarding the elimination of IMI during the dry period.

Effects of selective dry cow therapy on IMI prevalence at calving

In nine trials, IMI prevalence at calving was reported. Only one trial characteristic (whether an ITS was applied for healthy cows/quarters) was significant ($p<0.01$, tau2=0.01). Figure 10 presents the RR comparing risk of IMI at calving between SDCT and BDCT for each trial, as well as summaries RR for each category of ITS usage. For trials without ITS (n=5), the risk of IMI at calving was significantly higher for selectively treated cows/quarters than blanket treated cows/quarters (RR=1.57, 95% CI: 1.19 – 2.06), but substantial heterogeneity was still present within this category (I^2=60%). For trials using an ITS (n=4), the risk of IMI at calving was not different for selectively and blanket treated cows/quarters (RR=1.03, 95% CI: 0.97 – 1.09). For this latter category, no heterogeneity was seen (I^2=0%).

Effects of selective dry cow therapy on clinical mastitis incidence in the early lactation

Incidence risk of CM early in the following lactation was investigated in 8 trials. Two of them reported CM incidence at cow level and the other six at quarter level. Before commingling these studies together, it would have been interesting to investigate in a meta-regression the impact of reporting CM, the outcome, at cow vs. quarter level, but there were not enough trials where CM were reported at cow level. Among the other potential predictors, only the method used to identify infected cows/quarters at dry off could be tested in a meta-regression and it was not significant. Figure 11 presents the RR of CM incidence during the first 120 days of lactation between SDCT and BDCT for each trial, as well as a summary RR for all trials.

The risk of CM incidence during the first four months of lactation was not significantly different between selectively and blanket dry treated cows/quarters (RR=1.03, 95% CI: 0.65 – 1.64). However, there was an important heterogeneity among trials (I^2=83%). When we considered only the six trials where an ITS was used for healthy cows/quarters, the risk of CM was still not different between SDCT and BDCT (RR =0.84, 95% CI: 0.65 – 1.08), however, the heterogeneity was reduced to an almost null value (I^2=3%).

Effects of selective dry cow therapy on milk yield in the early lactation

Only five trials reported on milk yield during the first four months of the subsequent lactation. Figure 12 presents mean difference of milk production during the first four months of lactation after a SDCT approach, in comparison with a BDCT, for each trial, as well as a summary measure for all trials. There was no difference in milk yield during the first months of the subsequent lactation (MD=-0.24kg/d, 95% CI: -1.17 – 0.70) between SDCT and BDCT.

Effects of selective dry cow therapy on SCC in the early lactation

Five trials reported on SCC (transformed in ln SCC using the natural logarithm scale) during the first four months of the subsequent lactation. Figure 13 presents mean difference of ln SCC during the first months of lactation after a SDCT approach, in comparison with a BDCT, for each trial, as

well as a summary measure. There was no difference in ln SCC during the 0-120 DIM period of the subsequent lactation (MD=0.03, 95% CI: -0.09 – 0.15) between selective and BDCT.

Publication bias

Contour-enhanced funnel plots for each outcome of comparison between SDCT and BDCT are presented in Figure 14. However, because of the limited number of available trials, tests for funnel plot asymmetry could not be performed. Therefore, plots were evaluated visually, but it was not possible to identify putative missing studies.

Summary of evidence

Table 4 presents a GRADE evidence profile for the different outcomes comparing SDCT and BDCT. Our GRADE assessment indicated a high level of confidence for four of the six studied outcomes/comparisons: 1) risk of acquiring a new IMI in selective dry cow treated quarters/cows when an ITS was administered to healthy quarters; 2) prevalence of IMI, again when an ITS was administered to healthy quarters as part of the selective dry cow protocol; 3) milk yield in the subsequent lactation; and 4) ln SCC in the subsequent lactation.

Discussion

This systematic review was conducted to determine the efficacy of SDCT (antimicrobial treatment of infected quarters/cows solely) compared to BDCT (all quarters/all cows treated). It reports on SDCT as a potential alternative to BDCT. The main rationale for using a SDCT strategy is to reduce antimicrobial use. This, however, should be achieved, if possible, without any detrimental effect on udder health and milk production. Our results confirm that SDCT can help reduce the use of antimicrobials and that, without detrimental effects. However, this was only achieved when acquisition of new IMI in untreated quarters was prevented using an ITS.

A comparable effect of SDCT and BDCT was reported by a review reporting on the prevalence of IMI at calving when all cows received an ITS (Winder et al., 2019). The same review, in agreement with us, reported a difference between SDCT and BDCT, when an ITS was not used to protect untreated quarters/cows. The importance of the use of ITS at dry off was reported by other previous reviews (Dufour et al., 2019, Halasa et al., 2009b, Rabiee and Lean, 2013).

The current review also reported on acquisition and elimination of IMI during the dry period, and on CM, milk yield, and ln SCC during the subsequent lactation. For all these outcomes, SDCT and BDCT were equivalent, as long as an ITS was used for untreated quarters. However, all trials which reported on milk yield and ln SCC used an ITS. Thus, for those two outcomes, it was not possible to measure the effect of SDCT when an ITS is not used for untreated quarters/cows.

There were small numbers of trials in both ITS categories for all outcomes, but low or no heterogeneity was observed in the ITS category for all tested outcomes (new IMI, prevalence of IMI at calving, and CM during the first 4 months of the subsequent lactation). For trials not using ITS, there was a high risk of new IMI and of IMI at calving in cows/quarters assigned to a SDCT protocol, compared to BDCT, but heterogeneity between trials was still important in this category. This maintenance of heterogeneity may be due, not only to a small number of included trials, but also to other unmeasured factors which may affect the effect estimated (Winder et al., 2019).

For all trials, cow- or quarter-level data were used in meta-analysis and therefore, clustering of quarters by cow or cows by herd was not accounted for. However, by considering a random effect approach, we accounted for clustering of individuals within different studies.

Regarding the reduction of antimicrobial use in dairy cows at dry off, we conclude that when SDCT is applied, antimicrobial use could be reduced by 66% (95% CI: 49 – 80) compared to BDCT. For that outcome, a bimodal distribution was observed, with eight trials reporting proportions in the range of 43 – 68% and 2 trials with proportions of 81 and 96%. However, in these trials reporting proportions of 81 (Hassan et al., 1999) and 96% (Ward and Schultz, 1974), selection of treated quarters was based on a high N-acetylf-D-glucosaminidase (NAGase) value or the occurrence of clinical mastitis during 1 month prior to drying off, respectively. Moreover, 112 additional antimicrobial infusions during the dry period and early lactation were reported by Ward and Schultz (1974). In total, 37 positive quarters including 10 clinical mastitis were reported by Hassan et al. (1999) during the dry period in the selective group. These two latter SDCT approaches indeed resulted in very large reduction in antimicrobial usage at dry-off, but also in substantial usage of antimicrobials during the dry period.

Summary of evidence

Impact of selective dry cow therapy on preventing the acquisition of new intramammary infections during the dry period

Regarding prevention of IMI acquisition over the dry period, we conclude with a high level of confidence that SDCT is as efficient as BDCT when an ITS (65% bismuth subnitrate) is used for untreated healthy quarters/cows at dry off. The efficacy of ITS in the prevention of IMI has been reported in previous reviews (Dufour et al., 2019, Halasa et al., 2009b, Rabiee and Lean, 2013, Robert et al., 2006b). When an ITS was not used, we would conclude toward a higher risk of new IMI in SDCT compared to BDCT, but with a low level of confidence. These results suggest that, in the countries and through the different time period where these studies were conducted, the infection pressure during the dry period was too important for leaving quarters completely unprotected (i.e., without antimicrobial AND without ITS).

Regarding applying the selection at cow or quarter levels, we did not detect a difference between these SDCT approaches for IMI prevention. However, Halasa et al. (2009b) reported BDCT to be more protective of new IMI than SDCT when selection was based at quarter level (RR=2.01, 95% CI = 1.34, 3.02), but to no significant difference when selection was based at cow level (RR=0.52, 95% CI: 0.12 – 2.31). In this latter review, however, SDCT protocols of included studies did not include an ITS for untreated, healthy quarters or cows.

Impact of selective dry cow therapy on elimination of existing intramammary infections during the dry period

Regarding the elimination of existing IMI present at dry off, we conclude with a moderate level of confidence toward comparable efficiency of SDCT and BDCT. For that comparison, our level of confidence was mainly affected by the multimodal distribution observed for RR point estimates, with one trial reporting RR estimate of 1.28 (Roguinsky and Serieys, 1977), one trial with RR estimate of 0.52 (Ward and Schultz, 1974) and eight trials with RR estimates in the 0.80–1.02 range. However, heterogeneity for this comparison was low (I^2=32.8%) and the predicted RR interval was the same as the confidence interval of the effect size from the random effect model (0.96 – 1.03). A similar efficiency between SDCT and BDCT was also reported by Halasa et al.

(2009a). When Ward and Schultz (1974) was omitted, the RR was the same (RR=0.99, 95% CI: 0.96 – 1.03), but no heterogeneity was still seen in the analysis (I^2=0).

Impact of selective dry cow therapy on intramammary infection prevalence at calving

Regarding IMI prevalence at calving we concluded with a high level of confidence regarding the comparable efficiency of SDCT and BDCT, when an ITS (65% bismuth subnitrate) was used for untreated healthy quarters/cows. The same conclusion was reported by Winder et al. (2019).

Conversely, when an ITS was not used, we had a low confidence in our general conclusions. The level of confidence was mainly affected by the bimodal distribution observed for RR point estimates and by a very serious risk of bias. Almost all trials included in this comparison were older (published between 1974 and 1999) and many of the important information on randomization (e.g., random sequence generation, allocation concealment) were not reported. As it was also reported by Winder et al. (2019), when an ITS was not used, there was an increased risk of IMI at calving for SDCT compared to BDCT and a substantial heterogeneity was noted in this subgroup. The presence of a high residual heterogeneity indicates that there is more than one effect within the trials where and ITS was not used. The predicted RR interval within this sub-group was 0.74 – 2.93.

Impact of selective dry cow therapy on clinical mastitis incidence early in the subsequent lactation

We have a moderate level of confidence regarding the equivalence of SDCT compared to BDCT for the reduction of CM in the following lactation. The level of confidence was affected by a bimodal distribution observed for the estimated RR. However, when we exclude two trials where an ITS was not used for untreated healthy quarters/cows at dry off, the heterogeneity was very low. The importance of ITS in the reduction of CM incidence in the subsequent lactation was reported by previous reviews (Dufour et al., 2019, Rabiee and Lean, 2013).

Impact of selective dry cow therapy on milk yield and ln SCC during the subsequent lactation

Concerning milk yield and ln SCC during the subsequent lactation, we conclude with a high level of confidence regarding the comparable efficiency of SDCT and BDCT. However, only trials published between 2014 and 2020 and where ITS were used for untreated healthy quarters/cows at dry off were included in this comparison. None of the previous reviews reported on these two outcomes. In fact, those outcomes were not commonly reported in older studies. However, one of the included trials (Rindsig et al., 1978) reported SCC, but on an arithmetic scale which could not be compared with the logarithmic scale. We were not able to reach the authors to get these latter data on a logarithmic scale.

Comparisons with published reviews

The fact that the Winder et al. (2019) review was conducted concurrently to our review provided an opportunity for comparing how our different methodologies affected the presented results. The most striking difference between reviews are the outcomes analyzed. The main rationale for adopting selective dry cow treatment is the associated reduction in use of antimicrobials. Quantifying this potential reduction was, in our opinion, essential. Likewise, risk of CM, milk yield, and SCC in the early next lactation are also important parameters to quantify, to better inform producers considering moving to a selective treatment approach. Finally, although IMI incidence and elimination rates are somewhat captured by measuring IMI prevalence at calving, reporting on these indices provide a better understanding of the underlying biological processes. Our analyses indeed confirmed that the increased IMI prevalence at calving in SDCT protocols when an ITS is not used, was mainly caused by an increased IMI incidence in untreated quarters.

Beyond the different outcomes presented, our different methodologies also affected articles selection. Three articles included in Winder et al. (2019) were not included in our review. The first article (Seeth et al., 2017) was excluded from our review because the antimicrobials used were not specified. Furthermore, it was not clear whether infected cows in the selective group received the same antimicrobial as cows in the blanket group. When the first author was contacted, he confirmed that each farm used the intramammary antibiotic which was normally used before the

trial, but he could not confirm that cows of the same herd and allocated to the selective or blanket groups received the same antimicrobial, as the antimicrobials used could have been modified by a farmer during the study. The second article (Robinson et al., 1983) was excluded, as we considered that cows in the SDCT and BDCT groups were managed differently. In fact, in this latter study, cows in the BDCT group were teat dipped after each milking, while in the SDCT group, they were not teat dipped. Thus, the Robinson et al. (1983) study actually compared blanket dry cow therapy with teat disinfection vs. selective dry cow therapy and no teat disinfection. Moreover, Winder et al. (2019) included the Serieys and Roguinsky (1975) paper, while our review considered the Roguinsky and Serieys (1977) paper. These two papers reported on results of a same trial. The 1977 paper was judged more complete by our team and was, therefore, chosen for inclusion. The results presented in the 1975 and 1977 papers differed slightly and this resulted in Winder et al. (2019) using 23/82 quarters with an IMI at calving for blanket treated cows for the Roguinsky's study while our review considered 23/72 infected quarters at calving for blanket treated cows for that same study. Finally, one paper (Rowe et al., 2020a) published after the publication of the Winder et al. (2019) review was included in our review and used for comparing prevalence of IMI at calving.

Another difference between our review and that of Winder et al. (2019) was observed in the numbers extracted from the Cameron et al. (2014) study. In their review, Winder et al. (2019) mentioned 164/1130 and 160/1157 quarters with a prevalent IMI at calving for SDCT and BDCT groups, respectively. These numbers were incorrectly extracted in the Winder et al. (2019) review. In the Cameron et al. (2014) paper, these numbers are indeed presented, but they represented the new IMI risk over the dry period, not the post-calving IMI risk, which were presented in a different table. These numbers were 179/1130 and 177/1157 quarters with a prevalent IMI at calving for SDCT and BDCT groups, respectively.

Overall, these differences in selected studies and in data extraction between reviews resulted in very small differences in the estimated summary measures. Using data from 3750 quarters, Winder et al. (2019) reported a summary risk ratio (95% CI) of 1.09 (0.92 – 1.28) when comparing risk of IMI at calving using selective dry cow therapy with a teat sealant for untreated quarters compared to blanket dry cow therapy. Using data from 8045 quarters, we reported a risk ratio of

1.03 (0.97 – 1.09). On the other hand, we were also able to report on reduction of antimicrobial usage, IMI incidence risk, IMI elimination risk, as well as CM incidence, milk yield, and ln SCC in the beginning of the subsequent lactation.

Beyond the Winder et al. (2019) review, two other previously published meta-analyses (Halasa et al., 2009a, Halasa et al., 2009b) have investigated the comparison of SDCT vs. no dry cow treatment or SDCT vs. BDCT for the prevention of new IMI and elimination of existing IMI during dry period. In our review, only studies comparing SDCT and BDCT were retained. Thus, only a small number of articles used in the Halasa et al. (2009a) and Halasa et al. (2009b) reviews are included in this review (Browning et al., 1994, Hassan et al., 1999, Rindsig et al., 1978, Williamson et al., 1995). Moreover, none of the studies included in the comparison of SDCT and BDCT (Browning et al., 1994, Hassan et al., 1999, Rindsig et al., 1978, Robinson et al., 1988, Williamson et al., 1995) had used ITS for untreated, healthy quarters or cows.

Limitations

A small number of trials were included in our review. Those trials were published over a wide period of time (1974–2020). Herd-level inclusion criteria were not reported for six trials. For trials which did, herds were selected with a low BTSCC (<250,000 cells/mL of milk) (Cameron et al., 2015, Cameron et al., 2014, Kabera et al., 2020, Rowe et al., 2020a, b) or a wide range in BTSCC (100,000 – 400,000 cells/mL of milk) (Browning et al., 1990, Browning et al., 1994). Moreover, Cameron et al. (2014) and Cameron et al. (2015) reported on cows with a SCC < 200,000 cells/mL on last 3 DHI tests and no CM on the same period.

Most reviewed studies (mostly the more recent ones) and in particular studies where ITS was used for healthy and untreated quarters were conducted in herds with a relatively low bulk tank SCC < 250,000 cells/mL. For herds with higher bulk milk SCC, there would probably be a higher prevalence of IMI at dry-off and especially a higher prevalence of contagious pathogens. Thus, there might be an increased risk of IMI during the dry period for quarters that were not treated at dry-off, regardless of receiving an ITS. So, these review´s results should be extrapolated to low SCC herds (BTSCC < 250,000 cells/mL) only.

There was also differences in the definition of IMI used across different trials, and the time when the post-calving samples were collected also varied between studies (Table 3). These differences in IMI definition could be one of the important causes for the heterogeneity of effect observed between studies.

We initially planned to investigate the effect of randomization (randomized vs. non-randomized trials) in our meta-regressions. However, there was no information on randomization for four studies (Browning et al., 1990, Browning et al., 1994, Hassan et al., 1999, Roguinsky and Serieys, 1977, Williamson et al., 1995). They reported that subjects were allocated randomly, but the description of the randomization process was not detailed. In our descriptive work, these studies were, therefore, classified simply as controlled trials. These studies with no mention of randomization were, however, mostly older studies. Perhaps, at that time, it was not common to mention randomization in the text. Thus, it is unclear whether these studies were truly non-randomized or if the information on randomization was simply lacking in the text. To avoid inappropriate categorization, we did not conduct meta-regression based on reporting or not a randomization.

Meta-regression suggested that the use of teat sealants for quarters/cows not treated with an antimicrobial could explain part of the heterogeneity in the original analysis and reduces the negative impact of SDCT on udder health and milk production in the subsequent lactation. More research would be needed to investigate other factors explaining heterogeneity in the effect estimates.

Another potential limitation was the language restriction, as only articles in English and French were evaluated for inclusion in our review. Thus, we could hypothesize that additional articles would possibly have been included if this restriction was not applied. Also, because of a small number of included trials in each comparison, the potential publication bias could not be thoroughly investigated.

Conclusion

From the available literature, we can conclude that, for low SCC herds (BTSCC < 250,000 cells/mL), SDCT is as efficient as BDCT for curing existing IMI at dry off, preventing new IMI during the dry period, and preventing CM in the beginning of the subsequent lactation if ITS (65% bismuth subnitrate) are used for healthy, untreated quarters/cows. Moreover, milk yield and ln SCC in the beginning of the subsequent lactation would not differ between quarters treated using a selective or a blanket treatment approach. Finally, we can conclude that the use of SDCT would have an important impact on the use of antimicrobials at dry off in dairy cows.

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Author contributions

The first author coordinated the team and was responsible for searching articles from databases and drafting the manuscript. MA was responsible for searching articles from conference proceedings. All authors contributed to the development of the review protocol and selection of

eligible articles. FK, JPR and SD were responsible for extracting data, assessing risk of bias and for analyses. All authors reviewed and provided feedback on the manuscript.

Conflict of interest

None of the authors has conflicts to declare. The funders have no role in the design and conduct of the study, data collection and analysis, or interpretation of the data.

References

Afifi, M., Kabera, F., Stryhn, H., Roy, J. P., Heider, L. C., Godden, S., Montelpare, W., Sanchez, J. & Dufour, S. (2018). Antimicrobial-based dry cow therapy approaches for cure and prevention of intramammary infections: a protocol for a systematic review and meta-analysis. *Anim. Health Res. Rev.*, 19, 74-78.

Bradley, A. J. & Green, M. J. (2000). A Study of the Incidence and Significance of Intramammary Enterobacterial Infections Acquired During the Dry Period. *J. Dairy Sci.*, 83, 1957-1965.

Bradley, A. J. & Green, M. J. (2004). The importance of the nonlactating period in the epidemiology of intramammary infection and strategies for prevention. *Vet. Clin. North Am. Food Anim. Pract.*, 20, 547-568.

Browning, J. W., Mein, G. A., Barton, M., Nicholls, T. J. & Brightling, P. (1990). Effects of Antibiotic-Therapy at Drying Off on Mastitis in the Dry Period and Early Lactation. *Aust. Vet. J.*, 67, 440-442.

Browning, J. W., Mein, G. A., Brightling, P., Nicholls, T. J. & Barton, M. (1994). Strategies for Mastitis Control - Dry Cow Therapy and Culling. *Aust. Vet. J.*, 71, 179-181.

Cameron, M., Keefe, G. P., Roy, J. P., Stryhn, H., Dohoo, I. R. & Mckenna, S. L. (2015). Evaluation of selective dry cow treatment following on-farm culture: Milk yield and somatic cell count in the subsequent lactation. *J. Dairy Sci.*, 98, 2427-2436.

Cameron, M., Mckenna, S. L., Macdonald, K. A., Dohoo, I. R., Roy, J. P. & Keefe, G. P. (2014). Evaluation of selective dry cow treatment following on-farm culture: Risk of postcalving

intramammary infection and clinical mastitis in the subsequent lactation. *J. Dairy Sci.*, 97, 270-284.

Dufour, S., Wellemans, V., Roy, J. P., Lacasse, P., Ordonez-Iturriaga, A. & Francoz, D. (2019). Non-antimicrobial approaches at drying-off for treating and preventing intramammary infections in dairy cows. Part 1. Meta-analyses of efficacy of using an internal teat sealant without a concomitant antimicrobial treatment. *Anim. Health Res. Rev.*, 20, 86-97.

Green, M. J., Green, L. E., Medley, G. F., Schukken, Y. H. & Bradley, A. J. (2002). Influence of Dry Period Bacterial Intramammary Infection on Clinical Mastitis in Dairy Cows. *J. Dairy Sci.*, 85, 2589-2599.

Guyatt, G. H., Oxman, A. D., Kunz, R., Brozek, J., Alonso-Coello, P., Rind, D., Devereaux, P. J., Montori, V. M., Freyschuss, B., Vist, G., Jaeschke, R., Williams, J. W., Jr., Murad, M. H., Sinclair, D., Falck-Ytter, Y., Meerpohl, J., Whittington, C., Thorlund, K., Andrews, J. & Schünemann, H. J. (2011). GRADE guidelines 6. Rating the quality of evidence--imprecision. *J Clin Epidemiol*, 64, 1283-93.

Halasa, T., Nielen, M., Whist, A. C. & Osteras, O. (2009a). Meta-analysis of dry cow management for dairy cattle. Part 2. Cure of existing intramammary infections. *J. Dairy Sci.*, 92, 3150-3157.

Halasa, T., Osteras, O., Hogeveen, H., Van Werven, T. & Nielen, M. (2009b). Meta-analysis of dry cow management for dairy cattle. Part 1. Protection against new intramammary infections. *J. Dairy Sci.*, 92, 3134-3149.

Hassan, Z., Daniel, R. C. W., O'boyle, D. & Frost, A. J. (1999). Effects of dry cow intramammary therapy on quarter infections in the dry period. *Vet. Rec.*, 145, 635-639.

Higgins, J. P. T., Sterne, J., Savović, J., Hróbjartsson, A., Boutron, I., Reeves, B. & Eldridge, S. (2016). A revised tool for assessing risk of bias in randomized trials. In: CHANDLER, J., MCKENZIE, J., BOUTRON, I. & WELCH, V. (eds.) *Cochrane Database Syst. Rev.*; *(10 Suppl 1)*. Oxford: John Wiley & Sons, Ltd., pp. 29–31.

Huxley, J. N., Green, M. J., Green, L. E. & Bradley, A. J. (2002). Evaluation of the efficacy of an internal teat sealer during the dry period. *J. Dairy Sci.*, 85, 551-561.

Kabera, F., Dufour, S., Keefe, G., Cameron, M. & Roy, J. P. (2020). Evaluation of quarter-based selective dry cow therapy using Petrifilm on-farm milk culture: A randomized controlled trial. *J. Dairy Sci.*, 103, 7276-7287.

Knapp, G. & Hartung, J. (2003). Improved tests for a random effects meta-regression with a single covariate. *Stat Med*, 22, 2693-710.

Langan, D., Higgins, J. P. T., Jackson, D., Bowden, J., Veroniki, A. A., Kontopantelis, E., Viechtbauer, W. & Simmonds, M. (2019). A comparison of heterogeneity variance estimators in simulated random-effects meta-analyses. *Res Synth Methods*, 10, 83-98.

Moher, D., Liberati, A., Tetzlaff, J. & Altman, D. G. (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *J Clin Epidemiol.*, 62, 1006-1012.

Neave, F. K., Dodd, F. H., Kingwill, R. G. & Westgarth, D. R. (1969). Control of Mastitis in the Dairy Herd by Hygiene and Management. *J. Dairy Sci.*, 52, 696-707.

Patel, K., Godden, S. M., Royster, E. E., Timmerman, J. A., Crooker, B. A. & Mcdonald, N. (2017). Pilot study: impact of using a culture-guided selective dry cow therapy program targeting quarter-level treatment on udder health and antibiotic use. *Bov. pract.*, 51, 48-57.

Rabiee, A. R. & Lean, I. J. (2013). The effect of internal teat sealant products (Teatseal and Orbeseal) on intramammary infection, clinical mastitis, and somatic cell counts in lactating dairy cows: A meta-analysis. *J. Dairy Sci.*, 96, 6915-6931.

Rindsig, R. B., Rodewald, R. G., Smith, A. R. & Spahr, S. L. (1978). Complete versus selective dry cow therapy for mastitis control. *J. Dairy Sci.*, 61, 1483-1497.

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

Robinson, T. C., Jackson, E. R. & Marr, A. (1983). Within herd comparison of teat dipping and dry cow therapy with only selective dry cow therapy in six herds. *Vet Rec*, 112, 315-9.

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

Roguinsky, M. & Serieys, F. (1977). Treatment of mastitis in dry cows: treatment of all cows vs treatment of infected cows only (author's transl). *Ann. Rech. Vet.*, 8, 327-331.

Rowe, S. M., Godden, S. M., Nydam, D. V., Gorden, P. J., Lago, A., Vasquez, A. K., Royster, E., Timmerman, J. & Thomas, M. J. (2020a). Randomized controlled non-inferiority trial investigating the effect of 2 selective dry-cow therapy protocols on antibiotic use at dry-off and dry period intramammary infection dynamics. *J. Dairy Sci.*.

Rowe, S. M., Godden, S. M., Nydam, D. V., Gorden, P. J., Lago, A., Vasquez, A. K., Royster, E., Timmerman, J. & Thomas, M. J. (2020b). Randomized controlled trial investigating the effect of 2 selective dry-cow therapy protocols on udder health and performance in the subsequent lactation. *J. Dairy Sci.*

Sanford, C. J., Keefe, G. P., Dohoo, I. R., Leslie, K. E., Dingwell, R. T., Descoteaux, L. & Barkema, H. W. (2006). Efficacy of using an internal teat sealer to prevent new intramammary infections in nonlactating dairy cattle. *Javma-Journal of the American Veterinary Medical Association*, 228, 1565-1573.

Seeth, M. T., Wente, N., Paduch, J. H., Klocke, D., Vries, E. M. D., Hoedemaker, M. & Kromker, V. (2017). Different selective dry cow therapy concepts compared to blanket antibiotic dry cow treatment. *Tierarztl Prax Ausg G Grosstiere Nutztiere*, 45, 343-349.

Serieys, F. & Roguinsky, M. (1975). Comparative tests with dry cow therapy for some or all cows in a herd. *Annual Bulletin, International Dairy Federation*, 349-351.

Vanhoudt, A., Van Hees-Huijps, K., Van Knegsel, A. T. M., Sampimon, O. C., Vernooij, J. C. M., Nielen, M. & Van Werven, T. (2018). Effects of reduced intramammary antimicrobial use during the dry period on udder health in Dutch dairy herds. *J. Dairy Sci.*, 101, 3248-3260.

Ward, G. E. & Schultz, L. H. (1974). Incidence and control of mastitis during the dry period. *J. Dairy Sci.*, 57, 1341-1349.

Williamson, J. H., Woolford, M. W. & Day, A. M. (1995). The Prophylactic Effect of a Dry-Cow Antibiotic against Streptococcus-Uberis. *N. Z. Vet. J.*, 43, 228-234.

Winder, C. B., Sargeant, J. M., Kelton, D. F., Leblanc, S. J., Duffield, T. F., Glanville, J., Wood, H., Churchill, K. J., Dunn, J., Bergevin, M. D., Dawkins, K., Meadows, S. & O'connor, A. M. (2019). Comparative efficacy of blanket versus selective dry-cow therapy: a systematic review and pairwise meta-analysis. *Anim. Health Res. Rev.*, 20, 217-228.

Tableau 1. – Characteristics of 13 articles representing 12 trials included in the systematic review comparing selective dry-cow therapy and blanket dry-cow therapy for curing and preventing intramammary infections.

Article	Country	Study design	#[1] herds	# cows	# quarters	Inclusion criteria
Ward and Schultz, 1974	USA	CT[2]	4	402	1600	No criteria
Roguinsky et Serieys, 1977	France	CT	1	40	159	NR[3]
Rindsig et al., 1978	USA	CT	1	232	928	NR
Browning et al., 1990 and 1994	Australia	CT	12	1044	4176	BTSCC[4] 100,000 – 400,000 cells/mL; cow's expected dry period ≥2 months; and < 4 infected quarters at dry-off.
Williamson et al., 1995	New Zealand	CT	4	371	NR	NR
Hassan et al., 1999	Australia	CT	3	150	600	NR
Cameron et al., 2014 and 2015	Canada	RCT[5]	16	603	2287	BTSCC < 250,000 cells/mL; cow's SCC[6] < 200,000 cells/mL on last 3 DHI[7] tests; no CM[8] on the same period; cow's expected dry period 30 to 90 d; cow had no antimicrobial treatment in the last 14 d; all quarters of the cow had CMT[9] < 2 on day prior to drying off.
Patel et al., 2017	USA	RCT	1	56	224	Four functional quarters; no antibiotic or anti-inflammatory medication during the 14-d period prior to dry off; clinically healthy; no signs of CM at enrollment or on the day of dry off; expected dry period 30 to 90 d.
Rowe et al., 2020a, b	USA	RCT	7	1243	5100	Herd size sufficient to dry off ≥15 cows per week; BTSCC < 250,000 cells/mL; record CM, culling, and death events; cow's expected dry period 30 to 90 d; no antibiotic or anti-inflammatory treatment within 14 d; no CM; no lameness (> 3/5) or poor body condition (< 2/5).
Kabera et al., 2020	Canada	RCT	9	569	2142	BTSCC < 250,000 cells/mL; no CM or antimicrobial treatment during 14 d prior to dry off; and cow's expected dry period 35 to 75 d.

[1]Number of units analysed; [2]Controlled Trial (no randomization reported); [3]Not Reported; [4]Herd mean 12-month Bulk Tank Somatic Cell Count; [5]Randomized Controlled Trial; [6]Somatic cell counts; [7]Dairy herd improvement; [8]Clinical mastitis; [9]California mastitis test.

Tableau 2. – Treatment regimens and outcomes studied in 13 articles representing 12 trials included in a systematic review comparing selective dry-cow therapy (SDCT) and blanket dry-cow therapy (BDCT).

Article	SDCT description					Outcomes measured				
	Method for identifying units to treat	Level[1]	Threshold for treatment	Tx[2] if +	Tx[3] if -	% with no ATB[4]	New IMI[5]	Elimination of IMI[6]	IMI[7]	Others in next lactation
Ward et Schultz, 1974	CM[8]	Q	≥1 CM in last month	Neomycin sulfate	No Tx	96.1	Yes	Yes	Yes	CM
Roguinsky & Serieys, 1977	CMT	C	≥1 quarter with CMT[9] ≥ 3 in last month	Cloxacillin or Penicillin and streptomycin (half of cows received each treatment)	No Tx	68.2	Yes	Yes	Yes	None
Rindsig et al., 1978	SCC[10], CMT, and CM	C	Cow SCC >500,000 cells/mL or CMT ≥2 in any quarter or ≥1 CM	Penicillin and Streptomycin	No tx	42.9	Yes	Yes	Yes	SCC
Browning et al., 1990 and 1994	Lab-based milk culture	Q	NR[11]	Benzathine cloxacillin	No Tx	67.5	Yes	Yes	Yes	CM
Williamson et al., 1995	Lab-based milk culture	Q	NR	Cephalonium	No Tx	NR	Yes	No	No	CM
*Hassan et al., 1999	N-acetylf-D-glucosaminidase	Q	High NAGase on a sample taken 24 h before dry-off	Benzathine cloxacillin	No Tx	81.1	No	No	Yes	CM
Cameron et al., 2014 and 2015	Aerobic count Petrifilm®	C	≥50 CFU/mL[12] in composite milk	Ceftiofur hydrochloride and ITS[13]	ITS	45.6	Yes	Yes	Yes	MY[14], CM, SCC
Patel et al., 2017	Minnesota Easy culture system	Q	≥100 CFU/mL in quarter milk	Ceftiofur hydrochloride + ITS	ITS	48.1	Yes	Yes	Yes	CM
Kabera et al., 2020	Aerobic count Petrifilm®	Q	≥50 CFU/mL in quarter milk	Penicillin G Procaine and Novobiocin	ITS	57.4	Yes	Yes	No	MY, CM, SCC
	Aerobic count Petrifilm®	Q	≥50 CFU/mL in quarter milk	Penicillin G Procaine and Novobiocin+ITS	ITS	58.6	Yes	Yes	No	MY, CM, SCC
Rowe et al., 2020a, b	Minnesota Easy® 4Cast® plate	Q	≥100 CFU/mL in quarter milk	Ceftiofur hydrochloride + ITS	ITS	55.5	Yes	Yes	Yes	MY, CM, SCC
	Algorithm (SCC+CM)	C	≥2 CM during lactation or any DHIA[15] test with SCC > 200,000 cells/mL during lactation	Ceftiofur hydrochloride + ITS	ITS	55.2	Yes	Yes	Yes	MY, CM, SCC

[1]Selection for treatment applied at cow (C) or quarter-level (Q); [2]Treatment for infected cow/quarter; [3]Treatment for uninfected cow/quarter; [4]Percentage of antimicrobial use reduction; [5]new intramammary infections during dry period; [6]Elimination of intramammary infections during dry period; [7]Prevalence of intramammary infections at calving; [8]Clinical mastitis history in current lactation; [9]California mastitis test; [10]Somatic cell counts; [11]Not reported; [12]Colony forming units per milliliter; [13]Internal Teat Sealant (65% bismuth subnitrate); [14]Milk yield; [15]Dairy Herd Improvement Association; [*]This study had both a positive and a negative control group.

Tableau 3. – Follow-up period and definitions of intramammary infection (IMI) at dry off and calving, of new IMI (NIMI) and of elimination of IMI (EIMI) during dry period in 13 articles representing 12 trials included in a systematic review comparing selective dry-cow therapy and blanket dry-cow therapy.

Authors/year	IMI	EIMI	NIMI	Follow up period
Ward & Schultz, 1974	(1) A microorganism was isolated from two consecutive samples or (2) a microorganism must be isolated once and a leucocyte count of the foremilk must be above 1,000,000 cells/mL and/or a CMT[1] score of 2 + or 3 +. Samples were taken within 1 week, about 2 weeks and about 4 weeks after calving	A microorganism that was present at dry off was not isolated from any samples taken after calving	Not reported	CM[2]: 30 DIM Milk yield: Not followed SCC[3]: 30-60 DIM
Roguinsky & Serieys, 1977	Isolation of one or more pathogens in the first monthly milk sample after calving	Absence at calving of pathogen isolated at dry off or isolation of a different pathogen	Isolation of a pathogen which was not present at dry off	CM: Not followed Milk yield: Not followed SCC: Not followed
Rindsig et al., 1978	1) a microorganism was isolated from two consecutive samples	A microorganism was eliminated if it has not been	1) a microorganism was isolated in both post-	CM: Not followed

	taken within 1 week and at 2 weeks post-calving, or 2) a microorganism was isolated once and CMT score ≥ +2 or somatic cell number ≥ $1x10^6$cells/mL. Corynebacterium bovis was not considered a pathogen and was excluded in determining infections and rates of infection	isolated from any samples taken after calving	calving samples with no microorganism prior to drying off, or 2) the microorganism post-calving differed from the microorganism prior to drying off	Milk yield: Not followed SCC: 1 – 56 DIM
Browning et al., 1990	Two or three consecutive milk samples contained the same major pathogen (samples taken within 12 h of calving and at the next two consecutive milking)	Not applicable	Infections found in previously uninfected quarters at drying off	CM: 5 months Milk yield: Not followed SCC: Not followed
Browning et al., 1994	Not reported	Not reported	An infection that was identified at calving or during lactation in a quarter that had been uninfected at the previous drying off	CM: 5 months Milk yield: Not followed SCC: Not followed

Williamson et al., 1995	Same organism cultured from both foremilk duplicate samples taken 1–4 days post-calving	Not reported	Not applicable	CM: 8 months Milk yield: Not reported SCC: Not reported
Hassan et al., 1999	Isolation of pathogen on culture of a sample taken at calving. *Corynebacterium bovis* and miscellaneous infections considered to be of minimal importance were excluded	Not applicable	Not applicable	CM: 3 weeks after calving Milk yield: Not followed SCC: Not followed
Cameron et al., 2014	≥100 CFU/mL[4] of milk of any pathogenic organism of interest at either of two samples taken at 3–4 and 5–18 DIM. For NAS[5], a definition of ≥200 CFU/mL was used	A pathogen isolated in the dry off sample was considered eliminated over the dry period if it was absent in both post calving samples (3-4 and 5-18 DIM)	A pathogen was cultured at calving on both samples (3-4 and 5-18 DIM) and that was not present at dry off	CM: 120 DIM. Milk yield: Not followed SCC: Not followed
Cameron et al., 2015	Not applicable	Not applicable	Not applicable	CM: Not followed Milk yield: 180 DIM SCC: 180 DIM

Patel et al., 2017	≥100 CFU/mL of milk of any pathogenic organism of interest, except for NAS and Bacillus spp where spp ≥200 CFU/mL and ≥500 CFU/mL were used, respectively	A pathogen isolated in the dry off sample was considered eliminated over the dry period if it was absent in the post calving sample If a quarter had a mixed infection at dry off (2 pathogens), the absence of both pathogens was required for that quarter to be considered eliminated	The presence of 1 or 2 new pathogens in the post calving sample that were not previously observed in the dry off sample	CM: 30 DIM Milk yield: Not followed SCC: Not followed
Kabera et al., 2020	≥100 CFU/mL of milk of any pathogenic organism of interest	A specific pathogen species found at dry off and absent in the first post-calving sample. If a quarter was infected with two pathogens at drying off, the absence of both pathogens was required at calving	A specific pathogen species not found in the drying off sample and present in the first post calving sample	CM: 1 – 120 DIM Milk yield: 1 – 120 DIM SCC: 1 – 120 DIM

Rowe et al., 2020a	Not applicable	Not applicable	Not applicable	CM: 1 – 120 DIM Milk yield: 1 – 120 DIM SCC: 1 – 120 DIM
Rowe et al., 2020b	≥100 CFU/mL of milk of any pathogenic organism of interest, except for NAS and Bacillus spp where ≥200 CFU/mL and ≥500 CFU/mL were used, respectively	A quarter with a species-level IMI present at enrollment that was not isolated in the post-calving sample	A quarter with a species-level IMI at calving that was not originally present in the enrollment sample	Not applicable

[1]California mastitis test; [2]Clinical mastitis; [3]Somatic cell counts; [4]Colony forming units per milliliter of milk; [5]No aureus Staphylococcus

Tableau 4. – GRADE evidence profile: comparison between selective dry cow therapy (SDCT) and blanket dry cow therapy (BDCT) for curing intramammary infections (IMI) at dry off and preventing new IMI during dry period.

Outcome and comparison	Quality assessment						Number of quarters (for IMI) or cows (for CM)		Relative risk (95% CI) or Mean difference (95% CI)	Quality
	# trials (design)	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	BDCT	SDCT		
IMI incidence										
[a]ITS to healthy Q/C	6 (RCT)	No serious	No serious	No serious	No serious	No serious	855/4,713	884/4,665	1.04 (0.95; 1.13)	+ + + + High
[b]No ITS to healthy Q/C	2 (RCT) 3 (CT)	Very serious	Serious	No serious	No serious	No serious	150/3,483	310/3,467	1.97 (1.52; 2.54)	+ + − − Low
Elimination of IMI	8 (RCT) 2 (CT)	No serious	Serious	No serious	No serious	No serious	1,194/1455	1,170/1,458	0.99 (0.96; 1.02)	+ + + − Moderate
IMI at calving										
ITS to healthy Q/C	4 (RCT)	No serious	No serious	No serious	No serious	No serious	847/4,032	866/4,013	1.02 (0.94; 1.11)	+ + + + High
No ITS to healthy Q/C	3(RCT) 2 (CT)	Very serious	Serious	No serious	No serious	No serious	394/3,638	631/3,617	1.48 (1.19; 1.84)	+ + − − Low

CM incidence	7 (RCT) 1 (CT)	No serious	Very serious	No serious	No serious	No serious	258/4,035	287/3,931	1.03 (0.65; 1.64)	+ + + − Moderate
Milk yield	5 (RCT)	No serious	No serious	No serious	No serious	No serious	NA	NA	-0.24 (-1.17; 0.70)[c]	+ + + + High
ln SCC	5 (RCT)	No serious	No serious	No serious	No serious	No serious	NA	NA	0.03, (-0.09, 0.15)[d]	+ + + + High

CM: clinical mastitis; CI: confidence interval; RCT: randomized control trial; CT: control trial (not randomized or randomization not reported); ln SCC: natural logarithm of somatic cell counts.

[a] where ITS was used for healthy quarters/cows at dry off;

[b] where ITS was not used for healthy quarters/cows at dry off;

[c] mean difference in milk yield (kg/day) or in SCC on natural logarithm scale during the first months of the subsequent lactation;

[d] mean difference in SCC on natural logarithm scale during the first months of the subsequent lactation;

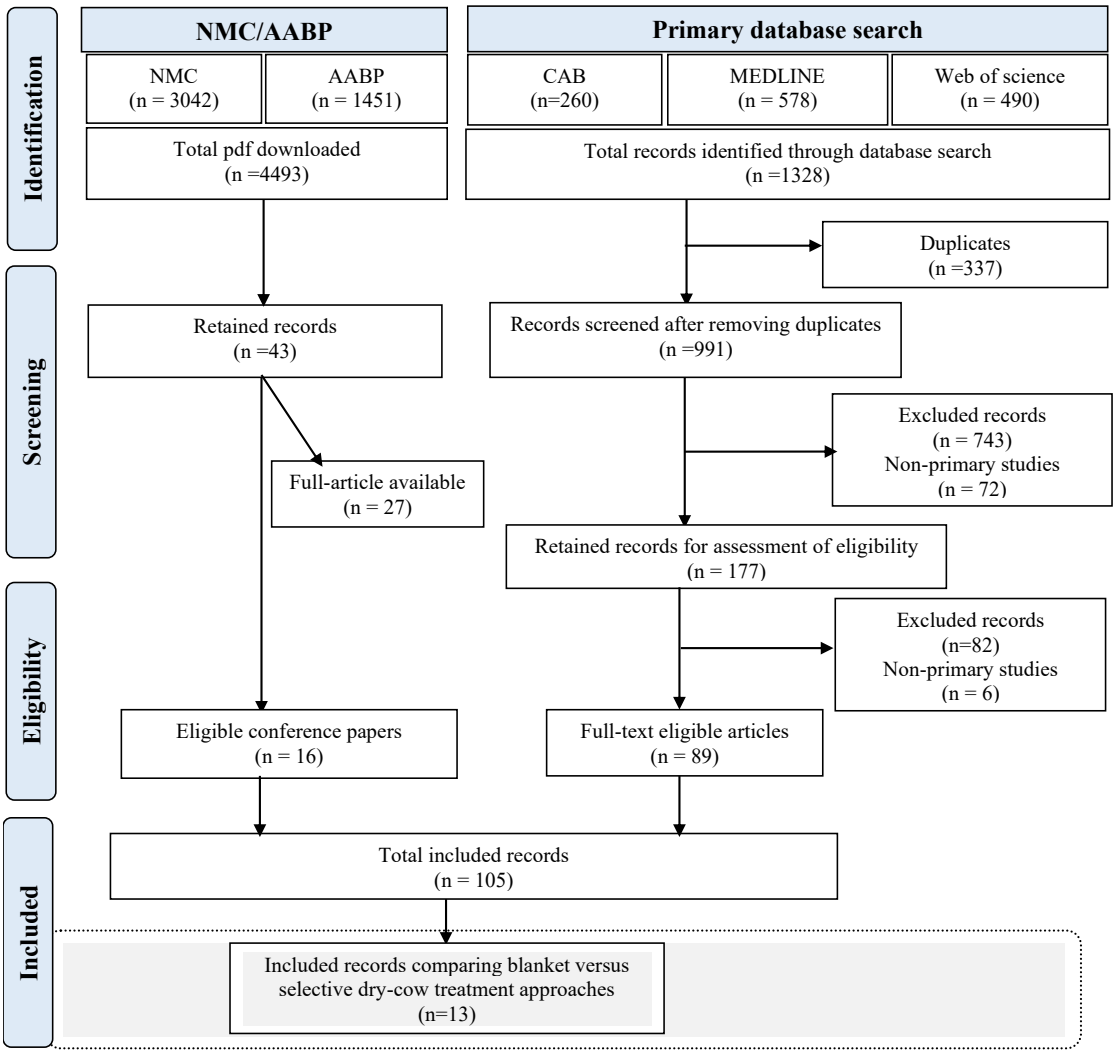


Figure 4. – Result of the different steps for searching and identifying relevant records for the systematic review and meta-analysis on antimicrobial-based dry cow therapy approaches. The search was conducted to answer three research objectives: (1) choice of antimicrobial at drying-off; (2) comparison of blanket versus selective dry cow treatment; and (3) complementing an antimicrobial treatment with a teat sealant. The grey box indicates results specific for objective (2), comparison of blanket versus selective dry cow treatment, the other two objectives will be presented in subsequent independent articles. Screening of references cited by the included articles was also conducted, but did not lead to the addition of eligible articles specific to the comparison of selective and blanket dry cow therapies. This latter part of the search strategy will be presented for the other two objectives in the subsequent associated articles.

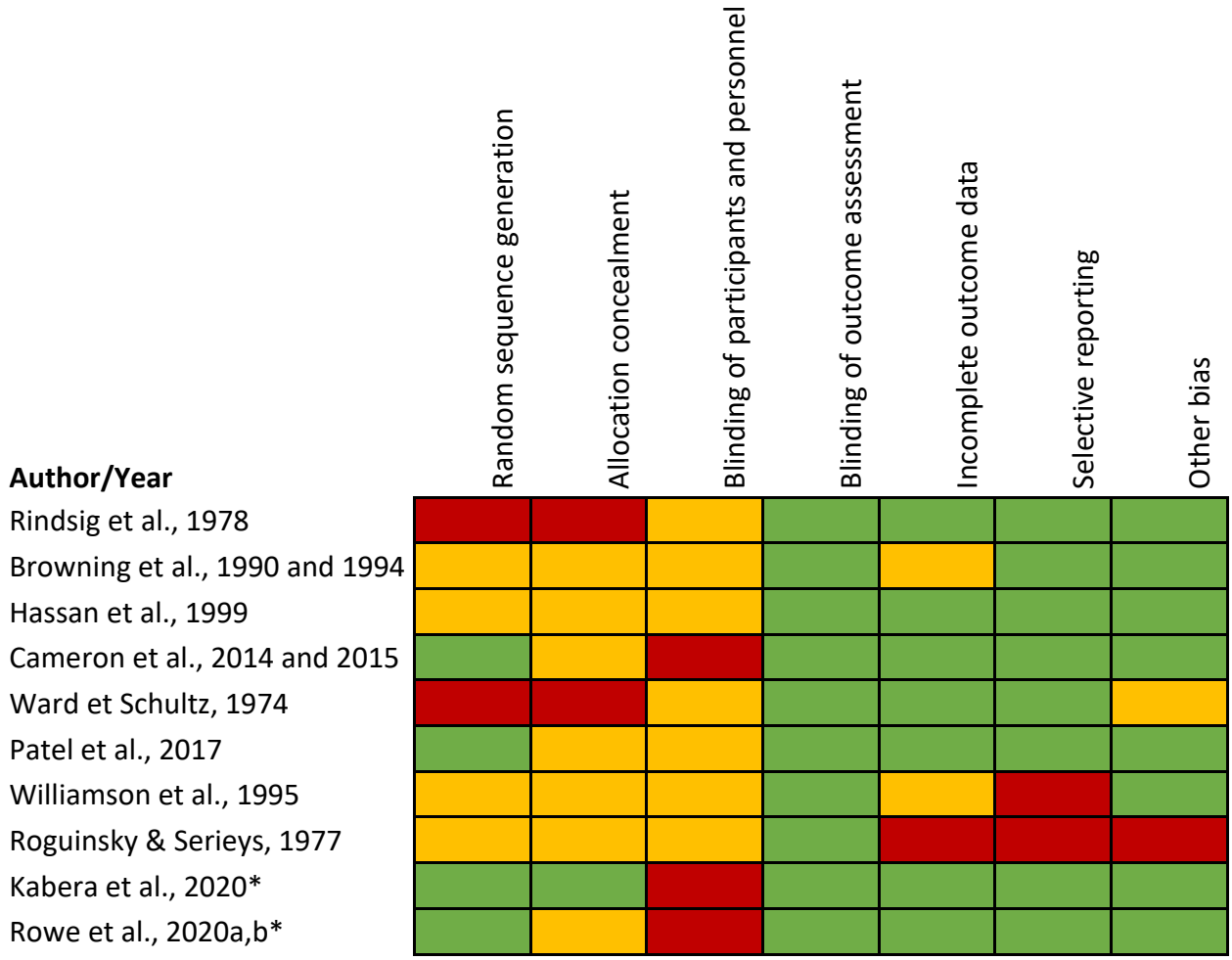


Figure 5. – Risk of bias for 12 trials reported in 13 articles included in a systematic review comparing selective dry-cow therapy and blanket dry-cow therapy for elimination and prevention of intramammary infections.

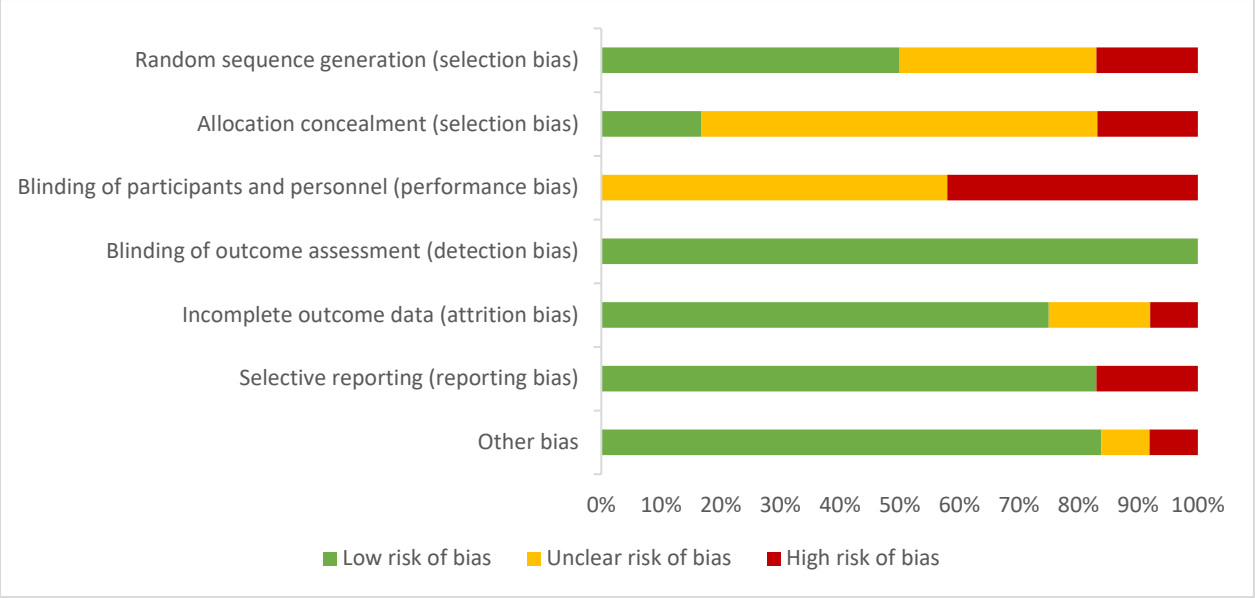


Figure 6. – Proportion of studies with a given risk of bias among 12 trials included in a systematic review comparing selective dry-cow therapy and blanket dry-cow therapy.

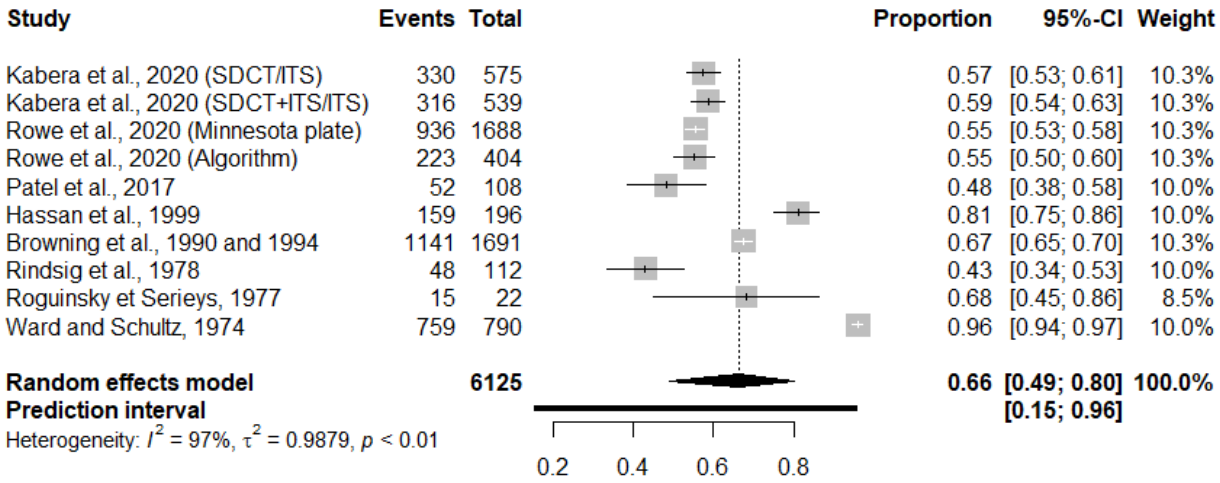


Figure 7. – Forest plots showing the proportion of antimicrobial use reduction.

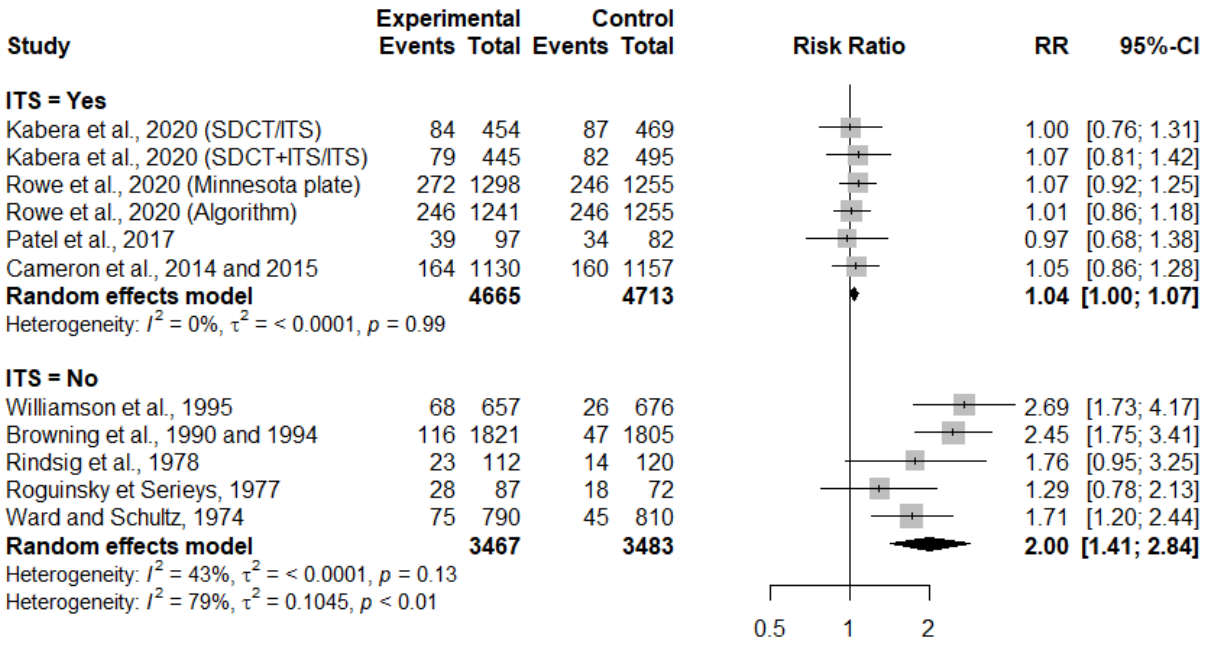


Figure 8. – Forest plots showing the effect of selective dry cow treatment compared to blanket dry cow therapy on risk of acquiring new IMI during dry period, grouped by studies where untreated cows/quarters with antimicrobial received an internal teat sealant (ITS = Yes) and those where they didn't receive an internal teat sealant (ITS = No).

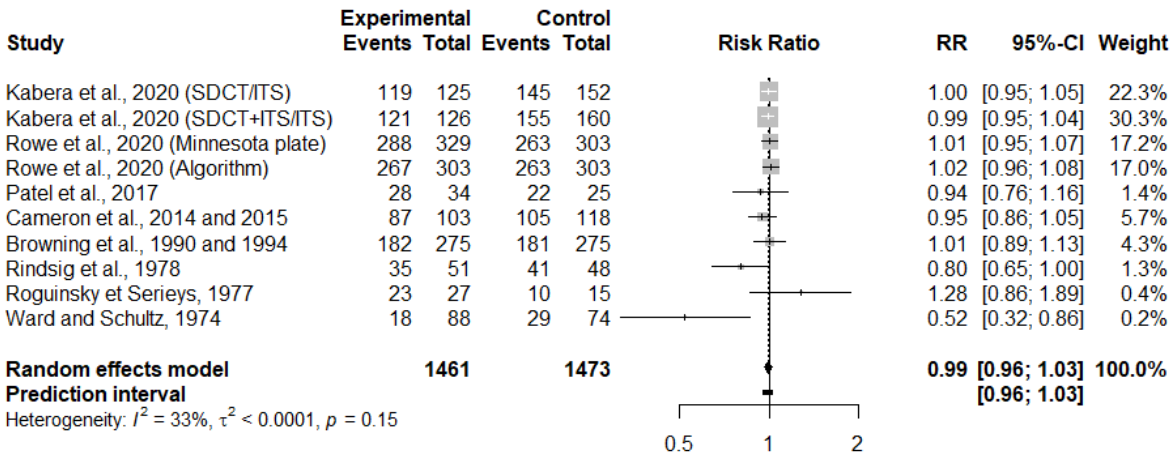


Figure 9. – Forest plots showing the effect of selective dry cow treatment compared to blanket dry cow therapy on risk of IMI elimination during dry period.

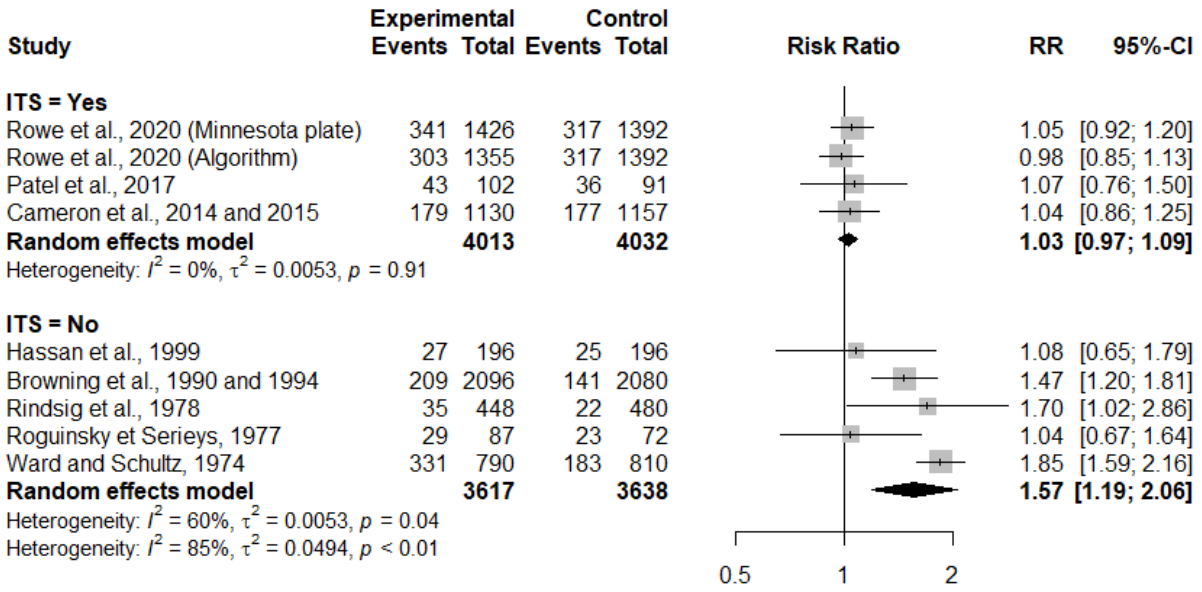


Figure 10. – Forest plots showing the effect of selective dry cow treatment compared to blanket dry cow therapy on risk of IMI prevalence at calving.

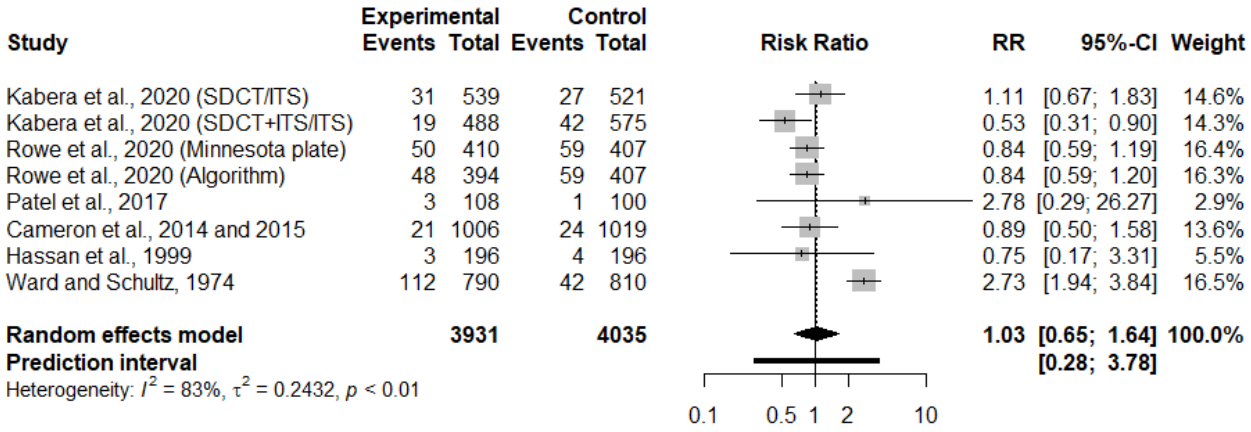


Figure 11. – Forest plots showing the effect of selective dry cow treatment compared to blanket dry cow therapy on risk of acquiring CM during the first four months of lactation.

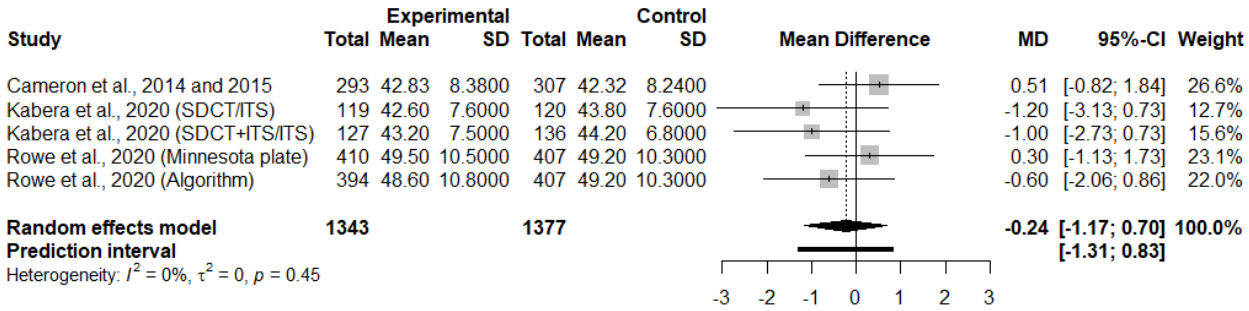


Figure 12. – Forest plot illustrating the mean difference in milk production (kg/day) during the first four months of lactation after a selective dry cow treatment approach, in comparison with a blanket dry cow therapy.

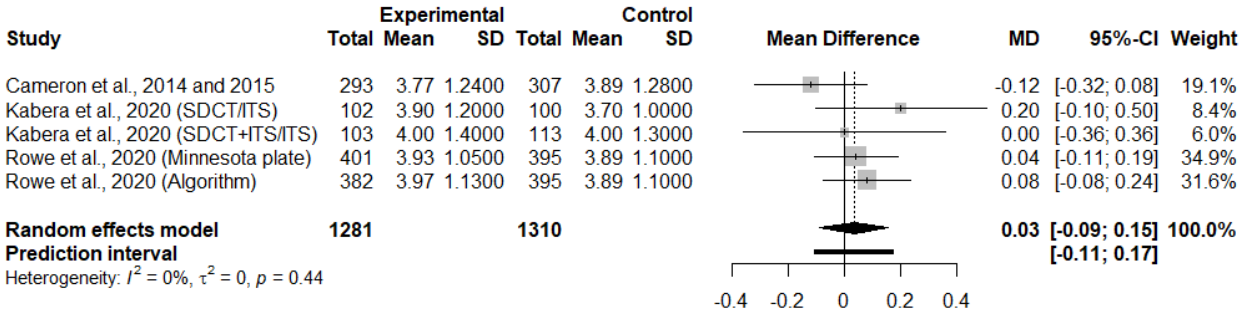
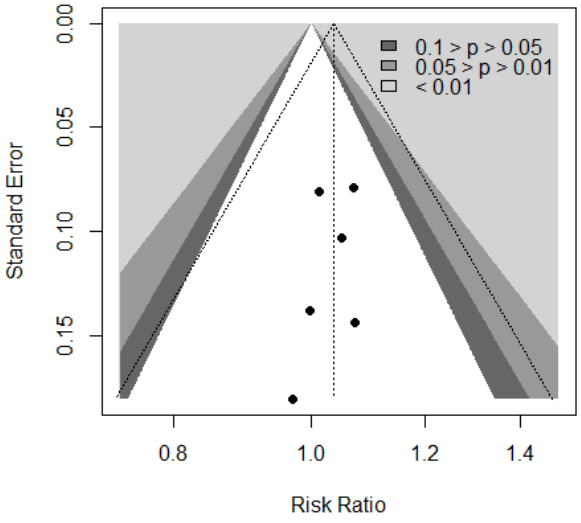
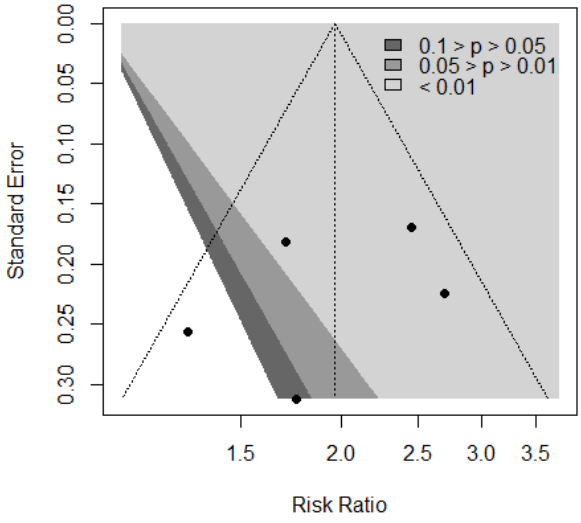


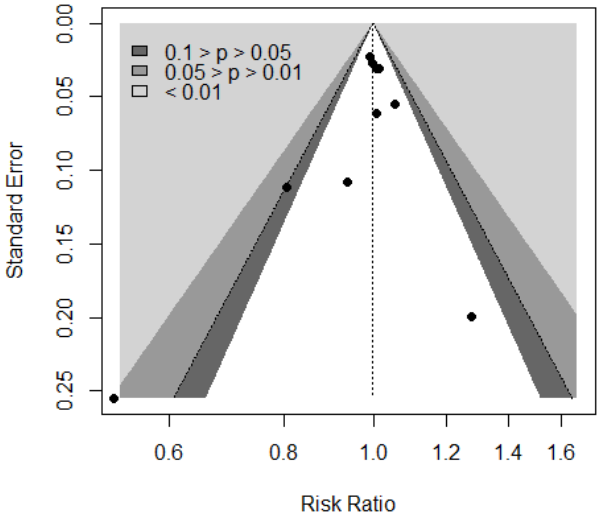
Figure 13. – Forest plot illustrating the mean difference in somatic cells counts (on a natural logarithm scale) during the first four months of lactation after a selective dry cow treatment approach, in comparison with a blanket dry cow therapy.



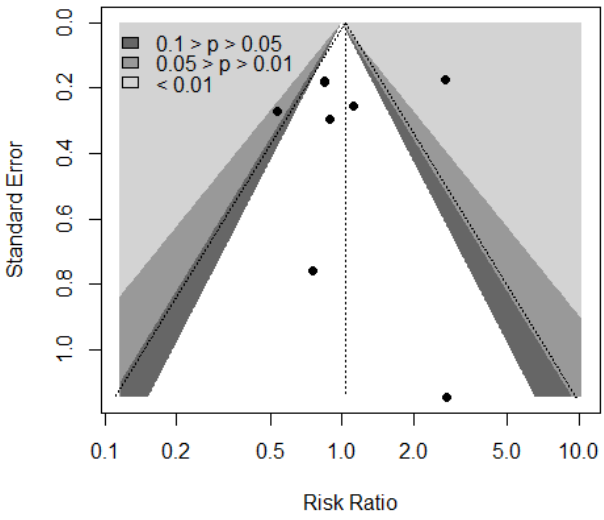
New infections during dry period, ITS



New infections during dry period, no ITS



Elimination of IMI during dry period



Clinical mastitis

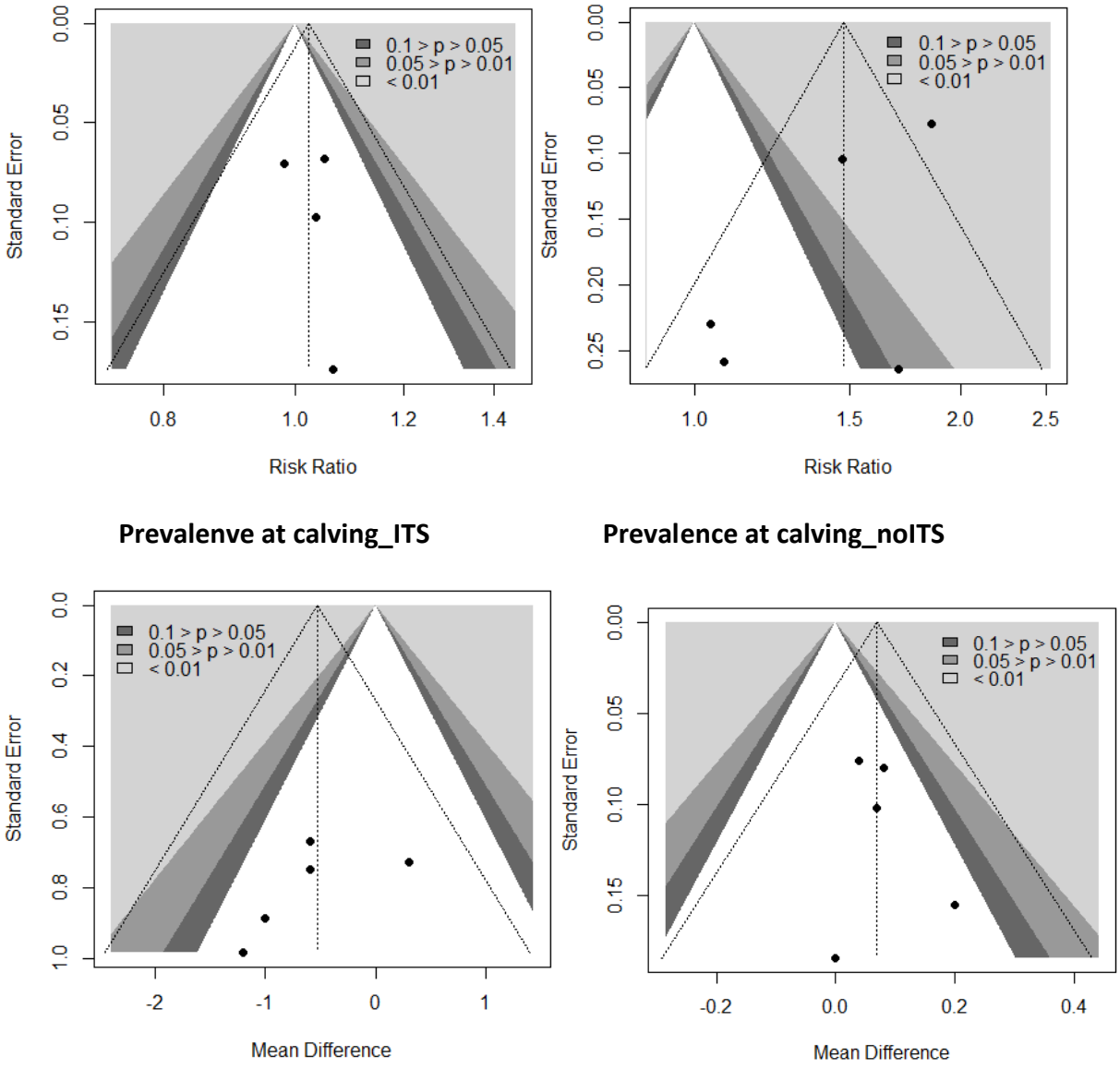


Figure 14. – Contour-enhanced funnel plots illustrating potential publication bias for five outcomes investigating the comparison of selective dry cow therapy and blanket dry cow therapy: 1) IMI incidence during dry period; 2) Elimination of IMI during dry period; 3) Clinical mastitis incidence during the first days of the subsequent lactation; 4) IMI prevalence at calving; 5) Milk yield during the first days of the subsequent lactation; 6) ln SCC during the first days of the subsequent lactation.

Chapitre 5 – Evaluation of a quarter-based selective dry cow therapy using Petrifilm® on-farm milk culture: A randomized controlled trial

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Abstract

The objective of this randomized controlled trial was to assess the efficacy of an on-farm culture system using Petrifilm® (3M, London, ON, Canada) for targeted treatment decisions at the quarter level at dry-off and its effects on dry period intramammary infections (IMI) and udder health and milk production in the subsequent lactation. A total of 569 cows (2,251 quarters) from 9 dairy herds with bulk tank somatic cell count <250,000 cells/mL in Québec, Canada, were systematically enrolled and randomly allocated to 4 groups: 2 quarter-based selective (QSDCT) groups, using results of quarter-milk culture on Petrifilm®, and 2 blanket dry cow therapy (BDCT) groups. The 2 QSDCT groups consisted of (1) antimicrobial to infected quarters and internal teat sealant (ITS) to

healthy quarters (QSDCT/ITS); and (2) antimicrobial and ITS to infected quarters and ITS to healthy quarters (QSDCT+ITS/ITS). The 2 BDCT groups were (1) antimicrobial alone to all quarters (BDCT); and (2) antimicrobial and ITS to all quarters (BDCT+ITS). Quarter milk samples were collected at dry-off and after calving for routine bacteriological culture at the laboratory to monitor IMI; data on milk production, somatic cell count, and clinical mastitis recorded up to 120 d in milk were retrieved from health and DHI records. The probability of avoiding antimicrobial treatment in QSDCT groups was estimated at 51.7% (95% CI: 39.2 – 64.3). There was no significant difference between the 4 treatment groups regarding acquisition of new IMI (15.9, 13.2, 15.8, and 15.1% probability for BDCT, BDCT+ITS, QSDCT/ITS, and QSDCT+ITS/ITS, respectively) or persistence of existing IMI (3.2, 2.1, 3.4, and 2.7% probability, respectively) over the dry period. In the subsequent lactation, there was no difference between groups regarding incidence of clinical mastitis (2.4, 3.7, 2.9, and 1.7% respectively for BDCT, BDCT+ITS, QSDCT/ITS, and QSDCT+ITS/ITS), mean milk somatic cell score (1.7, 2.0, 2.0, and 2.0 respectively), or mean daily milk production (43.8, 44.2, 43.2, and 42.6 kg/d, respectively) during the first 120 d in milk. In conclusion, QSDCT using the Petrifilm® on-farm culture system to detect infected quarters at dry-off is an interesting option to decrease antibiotic use without any negative effects on udder health or milk production in the first 120 d of the subsequent lactation compared with BDCT.

Key words: dry period, selective antibiotic treatment, on-farm culture, mastitis, intramammary infection.

Introduction

Blanket dry cow therapy (BDCT), where all quarters of all cows are treated with an antimicrobial at dry-off, was introduced many years ago (Neave et al., 1969) and is widely used by dairy farmers. This practice is used to increase the elimination of existing IMI at dry-off and prevent the occurrence of new IMI during the dry period. However, with changes in mastitis epidemiology and increasing public health concerns regarding the overuse of antimicrobials, selective dry cow therapy (SDCT) is a potential alternative to BDCT to reduce antimicrobial usage in dairies (Rindsig et al., 1978, Rindsig et al., 1979). When using a SDCT approach, antimicrobial treatment is reserved for cows or quarters known to have or suspected of having an IMI, with uninfected cows

and quarters not receiving antimicrobial treatment. In addition, internal teat sealants (ITS) have been shown to be a very effective nonantimicrobial alternative to prevent new IMI during the dry period (Huxley et al., 2002, Sanford et al., 2006a, Woolford et al., 1998).

The success of a SDCT approach can be measured by the relative reduction in antimicrobial usage at dry off, the absence of a negative effect on IMI incidence and elimination during the dry period, and udder health and production in the subsequent lactation. The economic impact of a SDCT approach is also an important factor, although, in some contexts, it may not be a primary concern. In fact, in some countries, prophylactic use of antimicrobials is prohibited. The success of a SDCT approach will be strongly influenced by the ability to determine correctly the infection status of the quarter or cow so that the appropriate treatment is applied at dry-off (Huxley et al., 2002, Robert et al., 2008, Torres et al., 2008). Since the introduction of SDCT, different methods of selecting cows with an IMI have been reported (Browning et al., 1990, Cameron et al., 2013, Torres et al., 2008), but previous lactation SCC alone or combined with the history of clinical mastitis (CM) is the most commonly used (Scherpenzeel et al., 2014, Torres et al., 2008). However, the accuracy of this diagnostic approach is not perfect, with sensitivity and specificity of approximately 70 and 64%, respectively (Torres et al., 2008). Selective dry cow therapy based on on-farm culture diagnostic methods has been reported (Cameron et al., 2013, Cameron et al., 2015, Cameron et al., 2014), with sensitivity and specificity of 85.2 and 73.2%, respectively, and without any negative effect on udder health during the subsequent lactation.

A SDCT protocol for low-SCC herds, using culture results from Petrifilm® (commercially available dehydrated culture media; 3M Petrifilm®, London, ON, Canada) to differentiate infected from healthy cows has been investigated (Cameron et al., 2013, Cameron et al., 2015, Cameron et al., 2014). This protocol resulted in a reduction of 22% in antimicrobial use at dry-off, with no negative effect on udder health or milk production in the next lactation. However, these authors reported that, based on bacteriological culture in the laboratory, 82.7% of quarters were healthy at dry-off and could have been left untreated with antimicrobials. Therefore, because many cows had only 1 or 2 infected quarters, we hypothesized that selection at the quarter level based on on-farm milk culture may further reduce antimicrobial use associated with the SDCT approach, without any harmful effect on udder health or milk production in the subsequent lactation. The objective

of the current study was to determine the reduction in antimicrobial use and compare dry period IMI elimination and incidence rates and udder health and production in the first 120 d of the subsequent lactation between groups of cows treated using a BDCT protocol compared with a quarter-based SDCT protocol using Petrifilm®.

Materials and methods

A randomized controlled trial was conducted to compare the effect of quarter-based SDCT to BDCT on IMI incidence and elimination during the dry period, and on SCC, CM incidence, and mean daily milk production (in kg/d) during the first 120d of the subsequent lactation. The research protocol was approved by the Animal Ethics Committee of the Université de Montréal (15-Rech-1774). The reporting guidelines for randomized controlled trials for livestock and food safety were used to design the study and report the results (O'Connor et al., 2010, Sargeant et al., 2010).

Participants

A convenience sample of 9 dairy herds in Québec, Canada, was selected. Eligible herds were selected based on proximity to the Faculty of Veterinary Medicine of the Université de Montréal, Québec, Canada, with a maximum distance of 150 km. These herds averaged 92 lactating cows (range of 50 to 200) and a 305-d average milk production of 9,841 kg (range of 9,050 to 11,369 kg). Herd inclusion criteria included (1) an average bulk tank SCC <250,000 cells/mL over the last 12 mo; (2) participation in a DHI program with regular milk testing; (3) willingness to commit to the project protocol; and (4) agreement to set dry-off day at 2-wk intervals to allow the research team to collect milk samples and assist in setting up the on-farm culture on the day before dry-off. From these farms, all cows that entered the dry period between July 2015 and May 2016 were considered for inclusion in the trial. Enrolled cows had no CM or antimicrobial treatment during 14 d before dry-off, an expected dry period of 35 to 75 d, and at least 3 functional quarters. Cows that failed to meet the inclusion criteria were not included in the randomized controlled trial and were treated according to routine farm procedures for dry-off.

Treatment Allocation

Before initiating the study, random numbers between 1 and 4 were generated by an investigator not involved with the enrollment of cows using a random number generator function (RANDBETWEEN function of the Excel 2013 software; Microsoft Corp., Redmond, WA). Sealed and numbered (1 through 600) envelopes were prepared, each containing a random number later used to assign each cow to 1 of the 4 treatment groups. At each farm visit, an animal health technician and the first author allocated enrolled cows into the 4 treatment groups. When more than one cow was to be dried off on a given day, cows were ordered as presented by the herd health software. Thus, the first envelope was assigned to the first cow on the list, the second envelope to the second cow on the list, and so on. Envelopes were opened only after confirmation of eligibility of the cow and before collecting milk samples. Farm staff were not involved in treatment allocation and therefore, they were unable to intentionally select a treatment group or keep a particular cow out of the study.

Interventions

The first and second groups of cows were positive control groups and were treated as follows: (1) intramammary infusion of antimicrobial alone (200,000 IU of Penicillin G Procaine and 400 mg of Novobiocin; Novodry Plus, Zoetis Canada, Kirkland, QC, Canada) to all quarters of all cows (BDCT); and (2) intramammary infusion of the same antimicrobial and an ITS (4 g of an ITS containing 65% wt/wt of bismuth subnitrate; Orbeseal, Zoetis Canada) to all quarters of all cows (BDCT+ITS). The third and fourth groups of cows were the quarter-based SDCT (QSDCT) groups. In these latter groups, healthy and infected quarters were first differentiated using Petrifilm® Aerobic Count plates (3M Petrifilm®) used in an on-farm culture system. Briefly, using an aseptic sampling technique (National Mastitis Council, 2017a), single quarter milk samples were collected and cultured using Petrifilm® Aerobic Count plates on the day before dry-off. Both the milk sample collection and Petrifilm® on-farm culture were performed by research team members. One milliliter of quarter milk was added to 9 mL of sterile water to make a 1:10 dilution. One milliliter of diluted milk was cultured on a Petrifilm® Aerobic Count plate and incubated on-farm at 35°C for 24 h in a TurboFan Hova-Bator (GQF Manufacturing, Savannah, GA). Considering a dilution factor of 1:10, a single colony-forming unit was equivalent to 10 cfu/mL of milk. Culture results

on Petrifilm® were read by the producer on the day of dry-off and quarters were classified as infected if ≥5 colonies (McCarron et al., 2009) were present on the Petrifilm® plate (equivalent to ≥50 cfu/mL of milk). Cows were considered healthy if <5 colonies could be visualized. In the third group (QSDCT/ITS), infected quarters were treated with an intramammary infusion of the previously described antimicrobial, and healthy quarters received only the described ITS. Thus, quarters of a given cow could be treated differently depending on infection status. In the last group (QSDCT+ITS/ITS), infected quarters received an intramammary infusion of the described antimicrobial and ITS, whereas healthy quarters received the ITS only, thus allowing for different treatment for the quarters of the same cow. Allocating cows to these 4 treatment groups allowed for a comparison of QSDCT and BDCT in herds not using ITS and where only an antimicrobial is used (QSDCT/ITS vs. BDCT) and for a comparison of QSDCT and BDCT in herds usually treating all quarters with antimicrobials and an ITS (QSDCT+ITS/ITS vs. BDCT+ITS).

All treatments were applied by farm personnel immediately after the last milking at dry-off, following the procedures recommended by the Canadian Bovine Mastitis and Milk Quality Research Network's factsheet on administration technique for intramammary treatment in dairy cows (CBMQRN, 2010). At the farm level, treatments were recorded to verify accordance with the study protocol. Producers had to complete one form per cow regarding the Petrifilm® results and the treatment administered per quarter. Also, the research team had to verify after enrollment of each cow in the study that they were allocated to the correct group and that the interpretation of the Petrifilms by the producer was done correctly. To achieve this, Petrifilms were scanned using an automated 3M Petrifilm® reader (3M Canada, London, ON, Canada), which provided an automatized colony count. Farm personnel were not blinded to treatment groups, as they were responsible for administering treatments at dry-off.

Outcomes and Predictor

Outcomes

Four outcomes of interest were investigated, with the quarter as the statistical unit: (1) probability of not receiving an antimicrobial for quarters of cows randomized to a QSDCT; (2) probability of development of a new IMI over the dry period (for all groups); (3) probability of

persistence of an existing IMI over the dry period (for all groups); and (4) probability of experiencing ≥1 case of CM during the first 120 DIM of the subsequent lactation (for all groups). Two outcomes of interest were investigated, with the cow as the statistical unit: (1) mean daily milk production (in kg/d) during the first 120 DIM of the subsequent lactation, and (2) mean SCS during the first 120 DIM of the subsequent lactation.

Predictor

The cow's assigned treatment group (BDCT vs. BDCT+ITS vs. QSDCT/ITS vs. QSDCT+ITS/ITS) was the only predictor of interest and was used to describe the effect of QSDCT on udder health and milk production parameters.

Data Collection

One farm discontinued DHI testing during the course of the project. Data on milk production were, therefore, extracted from the farm milking system software (for the herd that discontinued DHI testing during the study), and monthly DHI data were used for the other 8 herds. In our analyses, we used the daily mean milk production (in kg) of the first 16 wk in milk for the farm from which we could get weekly milk production data and the daily mean milk production (in kg) of the first 4 DHI tests following calving for the other farms. Data on SCC during the first 120 DIM (4 milk tests) were extracted from monthly DHI data for all participating farms. Only cows with data from ≥2 milk tests (8 herds) or 8 wk of data (1 herd) were included in the final analysis of the effect of treatment group on milk production and SCS. Somatic cell count measurement (in cell/mL) of each test was converted to SCS using equation [1] (Shook, 1993), and the arithmetic mean of the different SCS measurement was then computed:

$$SCS = \frac{log_e(\frac{SCC}{100{,}000})}{0.6931} + 3 \qquad [1]$$

Milk Samples for Bacteriological Analyses

Using an aseptic sampling technique (National Mastitis Council, 2017a), for all enrolled cows, single quarter milk samples were collected on all quarters on (1) the day before dry-off (S1; collected by research team); (2) d 3 to 4 after calving (S2; collected by farm personnel); (3) d 5 to 18 after calving (S3, collected by research team); and (4) for all CM cases occurring between

calving and 120 DIM (S4, collected by farm personnel). Also, health records (DSAHR, animal health management software, Association des Médecins vétérinaires praticiens du Québec, Saint Hyacinthe, Québec, Canada) were used to obtain information on CM cases that were not reported by farmers on the project documents. The S2 and S4 samples were kept frozen at −20°C at the farm until the next farm visit (occurring every other week). Milk samples collected by the research team were placed on ice and transported to the laboratory at the Faculty of Veterinary Medicine of the Université de Montréal. They were frozen at −20°C before monthly shipment to the Maritime Quality Milk research laboratory at the University of Prince Edward Island, where culture and bacterial identification were conducted. Laboratory personnel were blinded to treatment allocation when conducting bacteriological analyses.

Laboratory Bacteriological Culture and Identification of Pathogens

Milk Bacteriological Culture

Milk samples were thawed and cultured in the Maritime Quality Milk research laboratory using standardized methods outlined in the Laboratory Handbook on Bovine Mastitis (National Mastitis Council, 2017b). Briefly, disposable plastic loops were used to streak 0.01 mL of milk on bi-plates containing half Columbia agar + 5% sheep blood and half MacConkey agar. Plates were incubated at 35°C and examined for bacterial growth after 24 and 48 h. Colonies were tentatively identified as staphylococci, streptococci, coliforms, or other pathogens based on colony growth characteristics, morphology, pattern of hemolysis, catalase reaction, and Gram stain. For each positive sample, the number of colony-forming units per 0.01 mL of milk was enumerated up to a maximum of 10 colonies. We attempted to identify all phenotypically distinct microorganisms recovered on a plate, regardless of the number of colonies. However, samples with ≥3 phenotypically different colony types were classified as contaminated and, in that case, bacterial identification was not attempted. Nevertheless, if a contaminated sample had one or more hemolytic colonies (suspected to be Staphylococcus aureus), the hemolytic colonies were enumerated and analyzed further. If Staph. aureus was isolated in a contaminated sample, the quarter was considered infected (vs. contaminated). Yeast and Prototheca spp. were recorded based on Gram stain results. Colonies of bacteria were subcultured individually on blood agar

plates to obtain pure cultures for further classification using matrixassisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Description of MALDI-TOF MS analyses

Final isolate identification was carried out using the direct transfer method (MALDI Biotyper 3.1 User Manual, Bruker Daltonics Inc., Billerica, MA). Briefly, a single-use, 15-cm sterile wooden applicator stick was used to lift material from a well-isolated bacterial colony followed by smearing a thin film of colony material onto a ground steel MSP 96-spot target (Bruker Daltonics Inc.). The spots were allowed to air dry at room temperature. Subsequently, the spots were overlaid with 1.0 µL of a saturated solution of α-cyano-4-hydroxycinnamic acid matrix in 50% acetonitrile, 47.5% water, and 2.5% trifluoroacetic acid (Sigma-Aldrich Canada Inc., Oakville, ON, Canada) using single-use pipette tips and air-dried at room temperature.

All bacterial spectral captures and classifications were carried out using Bruker Daltonics Research Use Only microbial classification platform that included a Microflex LT mass spectrometer, flexControl software (version 3.4), MALDI Biotyper Real-Time Classification, Offline Classification (version 3.1) with a 5,627 Main Spectrum reference database library (MBT-BDAL-5627) and a custom non-aureus staphylococci library developed by Cameron et al. (2017).

After the acquisition of a sample's mass spectrum, the Biotyper software compares the spectrum to the reference spectra contained in the database. The software then displays matching identifications and computes a score ranging from 0.0 to 3.0, indicating the degree of similarity between the sample's spectrum and the reference spectrum. Except for non-aureus staphylococci species, identification scores were interpreted as per manufacturer’s recommendations as follows: a score of 2.0 to 3.0 was deemed as acceptable for species-level identification, a score of 1.7 to < 2.0 indicated confident identification to the genus level, and a score of <1.7 was considered a non-reliable identification. A cut-off score ≥1.7 was considered as a reliable threshold for the bacterial identification of staphylococci at species-level according to previous studies (Cameron et al., 2017, Cameron et al., 2018, Mahmmod et al., 2018).

Definition of IMI, New IMI, and Persistence of IMI Over the Dry Period

The presence of ≥1 cfu/0.01 mL of milk of any bacteria was considered sufficient to qualify a quarter as having an IMI (Dohoo et al., 2011). If an IMI by a specific pathogen species was present in the first postcalving sample (S2) and if that same pathogen species was not found in the drying-off sample (S1), then the quarter was considered to have experienced a new IMI during the dry period.

Because ITS are used mainly to prevent new IMI by environmental bacteria (IMIenv), we also investigated specifically new IMI by these types of pathogens (new IMIenv), by creating a second new IMI definition, which further excluded new IMI by contagious mastitis pathogens (mainly Corynebacterium bovis, Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma bovis, or other Mycoplasma species). All enrolled quarters, irrespective of their dry-off IMI status, were considered at risk of acquiring a new IMI or new IMIenv.

In contrast with new infections, only infected quarters at dry-off were considered at risk of IMI persistence over the dry period. A persistent IMI over the dry period was considered if a specific pathogen species found at dry-off (S1) was found in the first postcalving sample (S2). If a quarter was infected with 2 pathogens at S1, the presence of any of the 2 pathogens in S2 was interpreted as a persistent IMI.

Finally, to investigate the effect of using a single postcalving sample (S2) to estimate new IMI, new IMIenv, and persisting IMI, these outcomes were all also investigated, but using case-definitions where both first (S2) and second (S3) postcalving samples were considered and using parallel interpretation of the 2 postcalving samples (i.e., ≥1 positive test is interpreted as presence of an IMI).

Missing IMI Status

The information on new IMI or new IMIenv or IMI persistence was, therefore, missing, if (1) sample S1 was contaminated; or (2) if sample S2 was missing or contaminated. Moreover, the new IMI, new IMIenv, and IMI persistence statuses of a quarter over the dry period were considered undetermined if a bacteria could not be identified at the species level, such as

Staphylococcus spp. or Corynebacterium spp., at either or both dry-off and postcalving time points.

Statistical Analyses

The primary unit of statistical analyses was the quarter. Descriptive statistics of the different outcomes and the main predictor were first explored; then, univariable analysis of the effect of treatment group was carried out for the different outcomes. First, to estimate the reduction in antimicrobial use resulting from the QSDCT, we computed the probability of being treated with antimicrobials in quarters allocated to the 2 QSDCT groups. To achieve this, we used a generalized linear mixed regression model with a logit link and using adaptive Gauss-Hermite quadrature for estimation in Stata/IC 11.0 (StataCorp, College Station, TX). Joe (2008) evaluated different estimation procedures (e.g., Gauss-Hermite, Laplace) for generalized mixed model and reported that Gauss-Hermite quadrature was the most accurate. In that model, the outcome was simply having received an antimicrobial or not at drying-off. The model contained only an intercept and no fixed predictors, and cow and herd random intercepts were included. Then, we estimated the effect of treatment allocation on the following dichotomous outcomes: (1) odds of new IMI or new IMIenv over the dry period; (2) odds of IMI persistence over the dry period; and (3) odds of having CM between calving and 120 DIM. Generalized linear mixed models (one for each of the 4 described outcomes) with a logit link and cow and herd random intercepts were used. In these models, the sole fixed predictor was treatment group. The odds of being treated with an antimicrobial, of new IMI/new IMIenv or IMI persistence or having CM between calving and 120 DIM were then converted into population-average estimates and further into a probability using the invert logit function using the Stata margin command.

A supplementary analysis was conducted to investigate the comparison of blanket treatment, in general (BDCT and BDCT+ITS combined) versus selective treatment in general (QSDCT/ITS and QSDCT+ITS/ITS combined). Also, we investigated the effect of treatment group on mean SCS and mean daily milk production at 120 DIM. For these quantitative outcomes, generalized linear mixed models with cow and herd random intercepts were used, with treatment group as the sole

fixed predictor. These models were estimated using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC).

For all models, the significance threshold was set at 0.05. A priori adjustments for multiple comparisons were considered using the Bonferroni method (results are not presented, as no significant differences were found between groups). Only subjects with complete information on all variables in the final model were included in the analyses (i.e., complete case analysis). For continuous outcomes, homoscedasticity and normality of residuals were evaluated by examination of residual plots.

The results obtained by the automated 3M Petrifilm® reader were used to evaluate the ability of producers to correctly identify infected quarters based on on-farm culture results. The level of agreement between the producer and the automated reader results was assessed using McNemar's test for paired data, followed by calculation of the kappa statistic.

Sample Size Estimation

Sample size calculations were conducted a priori using the POWER procedure of the SAS 9.4 software (SAS Institute Inc.). Assuming a risk of new IMI =0.31 per quarter for a dry period >45 d (Dufour and Dohoo, 2013), a type I error rate of 0.05, and a power of 90% to detect an odds ratio of 1.5, we estimated that a sample size of 140 cows (560 quarters) per treatment group was required. Stated differently, with this sample size, we would have 90% power to differentiate statistically a new IMI incidence of 0.31 (174/560) from one of 0.40 (226/560) IMI/quarter per dry period. Because follow-up losses, possible contamination of samples, and clustering of quarters in cow and cows in herds were ignored for those calculations, the true power was more likely to be less than 90% but still close to 80%. The randomization and treatment were done at the cow level but the unit for statistical analyses was the quarter.

Results

Cow Enrollment and Descriptive Statistics: Participants

Nine dairy farms with primarily Holstein cows were selected in Québec, Canada. In total, 569 cows (2,251 quarters) were recruited at dry-off between July 2015 and May 2016 (mean=63 cows/herd; range: 26–165 cows/herd) and were randomly allocated in the 4 treatment groups (average of 142 cows per group, range=133 to 153). Twenty-five quarters were not functional at drying-off and consequently did not receive any treatments. Figure 15 describes the recruitment and follow up of cows and quarters throughout the randomized controlled trial. The number of quarters as a function of group allocation, treatment administered at dry-off, and IMI status at dry-off, with descriptive statistics on daily milk production (in kg/d) and SCS of last DHI test before dry-off and during the first 120 DIM of the subsequent lactation are described in Table 5. Briefly, the 4 treatment groups did not differ at enrollment regarding IMI prevalence at dry-off (P=0.44), last DHI test SCS (P=0.63), or last DHI test daily milk production (P=0.28). On the pre-dry sample, 330/575 and 316/539 quarters were negative on Petrifilm® in the QSDCT/ITS and QSDCT+ITS/ITS groups, respectively, and therefore only received an ITS at drying-off. Thus, 646/1,114 (58%) of QSDCT quarters were not treated with antimicrobials. Prediction from the generalized linear mixed model indicated that 51.7% (95% CI: 39.2 – 64.3) of quarters would be left untreated when using QSDCT. Almost perfect agreement between Petrifilm® results obtained by the producer and those obtained by the automated Petrifilm® reader was observed, with a kappa value of 0.89 (95% CI: 0.86 – 0.92).

A total of 119 quarters had a CM between calving and 120 DIM, but farm personnel only collected milk samples before antimicrobial treatment for 66 of these. Out of the 66 samples collected, 37 (56.1%) yielded no growth. Pathogens retrieved from CM cases are presented in Table 6. Briefly, among the 29 quarters for which bacteria were isolated, the most frequent pathogens were Staphylococcus aureus (n=6/30 isolates), followed by Escherichia coli (n=3/30), Staphylococcus haemolyticus (n=3/30), and Streptococcus dysgalactiae (n=3/30).

Data on milk production following calving were missing for 66 cows (35 from a farm that discontinued DHI testing during the course of the study). Thus, the final analysis of the effect of

treatment on milk production during the subsequent 120 DIM included 502 cows. One farm discontinued regular milk control during the project; thus, we failed to obtain SCC data for all cows. Data on SCS during the first 120 DIM were missing for 170 cows (including 138 cows from the farm that discontinued DHI testing during the course of the study). Thus, the final analysis of SCS included data from 398 cows.

Outcomes and Estimations

New IMI, Persistence of IMI, and CM in First 120 DIM of Next Lactation

Results from the multilevel logistic regression models indicated that treatment group was not significantly associated with new IMI (P=0.74), new IMIenv (P=0.78), IMI persistence over the dry period (P=0.86), or occurrence of CM during the first 120 d of the next lactation (P=0.18). Model-estimated new IMI and new IMIenv cumulative incidences, IMI persistence prevalence, and CM cumulative incidence are reported in Table 7. Pathogens retrieved at dry-off and from dry period new IMI or persistent IMI for different treatment groups are presented in Table 8.

When collapsing all cows into 2 treatment groups (blanket vs. selective), again we did not observe significant differences for new IMI (P=0.63), new IMIenv (P=0.64), IMI persistence over the dry period (P=0.72), or occurrence of CM during the first 120 d of the next lactation (P=0.25). Finally, conclusions of the study regarding IMI results were not affected whether only the first (S2) or both (S2 and S3) postcalving samples were considered in the case definition.

Mean Daily Milk Production in the Subsequent 120 DIM

The distribution of mean daily 120 DIM milk production per treatment group is illustrated in Table 5. We did not observe a significant effect of the treatment group on milk production during 120 DIM after calving (P=0.35). Similarly, no significant effect (P=0.20) was observed when we compared the group of cows selectively treated and those that received blanket dry cow therapy (selective vs. blanket).

Somatic Cell Score

The distribution of mean 120 DIM SCS per treatment group is illustrated in Table 5. No significant effect of treatment was observed on SCS during 120 DIM after calving when considering each

treatment group individually (P=0.74) or cows selectively treated versus blanket dry cow therapy (P=0.57).

Discussion

Reduction in Antimicrobial Treatment

The current study showed that it is possible to achieve substantial reductions in the use of antimicrobials, with comparable udder health and milk production indicators, when using a QSDCT approach with an ITS to protect untreated quarters compared with a BDCT approach. The application of the Petrifilm® on-farm culture system at the quarter level resulted in a greater observed reduction of unnecessary antimicrobial use (58%) compared with the reduction of 22% that was achieved when applied at the cow level (Cameron et al., 2014). With a QSDCT, if only 1 or 2 quarters are infected, only those are treated. However, previous studies reported on the interdependence of quarters within a cow for the acquisition of new IMI during the dry period, supporting the application of antimicrobials to all quarters of a cow (Berry et al., 2003, Browning et al., 1990, Robert et al., 2006a). However, in these earlier studies, an ITS was not used to protect uninfected quarters. In fact, other studies reported that, regarding the acquisition of new IMI during dry-off, interdependence of quarters within a cow may be reduced when efficient prevention methods are applied to reduce the risk of new IMI (Berry et al., 2003, Robert et al., 2006a).

A substantial reduction in the use of antimicrobials (50%) has been reported by Scherpenzeel et al. (2014) with a SDCT program in low-SCC cows. However, they reported a higher incidence of new IMI during the dry period and CM during the first days (up to 100 DIM) of the next lactation.

In the current study, producers were able to accurately read the Petrifilm®, given that the level of agreement between them and the automated reader of Petrifilm® results was almost perfect (Landis and Koch, 1977). The interpretation of the Petrifilm® Aerobic Count plate is facilitated by the presence of a bright pink color indicative of the presence of colonies.

Prevalence of IMI at Dry-Off

The prevalence of IMI at dry-off reported in this study was similar to that reported by Godden et al. (2003) and Torres et al. (2008), but greater than the prevalence reported in other studies (Arruda et al., 2013, Cameron et al., 2014, Pantoja et al., 2009). Differences may be explained by differences in the definition of IMI or methodology among studies (e.g., selection criteria of herds or cows) or management within herds. For instance, a prevalence of 12.8% was reported by Pantoja et al. (2009) when IMI was defined as the presence of ≥3 colonies in 0.01 mL of milk for any given pathogen.

The study by Cameron et al. (2014) considered, in addition to low bulk tank SCC herds, low SCC at the cow level (SCC <200,000 cells/mL on the last 3 milk tests before drying off). Concerning SCC, we did not set a criterion when selecting cows to include in the current study. Thus, the potential inclusion of high-SCC cows in this study may explain this difference in the prevalence of infections at dry-off compared with that reported by Cameron et al. (2014). In fact, a proportion of 37% of high-SCC cows (i.e., cows with a monthly SCC >200,000 cells/mL for 3 last milk tests before dry-off) was reported by Cameron et al. (2014) for herds with a bulk tank SCC <250,000 cells/mL.

Effect of QSDCT Using Petrifilm® On-Farm Culture

The current study found no effect of treatment on risk for the development of new IMI or new IMIenv during the dry period or the persistence of IMI over the dry period or CM in early lactation up to 120 DIM or on SCC and average milk production during the subsequent lactation up to 120 DIM. Our results are in accordance with Cameron et al. (2014) and Cameron et al. (2015), who reported comparable results of SDCT and BDCT when selection was made at the cow level. Similarly, Vasquez et al. (2018) and Rajala-Schultz et al. (2011) could not highlight any significant negative effect on udder health between BDCT and SDCT when using culture-independent selection criteria (DHI SCC data, CM history) to identify cows to be treated in the SDCT group.

In contrast to the current study, other studies on SDCT reported a higher risk of new IMI incidence or CM or an elevated SCC in selectively dry-treated cows compared with groups of cows receiving BDCT (Berry and Hillerton, 2002b, Rindsig et al., 1978, Scherpenzeel et al., 2014). However, in those earlier studies, cows not receiving antimicrobials were left untreated and without an ITS.

Consequently, they were not protected from new IMI during the dry period. The efficacy of ITS in the prevention of new IMI over the dry period has been reported previously (Dufour et al., 2019, Huxley et al., 2002, Woolford et al., 1998). Regarding SCC in early lactation, other studies reported no difference between ITS and antimicrobial treatment when they were used in cows with a low SCC before drying off (Green et al., 2008, Sanford et al., 2006a).

Previous studies (Cook et al., 2005, Huxley et al., 2002, Woolford et al., 1998) reported that the additional protective effect of ITS, when used in conjunction with an antimicrobial, was not demonstrated in low-SCC cows. However, this combination has been recommended in high-SCC herds where cows are at high risk of new IMI during the dry period (Cook et al., 2005). A significant effect was also reported by Godden et al. (2003) and Mütze et al. (2012). In contrast, the current study did not show a significant difference between the use of an antimicrobial alone and its combination with an ITS at dry-off. This difference may be explained by the level of SCC of selected cows or herds, or by the length of the dry period. We may expect to detect a greater benefit of a combination of antimicrobial and ITS with longer dry periods. In fact, Berry and Hillerton (2007) reported a significantly lower incidence of new IMI during the dry period for cows receiving the combination treatment compared with antimicrobial alone, but the difference was not significant when the dry period was <10 wk.

Post Hoc Power Estimation

Post hoc power calculations were conducted using the observed risk of new IMI (0.15 new IMI/quarter), and the approximate number of available observations (around 450 observations per group). With these numbers, the current study had 90% power to detect an odds ratio ≥1.7 (or ≤0.59). Stated differently, with this sample size, we had 90% power to differentiate statistically a new IMI incidence of 0.15 (69/450) from one of 0.23 (104/450) IMI/quarter.

Limitations of the Study

One farm discontinued DHI testing over the course of the project and as a result, we were unable to obtain SCC data for the remaining study period. However, when statistical analyses for the effect of treatment group on SCC were performed both with and without the herd with missing data, the results and conclusions did not change substantially. After excluding that herd, we

observed mean SCS of 1.7, 2.0, 1.9, and 1.9 for the BDCT, BDCT+ITS, QSDCT/ITS, and QSDCT+ITS/ITS groups, respectively. For the effect of treatment group on milk production during the first 120 d of the subsequent lactation, a mean daily production over 17 wk (1 farm without monthly DHI testing) and a mean of 4 monthly test days (8 farms) were used in our analyses. We assumed that 17 wk is roughly equivalent to the 4 monthly test days. Moreover, because cows were randomly assigned to the 4 groups within each herd, the slight measurement difference between 17-wk measures and 4-mo measures should be balanced across groups.

In the current study, we were able to follow most of the typical guidelines recommended for randomized control trials. However, producers could not be blinded to treatment group assignment. The fact that producers were not blinded to treatment groups possibly affected follow-up of quarters or cows. For instance, it is possible that producers did follow more closely quarters or cows that did not receive an antimicrobial at dry-off, especially if they were expecting a detrimental health event in these animals. To ensure equal reporting in all 4 groups, at every visit on the farms, the research team asked dairy producers to make sure that all CM events were recorded. The other outcomes measured in this study were all objectives outcomes and, thus, the values reported would not be affected by the absence of blinding. Nevertheless, it is also possible that our dairy producers altered the level of care of their cows according to treatment group.

In our study, an IMI was defined by the presence of ≥1 cfu/0.01 mL of milk of any bacteria, as our objective was to identify as many infections as possible (Dohoo et al., 2011). This IMI definition was suggested to have high sensitivity and almost perfect specificity for most pathogens (Dohoo et al., 2011). Using ≥1 cfu/0.01 mL as a case definition, however, would result in a lower specificity than a ≥2 cfu/0.01 mL definition for some pathogens, such as the non-aureus staphylococci, but would yield substantially higher sensitivity (Dohoo et al., 2011). Nevertheless, for these latter pathogens, Haine et al. (2018) highlighted that, in a cohort study using the ≥1 cfu/0.01 mL case definition, the resulting bias would be toward the null value, and that using IMI definitions that are less sensitive and more specific (such as the ≥2 cfu/0.01 mL definition) would do very little to mitigate this bias when measuring association with an exposure (such as in the current study). Note that *Streptococcus agalactiae* and *Mycoplasma* species were not recovered in the current study. Because samples were frozen for, on average, 1 mo before culture, and because of the

culture techniques used, we were not able to isolate *Mycoplasma* species. On the other hand, *Streptococcus agalactiae*, *Mycoplasma bovis*, and other *Mycoplasma* species are relatively uncommon in Canada (Bauman et al., 2018, Francoz et al., 2012, Olde Riekerink et al., 2006). Freezing may also have affected recovery of other pathogens, such as *E. coli* (Schukken et al., 1989). Another limitation of the current study was the absence of molecular characterization of our bacterial isolates to ensure that isolates recovered at dry-off and calving were similar, when estimating persistence of IMI across the dry period.

At the end of the project, several producers were interested in continuing to apply the selective dry cow treatment by themselves. However, long-term follow up to monitor implementation was not performed. Nevertheless, 5 herds were clients of the bovine ambulatory clinic of the Faculty of Veterinary Medicine of the Université de Montréal and were therefore visited regularly after the end of the study. Of these 5, 4 continued selective dry cow therapy after the end of the project. However, 3 yr later, only 1 producer is still doing it. Two producers stopped after 1 yr due to lack of time (increase herd size, built new facilities, not enough staff) and one producer stopped after 2 yr because the employee in charge of this procedure left the business. Selective dry cow therapy requires more time and a certain expertise to identify cows or quarters to be treated compared with blanket dry cow therapy. Moreover, performing milk culture may be a challenge for some dairy producers. Therefore, selective dry cow therapy based on milk culture is easier to implement in herds with a good level of management and motivated personnel.

Conclusion

A very substantial reduction in antimicrobial use was achieved using a QSDCT program relying on Petrifilm® milk culture results, without any negative effects on udder health or milk production during the subsequent lactation. Compared with BDCT, a reduction in antimicrobial use of 58% was achieved.

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References

Arruda, A. G., S. Godden, P. Rapnicki, P. Gorden, L. Timms, S. S. Aly, T. W. Lehenbauer, and J. Champagne. 2013. Randomized noninferiority clinical trial evaluating 3 commercial dry cow mastitis preparations: I. Quarter-level outcomes. *J. Dairy Sci.* 96:4419–4435. https: / / doi .org/ 10 .3168/ jds .2012 -6461.

Bauman, C. A., H. W. Barkema, J. Dubuc, G. P. Keefe, and D. F. Kelton. 2018. Canadian National Dairy Study: Herd-level milk quality. *J. Dairy Sci.* 101:2679–2691. https: / / doi .org/ 10 .3168/ jds.2017 -13336.

Berry, E. A., and J. E. Hillerton. 2002. The effect of selective dry cow treatment on new intramammary infections. *J. Dairy Sci.* 85:112–121. https: / / doi .org/ 10 .3168/ jds .S0022 -0302(02)74059 -9.

Berry, E. A., and J. E. Hillerton. 2007. Effect of an intramammary teat seal and dry cow antibiotic in relation to dry period length on postpartum mastitis. *J. Dairy Sci.* 90:760–765. https: / / doi .org/ 10.3168/ jds .S0022 -0302(07)71560 -6.

Berry, E. A., W. T. Johnston, and J. E. Hillerton. 2003. Prophylactic effects of two selective dry cow strategies accounting for interdependence of quarter. *J. Dairy Sci.* 86:3912–3919. https: / / doi .org/10 .3168/ jds .S0022 -0302(03)73999 -X.

Browning, J. W., G. A. Mein, M. Barton, T. J. Nicholls, and P. Brightling. 1990. Effects of antibiotic therapy at drying off on mastitis in the dry period and early lactation. *Aust. Vet. J.* 67:440–442. https:/ / doi .org/ 10 .1111/ j .1751 -0813 .1990 .tb03055 .x.

Cameron, M., G. P. Keefe, J. P. Roy, I. R. Dohoo, K. A. MacDonald, and S. L. McKenna. 2013. Evaluation of a 3M Petrifilm on-farm culture system for the detection of intramammary infection at the end of lactation. *Prev. Vet. Med.* 111:1–9. https: / / doi .org/ 10.1016/ j .prevetmed .2013 .03 .006.

Cameron, M., G. P. Keefe, J. P. Roy, H. Stryhn, I. R. Dohoo, and S. L. McKenna. 2015. Evaluation of selective dry cow treatment following on-farm culture: Milk yield and somatic cell count in the subsequent lactation. *J. Dairy Sci.* 98:2427–2436. https: / / doi .org/10 .3168/ jds .2014 -8876.

Cameron, M., S. L. McKenna, K. A. MacDonald, I. R. Dohoo, J. P. Roy, and G. P. Keefe. 2014. Evaluation of selective dry cow treatment following on-farm culture: Risk of postcalving intramammary infection and clinical mastitis in the subsequent lactation. *J. Dairy Sci.* 97:270–284. https: / / doi .org/ 10 .3168/ jds .2013 -7060.

Cameron, M., Barkema, H. W., De Buck, J., De Vliegher, S., Chaffer, M., Lewis, J. & Keefe, G. P. 2017. Identification of bovine-associated coagulase-negative staphylococci by matrix assisted laser desorption/ionization time-of-flight mass spectrometry using a direct transfer protocol. *J. Dairy Sci.*, 100, 2137-2147.

Cameron, M., Perry, J., Middleton, J. R., Chaffer, M., Lewis, J. & Keefe, G. P. 2018. Short communication: Evaluation of MALDI-TOF mass spectrometry and a custom reference spectra

expanded database for the identification of bovine-associated coagulase-negative staphylococci. *J. Dairy Sci.*, 101, 590-595.

CBMQRN. 2010. Administration technique of intramammary treatment in dairy cows. Canadian Bovine Mastitis and Milk Quality Research Network. Accessed November 01, 2021. http://www.reseaumammite.org/tactic/wp-content/uploads/2020/02/FR-Fiche-Infusion-intramammaire.pdf

Cook, N. B., D. A. Pionek, and P. Sharp. 2005. An assessment of the benefits of Orbeseal (R) when used in combination with dry cow antibiotic therapy in three commercial dairy herds. *Bov. Pract.* 39:83–94.

Dohoo, I. R., J. Smith, S. Andersen, D. F. Kelton, and S. Godden. 2011. Diagnosing intramammary infections: Evaluation of definitions based on a single milk sample. *J. Dairy Sci.* 94:250–261. https: / / doi .org/ 10 .3168/ jds .2010 -3559.

Dufour, S., and I. R. Dohoo. 2013. Short communication: Relationship between herd intramammary infection incidence and elimination rate during the dry period. *J. Dairy Sci.* 96:1672–1676. https: / / doi .org/ 10 .3168/ jds .2012 -5899.

Dufour, S., V. Wellemans, J. P. Roy, P. Lacasse, A. Ordonez-Iturriaga, and D. Francoz. 2019. Non-antimicrobial approaches at drying-off for treating and preventing intramammary infections in dairy cows. Part 1. Meta-analyses of efficacy of using an internal teat sealant without a concomitant antimicrobial treatment. *Anim. Health Res. Rev.* 20:86–97. https: / / doi .org/ 10 .1017/ S1466252319000070.

Francoz, D., L. Bergeron, M. Nadeau, and G. Beauchamp. 2012. Prevalence of contagious mastitis pathogens in bulk tank milk in Quebec. *Can. Vet. J.* 53:1071–1078.

Godden, S., P. Rapnicki, S. Stewart, J. Fetrow, A. Johnson, R. Bey, and R. Farnsworth. 2003. Effectiveness of an internal teat seal in the prevention of new intramammary infections during the dry and early-lactation periods in dairy cows when used with a dry cow intramammary antibiotic. *J. Dairy Sci.* 86:3899–3911. https: / / doi .org/ 10 .3168/ jds .S0022 -0302(03)73998 -8.

Green, M. J., A. J. Bradley, G. F. Medley, and W. J. Browne. 2008. Cow, farm, and herd management factors in the dry period associated with raised somatic cell counts in early lactation. *J. Dairy Sci.* 91:1403–1415. https: / / doi .org/ 10 .3168/ jds .2007 -0621.

Haine, D., I. Dohoo, D. Scholl, and S. Dufour. 2018. Diagnosing intramammary infection: Controlling misclassification bias in longitudinal udder health studies. *Prev. Vet. Med.* 150:162–167. https: / / doi.org/ 10 .1016/ j .prevetmed .2017 .11 .010.

Huxley, J. N., M. J. Green, L. E. Green, and A. J. Bradley. 2002. Evaluation of the efficacy of an internal teat sealer during the dry period. *J. Dairy Sci.* 85:551–561. https: / / doi .org/ 10 .3168/ jds .S0022 -0302(02)74108 -8.

Joe, H. 2008. Accuracy of Laplace approximation for discrete response mixed models. *Comput. Stat. Data Anal.* 52:5066–5074. https: / / doi .org/ 10 .1016/ j .csda .2008 .05 .002.

Landis, J. R., and G. G. Koch. 1977. The measurement of observer agreement for categorical data. *Biometrics* 33:159–174. https: / / doi .org/ 10 .2307/ 2529310.

McCarron, J. L., G. P. Keefe, S. L. McKenna, I. R. Dohoo, and D. E. Poole. 2009. Laboratory evaluation of 3M Petrifilms and University of Minnesota Bi-plates as potential on-farm tests for clinical mastitis. *J. Dairy Sci.* 92:2297–2305. https: / / doi .org/ 10 .3168/ jds.2008 -1661.

Mahmmod, Y. S., Nonnemann, B., Svennesen, L., Pedersen, K. & Klaas, I. C. 2018. Typeability of MALDI-TOF assay for identification of non-aureus staphylococci associated with bovine intramammary infections and teat apex colonization. *Journal of Dairy Science*, 101, 9430-9438.

Mütze, K., W. Wolter, K. Failing, B. Kloppert, H. Bernhardt, and M. Zschöck. 2012. The effect of dry cow antibiotic with and without an internal teat sealant on udder health during the first 100 d of lactation: a field study with matched pairs. *J. Dairy Res.* 79:477–484. https: / / doi .org/ 10 .1017/ S0022029912000477.

National Mastitis Council. 2017a. Laboratory Handbook on Bovine Mastitis. National Mastitis Council Inc., New Prague, MN.

National Mastitis Council. 2017b. Chapter 1: Sample collection and handling. Laboratory Handbook on Bovine Mastitis. 3rd ed. National Mastitis Council Inc., New Prague, MN.

Neave, F. K., F. H. Dodd, R. G. Kingwill, and D. R. Westgarth. 1969. Control of Mastitis in the Dairy Herd by Hygiene and Management. *J. Dairy Sci.* 52:696–707. https: / / doi .org/ 10 .3168/ jds .S0022-0302(69)86632 -4.

O'Connor, A. M., J. M. Sargeant, I. A. Gardner, J. S. Dickson, M. E. Torrence, C. E. Dewey, I. R. Dohoo, R. B. Evans, J. T. Gray, M. Greiner, G. Keefe, S. L. Lefebvre, P. S. Morley, A. Ramirez, W. Sischo, D. R. Smith, K. Snedeker, J. Sofos, M. P. Ward, and R. Wills. 2010. The REFLECT statement: Methods and processes of creating reporting guidelines for randomized controlled trials for livestock and food safety. *J. Vet. Intern. Med.* 24:57–64. https: / / doi .org/ 10 .1111/ j .1939 -1676 .2009 .0441 .x.

Olde Riekerink, R. G., H. W. Barkema, S. Veenstra, D. E. Poole, R. T. Dingwell, and G. P. Keefe. 2006. Prevalence of contagious mastitis pathogens in bulk tank milk in Prince Edward Island. *Can. Vet. J.* 47:567–572.

Pantoja, J. C. F., C. Hulland, and P. L. Ruegg. 2009. Dynamics of somatic cell counts and intramammary infections across the dry period. *Prev. Vet. Med.* 90:43–54. https: / / doi .org/ 10 .1016/ j.prevetmed .2009 .03 .012.

Rajala-Schultz, P. J., A. H. Torres, and F. J. DeGraves. 2011. Milk yield and somatic cell count during the following lactation after selective treatment of cows at dry-off. *J. Dairy Res.* 78:489–499. https: / / doi .org/ 10 .1017/ S0022029911000690.

Rindsig, R. B., R. G. Rodewald, A. R. Smith, and S. L. Spahr. 1978. Complete versus selective dry cow therapy for mastitis control. *J. Dairy Sci.* 61:1483–1497. https: / / doi .org/ 10 .3168/ jds .S0022-0302(78)83753 -9.

Rindsig, R. B., R. G. Rodewald, A. R. Smith, N. K. Thomsen, and S. L. Spahr. 1979. Mastitis history, California Mastitis Test, and somatic cell counts for identifying cows for treatment in a selective dry cow therapy program. *J. Dairy Sci.* 62:1335–1339. https: / / doi.org/ 10 .3168/ jds .S0022 -0302(79)83421 -9.

Robert, A., N. Bareille, P. Roussel, B. Poutrel, V. Heuchel, and H. Seegers. 2006. Interdependence of udder quarters for new intramammary infection during the dry period in cows submitted to selective antibiotic therapy. *J. Dairy Res.* 73:345–352. https: / / doi .org/ 10 .1017/ S0022029906001981.

Robert, A., P. Roussel, N. Bareille, D. Ribaud, F. Serieys, V. Heuchel, and H. Seegers. 2008. Risk factors for new intramammary infections during the dry period in untreated dairy cows from herds using selective dry cow therapy. *Animal* 2:247–254.

Sanford, C. J., G. P. Keefe, I. R. Dohoo, K. E. Leslie, R. T. Dingwell, L. DesCôteaux, and H. W. Barkema. 2006. Efficacy of using an internal teat sealer to prevent new intramammary infections in nonlactating dairy cattle. *J. Am. Vet. Med. Assoc.* 228:1565–1573. https: / / doi .org/ 10 .2460/ javma .228 .10 .1565.

Sargeant, J. M., A. M. O'Connor, I. A. Gardner, J. S. Dickson, M. E. Torrence, I. R. Dohoo, S. L. Lefebvre, P. S. Morley, A. Ramirez, and K. Snedeker. 2010. The REFLECT statement: reporting guidelines for randomized controlled trials in livestock and food safety: explanation and elaboration. *J. Food Prot.* 73:579–603. https: / / doi .org/ 10 .4315/ 0362 -028X -73 .3 .579.

Scherpenzeel, C. G. M., I. E. M. den Uijl, G. van Schaik, R. G. M. Olde Riekerink, J. M. Keurentjes, and T. J. G. M. Lam. 2014. Evaluation of the use of dry cow antibiotics in low somatic cell count cows. *J. Dairy Sci.* 97:3606–3614. https: / / doi .org/ 10 .3168/jds .2013 -7655.

Schukken, Y. H., J. A. H. Smit, F. J. Grommers, D. Vandegeer, and A. Brand. 1989. Effect of freezing on bacteriologic culturing of mastitis milk samples. *J. Dairy Sci.* 72:1900–1906. https: / / doi .org/10 .3168/ jds .S0022 -0302(89)79309 -7.

Shook, G. E. 1993. Genetic improvement of mastitis through selection on somatic cell count. *Vet. Clin. North Am. Food Anim. Pract.* 9:563–577. https: / / doi .org/ 10 .1016/ S0749 -0720(15)30622 -8.

Torres, A. H., P. J. Rajala-Schultz, F. J. DeGraves, and K. H. Hoblet. 2008. Using dairy herd improvement records and clinical mastitis history to identify subclinical mastitis infections at dry-off. *J. Dairy Res.* 75:240–247. https: / / doi .org/ 10 .1017/ S0022029908003257.

Vasquez, A. K., D. V. Nydam, C. Foditsch, M. Wieland, R. Lynch, S. Eicker, and P. D. Virkler. 2018. Use of a culture-independent on-farm algorithm to guide the use of selective dry-cow antibiotic therapy. *J. Dairy Sci.* 101:5345–5361. https: / / doi .org/ 10 .3168/ jds .2017 -13807.

Woolford, M. W., J. H. Williamson, A. M. Day, and P. J. Copeman. 1998. The prophylactic effect of a teat sealer on bovine mastitis during the dry period and the following lactation. *N. Z. Vet. J.* 46:12–19. https: / / doi .org/ 10 .1080/ 00480169 .1998 .36044.

Tableau 5. – Distribution of quarters and their characteristics as function of group allocation in a randomized controlled trial evaluating a quarter-based selective dry cow therapy using Petrifilm® on-farm milk culture.

	Treatment group[1]			
	BDCT	**BDCT+ITS**	**QSDCT/ITS**	**QSDCT+ITS/ITS**
Treatment at drying-off (# of quarters)	529	604	575	539
Antimicrobial	529	0	245	0
Antimicrobial and internal teat sealant	0	604	0	223
Internal teat sealant alone	0	0	330	316
Proportion of quarters receiving an Antimicrobial[2] (%)	100	100	42.6	41.4
Prevalence of IMI at dry off[3] (%)	32.5[a]	32.0[a]	29.0[a]	27.0[a]
Last pre-dry DHI test				
Daily mean milk production (in kg/d)	23.7[a]	24.1[a]	25.5[a]	23.2[a]
SCS	2.7[a]	2.8[a]	2.5[a]	2.7[a]
Subsequent lactation 0-120 DIM				
Daily milk production (in kg/d)				
Mean	43.8[a]	44.2[a]	43.2[a]	42.6[a]
Interquartile range	39.4 – 47.9	39.5 – 47.8	37.8 – 47.7	37.6 – 46.7
Somatic cells score				
Mean	1.7[a]	2.0[a]	2.0[a]	2.0[a]
Interquartile range	0.7 – 2.4	0.6 – 3.0	0.6 – 3.2	0.6 – 2.9

[a]Values with the same letters within a row are not significantly different (P > 0.05).

[1]BDCT = blanket dry cow therapy; BDCT+ITS = blanket dry cow therapy and internal teat sealant; QSDCT/ITS = quarter-based selective dry cow therapy with antimicrobial for infected quarters and an internal teat sealant for healthy quarters; QSDCT+ITS/ITS = quarter-based selective dry cow therapy with antimicrobial and internal teat sealant for infected quarters and an internal teat sealant for healthy quarters.

[2]Treatment decision was based on milk culture on Petrifilm® aerobic count plates (3M Petrifilm®, London, ON, Canada) for QSDCT/ITS and QSDCT+ITS/ITS groups.

[3]Intramammary infections were defined using laboratory-based milk culture followed with MALDI-TOF identification.

Tableau 6. – Pathogens isolated per treatment group from the first cases of clinical mastitis occurring within 120 DIM for cows in four treatment groups: 1) blanket dry cow therapy (BDCT); 2) blanket dry cow therapy and internal teat sealant (BDCT+ITS); 3) quarter-based selective dry cow therapy with antimicrobial for infected quarters and an internal teat sealant for healthy quarters (QSDCT/ITS); 4) quarter-based selective dry cow therapy with antimicrobial and internal teat sealant for infected quarters and an internal teat sealant for healthy quarters (QSDCT+ITS/ITS).

Pathogen name	**BDCT**	**BDCT+ITS**	**QSDCT/ITS**	**QSDCT+ITS/ITS**	**Total**
Bacillus pumilus		1			1
Chryseobacterium species		1			1
Escherichia coli	1		2		3
Hafnia alvei		1			1
Klebsiella pneumoniae		2			2
Kocuria species	1				1
Other Gram Positive		1			1
Serratia liquefaciens			1		1
Serratia marcescens	1				1
Staphylococcus aureus	2	1	2	1	6
Staphylococcus haemolyticus	1	1		1	3
Staphylococcus xylosus				1	1
Streptococcus dysgalactiae	1	1	1		3
Streptococcus gallolyticus			1		1
Streptococcus uberis				1	1
Trueperella pyogenes	2				2
Yeast				1	1
Total	**9**	**9**	**7**	**5**	**30**

Tableau 7. – Number of quarters with IMI at dry-off, new infections (new IMI or new environmental IMI, IMIenv), persistence of IMI over the dry period, and occurrence of clinical mastitis (CM) during the first 120 DIM among the 4 treatment groups.

		Treatment group[2]				
Outcome	**Item[1]**	**BDCT**	**BDCT+ITS**	**QSDCT/ITS**	**QSDCT+ITS/ ITS**	**All groups**
IMI at dry off	Quarters at risk (no.)	529	604	575	539	2247
	Undetermined status (no.)	19	25	13	18	75
	Units analysed (no.)	510	579	562	521	2172
	Quarters with IMI (no.)	166	185	165	142	658
	Prevalence in % (95% CI)	31.0[a] (22.3, 39.7)	29.9[a] (21.7, 38.1)	28.5[a] (20.3, 36.6)	24.8[a] (17.2, 32.5)	28.6 (21.9, 35.2)
New IMI	Quarters at risk (no.)	529	604	575	539	2247
	Undetermined status (no.)	60	109	121	94	384
	Units analysed (no.)	469	495	454	445	1863
	Quarters with new IMI (no.)	87	82	84	79	332
	Cumulative incidence in % (95% CI)	15.9[a] (9.8, 22.1)	13.2[a] (7.9, 18.4)	15.8[a] (9.7, 22.0)	15.1[a] (9.2, 21.0)	15.0 (10.2, 19.7)
New IMIenv	Quarters at risk (no.)	529	604	575	539	2247
	Undetermined status (no.)	54	82	100	75	311
	Units analysed (no.)	475	522	475	464	1936
	Quarters with new IMIenv (no.)	77	82	74	63	296
	Cumulative incidence in % (95% CI)	13.4[a] (7.9, 19.0)	12.3[a] (7.3, 17.3)	13.1[a] (7.6, 18.5)	11.0[a] (6.2, 15.7)	12.4 (8.3, 16.6)
IMI persisting during the dry period	Quarters at risk (no.)	166	185	165	142	658
	Undetermined status (no.)	14	25	40	16	95
	Units analysed (no.)	152	160	125	126	563
	Quarters with persistent IMI (no.)	7	5	6	5	23
	Prevalence in % (95% CI)	3.2[a] (0.0, 6.6)	2.1[a] (0.0, 4.6)	3.4[a] (0.0, 7.3)	2.7[a] (0.0, 5.9)	2.8 (0.3, 5.4)
CM during 120 DIM	Quarters at risk (no.)	529	604	575	539	2247
	Undetermined status (no.)	8	29	36	32	105
	Units analysed (no.)	521	575	539	488	2142
	Quarters with CM (no.)	27	42	31	19	119
	Estimated incidence (%) (95% CI)	2.4[a] (0.8, 4.0)	3.7[a] (1.6, 5.8)	2.9[a] (1.1, 4.7)	1.7[a] (0.5, 3.0)	2.7 (1.4, 4.0)

[a]Values with the same letters within a row are not significantly different (P > 0.05).

[1]Reported prevalences and incidences along with 95% CI were computed using a generalized linear mixed model accounting for clustering of quarters by cows and by herds and then converted in population-average estimates and further into a probability using the invert logit function.

[2]BDCT = blanket dry cow therapy; BDCT+ITS = blanket dry cow therapy and internal teat sealant; QSDCT/ITS = quarter-based selective dry cow therapy with antimicrobial for infected quarters and an internal teat sealant for healthy quarters; QSDCT+ITS/ITS = quarter-based selective dry cow therapy with antimicrobial and internal teat sealant for infected quarters and an internal teat sealant for healthy quarters.

Tableau 8. – Pre-dry IMI status, dry period new IMI, and dry period persistent IMI for quarters of cows in four treatment groups: A) blanket dry cow therapy (BDCT); B) blanket dry cow therapy and internal teat sealant (BDCT+ITS); C) quarter-based selective dry cow therapy with antimicrobial for infected quarters and an internal teat sealant for healthy quarters (QSDCT/ITS); D) quarter-based selective dry cow therapy with antimicrobial and internal teat sealant for infected quarters and an internal teat sealant for healthy quarters (QSDCT+ITS/ITS).

	Dry off				**New infections**				**Persistent infections**			
Pathogen	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Aerococcus species	12	15	19	10	3	8	7	9				
Corynebacterium bovis	11	15	16	15	3		4	4	1			3
Other non-speciable *Corynebacterium*	10	10	20	12	9	5	4	13				
Escherichia coli				3	1	1	1	1				
Fungi and Yeast	1					1	1	1				
Staphylococcus aureus	9	11	11	10	3	5	7	7	1	3	2	
Staphylococcus auricularis	4	4	3	2	1		1					
Staphylococcus capitis	3	3	2	4			2	2				
Staphylococcus chromogenes	11	19	11	14	12	5	7	2				

Pathogen	Dry off				New infections				Persistent infections			
	A	B	C	D	A	B	C	D	A	B	C	D
Staphylococcus cohnii	2	10	9	5	5	12	1	2				
Staphylococcus epidermidis	7	8	5	9	1	2						1
Staphylococcus equorum	8	8	6	6	2	2	4	1				
Staphylococcus haemolyticus	21	23	20	13	7	2		3				
Staphylococcus hominis	9	3	8	2	1	3	2					
Staphylococcus saprophyticus	3	4	1	1	1	5	3	4				
Staphylococcus sciuri	3	8	7	7	5	5	2	4				
Staphylococcus simulans	6	1	4	5	4	1	6	3			2	
Staphylococcus xylosus	28	33	19	12	8	6	11	10	4	2	2	1
[1]Other Non-*aureus Staphylococci* (NAS)	4	2	2	1	3		2	2				
Other non-speciable *Staphylococci*	7	5	5	6	1	5	1	2				
Streptococcus dysgalactiae	2	1	2	2	2	2	3	2				
Other non-speciable *Streptococci*	2	1	2	2			1					
[2]Other Gram Negative		1		3	3	1	1	2				

Pathogen	Dry off				New infections				Persistent infections			
	A	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
[3]Other Gram Positive	16	13	14	9	14	16	14	10	1			
Total	**179**	**198**	**186**	**153**	**89**	**87**	**85**	**84**	**7**	**5**	**6**	**5**

[1]*Staphylococcus arlettae, Staphylococcus hyicus, Staphylococcus lentus, Staphylococcus pettenkoferi, Staphylococcus piscifermentans, Staphylococcus succinus, Staphylococcus vitulinus and Staphylococcus warneri.*

[2]*Acinetobacter* species*, Klebsiella* species*, Neisseria* species*, Oligella* species*, Pseudomonas* species *and Serratia* species*.*

[3]*Bacillus* species*, Brachybacterium* species*, Brevibacillus* species*, Curtobacterium flaccumfaciens, Enterococcus* species*, Kocuria* species*, Lactococcus* species*, Microbacterium* species*, Micrococcus luteus,* Other Gram Positive*, Paenibacillus lactis, Rothia nasimurium, Trueperella pyogenes, Weissella confuse.*

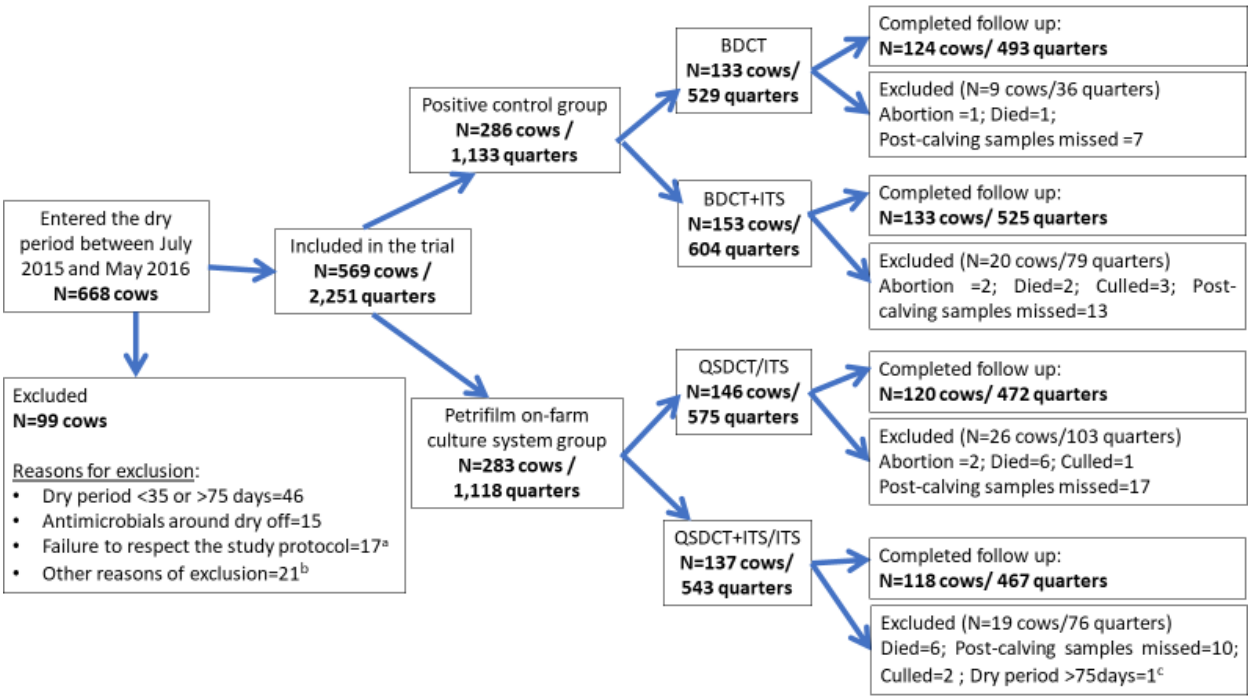

Figure 15. – Illustration of the enrollment and follow-up of cows in a randomized controlled trial comparing 4 groups: (1) blanket dry cow therapy (BDCT); (2) BDCT and internal teat sealant (BDCT+ITS); (3) quarter-based selective dry cow therapy with antimicrobial for infected quarters and an ITS for healthy quarters (QSDCT/ITS); (4) QSDCT with antimicrobial and ITS for infected quarters and an ITS for healthy quarters (QSDCT+ITS/ITS).

[a]Failure to respect the protocol included cows not treated according to the treatment group (e.g., a cow recruited in group 1 and treated as per group 2), cows that received dry cow treatment twice, or cows in which there was a delay between sampling date and dry-off (e.g., cows dried off a week after the sampling date). [b]Other reasons included breed other than Holstein, difficult to handle, contamination of pre-dry Petrifilm® (3M, London, ON, Canada) culture. [c]One cow was excluded a posteriori because the true length of the dry period was longer than the maximal acceptable value by a difference of more than 10 d.

Chapitre 6 –Bayesian estimation of diagnostic accuracy of somatic cell counts history and on-farm milk culture using Petrifilm® to identify quarters or cows that should be treated with antimicrobials in selective treatment protocols at dry off

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Abstract

Bayesian latent class models were used to estimate the test accuracy (sensitivity (Se), specificity (Sp), and predictive values (NPV and PPV)) of cow-level somatic cell counts (SCC) data, quarter-level Petrifilm® on-farm milk culture, and quarter-level standard milk bacteriology for the identification of quarters that should possibly be treated with antimicrobials at dry off in dairy cows. Data of 282 cows from 9 dairy herds in Québec, Canada, with bulk tank SCC < 250,000 cells/mL were used. Estimated median herd-prevalence of infections that should be treated was 16.2% (95% credibility interval (CI): 11.0–22.7). Se and Sp estimates for quarter-milk culture using

Petrifilm® were 82.2% (95%CI: 74.0–89.5) and 62.0% (95%CI: 58.6–65.6), respectively. Se and Sp for quarter-milk standard bacteriology were 67.4% (95%CI: 55.8–81.2) and 79.6% (95%CI: 76.4–83.0), respectively. Se and Sp of different SCC scenarios and thresholds were estimated. For first parity cows, using only the last Dairy Herd Improvement (DHI) test SCC with a threshold of 100,000 cells/mL appeared quite accurate, with Se, Sp, PPV, NPV and reduction of antimicrobial usage of 85.6% (95%CI: 69.6–95.6), 86.0% (95%CI: 80.0–91.7), 58.0% (95%CI: 42.3–74.2), 96.4% (95%CI: 91.3–99.0), and 75.3% (95%CI: 70.7–79.3), respectively. For cows of ≥ 2nd parity, using only the last DHI test SCC with a threshold of 200,000 cells/mL resulted in Se, Sp, PPV, NPV and reduction of antimicrobial usage of 75.3% (95%CI: 55.8–87.3), 84.0% (95%CI: 78.8–89.3), 47.2% (95%CI: 32.0–63.7), 94.7% (95%CI: 89.0–97.6), and of 77.0% (95%CI: 73.3–80.3), respectively. Adding quarter-level milk culture using Petrifilm® to cows identified as unhealthy using cow-level SCC data improved the test accuracy (mainly the PPV) and further reduced the use of antimicrobials. For instance, in ≥ 2nd parity cows, using only the last DHI SCC with a threshold of 200,000 cells/mL, adding a subsequent Petrifilm® test increased the reduction from 77.0% (95%CI: 73.3–80.3) to 89.5% (95%CI: 86.7–91.8). Considering the availability of SCC data, the easiness of using just the last DHI test, and the high NPV that could be achieved, producers may consider using just the last DHI test as a potential tool to identify cows that should be treated with antimicrobials at dry off. It may be used alone or in combination with quarter-level on-farm Petrifilm® milk culture on high SCC cows to further reduce the use of antimicrobials by identifying quarters that need to be treated.

Keywords: Diagnostic accuracy – Dairy cow – Milk culture – Somatic cell counts – Intramammary infection – selective treatment.

Introduction

Mastitis is an endemic disease in the dairy industry with important economic losses (Aghamohammadi et al., 2018, Halasa et al., 2007). Treatment of existing intramammary infections (IMI) at dry off and prevention of new IMI during the dry period constitute the principal reasons for antimicrobial usage on dairy farms, worldwide (Saini et al., 2012, Thomson et al.,

2008). In North America, the common practice, known as blanket dry cow therapy (BDCT), was to treat all quarters of all cows at dry off with an intramammary antimicrobial (Dufour et al., 2012).

However, with changes in mastitis epidemiology and increasing public health concerns regarding the use of antimicrobials and risk of antimicrobial resistance, selective dry cow therapy (SDCT) is a potential alternative to BDCT to reduce antimicrobial usage in dairy production (Cameron et al., 2014, Kabera et al., 2020, Rowe et al., 2020a). When using a SDCT approach, antimicrobial treatment is reserved for cows or quarters suspected of having an IMI, while uninfected cows or quarters do not receive antimicrobial treatment. In addition, internal teat sealants (ITS) have been shown to be a very effective nonantimicrobial alternative to prevent new IMI during the dry period for uninfected quarters/cows at dry off (Dufour et al., 2019, Sanford et al., 2006a, Woolford et al., 1998). The success of a SDCT approach will be strongly influenced by the ability to accurately determine the infection status of the quarter or cow so that the appropriate treatment is applied at dry off (Huxley et al., 2002, Robert et al., 2008, Torres et al., 2008).

Since SDCT was introduced, different methods of selecting infected cows or quarters have been reported: bacteriological culture in the laboratory (Browning et al., 1990, Browning et al., 1994, Robinson et al., 1988), somatic cell counts (SCC) and/or history of clinical mastitis (Rowe et al., 2020a, Torres et al., 2008, Vasquez et al., 2018), california mastitis test (Rindsig et al., 1978, Sanford et al., 2006b), N-acetyl-beta-D-glucosaminidase activity at dry off (Hassan et al., 1999), Petrifilm® (Cameron et al., 2014, Kabera et al., 2020) and Minnesota Easy 4Cast plate (Rowe et al., 2020a). Bacteriological culture in the laboratory is commonly used as a standard method for identifying IMI. However, logistic and financial considerations involved in sampling all quarters or all cows at the time of dry off may discourage its use in the selection of infected cows or quarters at dry off. On-farm culture system using Petrifilm® is easy to use, less costly than a standard culture, and has an important benefit of providing results in 24 hours, so producers would be able to apply targeted treatment decisions at dry off. It can be used on composite- (cow-level) (Cameron et al., 2014) or quarter-level milk samples (Kabera et al., 2020). Further, in herds enrolled in regular Dairy Herd Improvement (DHI) testing, data on SCC are available and could be used to differentiate infected from healthy cows at dry off, at no additional costs, and without additional delay. Quarter-level SCC data, however, are usually not available on most farms. Thus,

it is important to investigate the accuracy of Petrifilm® on-farm culture and/or of different approaches using cow-level SCC data in order to identify the best protocol for selecting quarters that should be treated with antimicrobials at dry off in dairy cows.

The first objective of the current study was to determine the diagnostic accuracy, using a Bayesian latent class model approach (LCM), of: 1) cow-level SCC data using different approaches and thresholds; 2) quarter-level Petrifilm®-based milk culture to identify quarters needing an antimicrobial treatment at dry off, while using 3) quarter-milk laboratory-based culture as a third reference test for comparison. The second objective was to describe the accuracy and predictive values of selection protocols based on a single vs. multiple tests.

Materials and methods

The STARD-BLCM statement (Standards for Reporting of Diagnostic accuracy studies that use Bayesian Latent Class Models) were used for reporting on the design, conduct, and results of the current study (Kostoulas et al., 2017).

Study Design and participants (herds and cows selection)

The study design and participants were described elsewhere (Kabera et al., 2020). Briefly, a convenience sample of 9 dairy herds with an average bulk tank SCC below 250,000 cells/mL over the last 12 months (i.e between June 2014 and July 2015) was selected in Québec, Canada. From these farms, all cows that entered the dry period between July 2015 and May 2016 were considered for inclusion in the trial. Enrolled cows had no clinical mastitis or antimicrobial treatment during 14 days prior to dry off, and an expected dry period of 35 – 75 days. As part of an ongoing randomized control trial, they were randomly allocated to four groups; two quarter-based selective (QSDCT) groups, using results of quarter milk culture on Petrifilm® and two blanket dry cow therapy (BDCT) groups (Kabera et al., 2020). In the current paper, we used the cow-level SCC, the quarter-level milk Petrifilm® on-farm bacteriology results, and the quarter-level milk laboratory-based bacteriological results from the two selective (QSDCT) groups.

Milk samples and data collection

Somatic cell counts and history of clinical mastitis

Data on milk SCC from the previous lactation were extracted for each cow from monthly DHI data for all participating farms. Data on clinical mastitis cases from the previous lactation were extracted from health records of each participating farms. One farm discontinued DHI testing over the course of the project and as a result, we were unable to get SCC data for the remaining study period. Therefore, on that farm, only cows with available DHI SCC data were considered for the analyses.

Petrifilm® on-farm culture

Using an aseptic sampling technique (National Mastitis Council, 2017a), single quarter milk samples were collected and cultured using Petrifilm® Aerobic Count plates on the day prior to dry off. Both the milk sample collection and Petrifilm® on-farm culture were performed by the research team members.

Laboratory bacteriological culture (reference test)

Using an aseptic sampling technique (National Mastitis Council, 2017a), single quarter milk samples were collected on all quarters on the day before dry off. Further descriptions are detailed in Kabera et al. (2020).

Test methods

Somatic cell counts

Three approaches were explored to classify cows at dry off based on cow-level SCC thresholds using: (1) only the last DHI milk recording before dry off; (2) the last three DHI tests before dry off; or (3) all available DHI tests during the lactation. When more than one DHI test was used, two different interpretations were compared: the mean of the tests above or below the threshold vs. each individual values above or below the threshold (i.e each individual test must be below the threshold for a cow to be qualified for an antimicrobial free treatment at dry off). Moreover, four SCC thresholds (> 50,000; > 100,000; > 150,000 and > 200,000 cells/mL) were evaluated, to

determine cows eligible for an antimicrobial treatment at dry off. Whenever a cow was above the SCC threshold for a given approach, all quarters of that cow were considered in need of being treated with antimicrobials.

Three minimal criteria had to be met for using DHI SCC data to define the status of a cow at dry off: (1) for all approaches, the last DHI test had to be conducted ≤ 50 days prior to dry off; (2) for the approach considering the results of the last 3 DHI tests before dry off, cows had to have a minimum of 3 DHI tests conducted during the lactation; (3) for the approach considering the results of all DHI tests during the lactation, cows had to have a minimum of 4 DHI tests conducted during the lactation. The SCC information was considered as missing whenever these criteria were not met.

Petrifilm® on-farm culture

One mL of quarter milk was added to nine mL of sterile water to make a 1:10 dilution. One mL of diluted milk was cultured on a Petrifilm® Aerobic Count plate and incubated on-farm at 35°C for 24 h in a TurboFan Hova-Bator (GQF Manufacturing, Savannah, GA).

On the day of dry off, producers read results of culture on Petrifilm®. Petrifilm® plates were kept frozen at −20°C at the farm until the next farm visit (occurring every other week). Then, they were placed on ice and transported to the laboratory at the Faculty of Veterinary Medicine of the Université de Montréal. They were kept frozen at −20°C before they were scanned. The research team verified that the interpretation of the Petrifilm® by the producer was done correctly for each cow in the study. To achieve this, Petrifilm® were scanned using an automated 3M Petrifilm® reader (3M Canada, London, ON, Canada), which provided an automatized colony count. An almost perfect agreement between Petrifilm® results obtained by the producer and those obtained by the automated Petrifilm® reader was observed, with a kappa value of 0.89 (95%CI: 0.86, 0.92) (Kabera et al., 2020). Therefore, for comparison with other tests, Petrifilm® results were used as read by producers.

The colony count threshold of ≥50 cfu/mL (presence of ≥5 colonies on Petrifilm®) was reported to maximize the sensitivity of Petrifilm® by McCarron et al. (2009) and Cameron et al. (2013) and was, therefore, used to defined a quarter that needed to be treated.

Laboratory bacteriological culture

Milk samples were thawed and cultured in the Maritime Quality Milk research laboratory (University of Prince Edward Island) using standardized methods outlined in the *Laboratory Handbook on Bovine Mastitis* (National Mastitis Council, 2017b). A more complete description of our bacteriological culture methods is provided in a previously published article (Kabera et al., 2020).

Colonies of bacteria were subcultured individually on blood agar plates to obtain pure cultures for further classification using matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). With the exception of *Staphylococcus* spp., identification scores were interpreted as per manufacturer's recommendations as follows: a score of 2.0 to 3.0 was deemed as acceptable for species-level identification, a score of 1.7 to <2.0 indicated confident identification to the genus level, and a score of <1.7 was considered a nonreliable identification. However, a cut-off score ≥1.7 was considered as a reliable threshold for species-level bacterial identification of *staphylococci*, specifically (Cameron et al., 2017, Cameron et al., 2018, Mahmmod et al., 2018). Laboratory technicians were blinded to the results obtained by the Petrifilm® on-farm culture system, SCC data, and historic of clinical mastitis (CM) of the cow.

The presence of ≥1 cfu/0.01 mL of milk of any bacteria was considered sufficient to qualify a quarter as having an IMI (Dohoo et al., 2011) at dry off. However, we considered that not all IMIs should be treated with antimicrobials at dry off. Quarters infected with bacterial species that are suspected to have a very low cure rate or with bacterial species that are not recognized as significant for udder health were not considered as needing an antimicrobial treatment. Table 9 lists the bacterial species identified, whether we considered that the affected quarter should be treated (National Mastitis Council, 2016), and the number of samples from which they were isolated.

Statistical Analyses

The laboratory bacteriological culture was considered as an imperfect reference test to determine the diagnostic test accuracy of cow-level SCC data using different approaches and thresholds and quarter-level Petrifilm®-based milk culture to identify eligible quarters or cows for an

antimicrobial treatment at dry off. Only complete cases (data available for Petrifilm®, standard bacteriology, and a given SCC approach and threshold) were considered in the analyses. For each SCC approach (last DHI test vs. last three DHI tests vs. all DHI tests), interpretation (mean value vs. individual values), and threshold (> 50,000; > 100,000; > 150,000 and > 200,000 cells/mL), a table presenting cross-tabulated results from the comparison of cow-level SCC vs. quarter-level milk culture on Petrifilm® vs. quarter-level standard milk culture was prepared. These different tables were used to inform the LCM. Moreover, these cross-tabulated tables were also further separated by parity (first vs. ≥ 2nd parity cows).

Latent class model

A LCM using a hierarchical prior for herd prevalence (Hanson et al., 2003) was fit within a Bayesian framework to estimate the sensitivity (Se) and specificity (Sp) of the three diagnostic tests for identifying quarters with an intramammary infection that should be treated with an antimicrobial at dry off as well as the mean herd-prevalence of quarters that should be treated at dry off. In the LCM model, we allowed the prevalence of cows or quarters that should be treated with antimicrobials at dry off to vary across the 9 herds (instead of a same prevalence for all herds). Also, we allowed for conditional dependence between Petrifilm® on-farm culture and standard bacteriological culture, since these two tests are both culture-based approaches. Conditional dependence was modelled by adding covariance terms between the Se and between the Sp of these two tests (Dendukuri and Joseph, 2001). Moreover, we allowed for Se and Sp of the SCC-based tests to vary as function of cow's parity (first vs. ≥ 2nd parity). We hypothesized that cow's parity could possibly affect SCC accuracy and parity was, therefore, considered as a covariate for all SCC-based approaches. Accuracy of the three diagnostic tests, however, were assumed to be the same in the 9 herds.

Prior information

The model yields sixty-three degrees of freedom (seven from each population) and contained 12 parameters to estimate: first parity cows SCC's Se and Sp, older cows SCC's Se and Sp, Petrifilm®'s Se and Sp, standard bacteriology Se and Sp, Covp (the positive covariance between Petrifilm® and standard bacteriology Se), Covn (the negative covariance between Petrifilm® and standard

bacteriology Sp), Mu (the mean herd-prevalence), and Psi (a measure of spread of the herd-prevalence distribution described by Hanson et al. (2003)). Epitools Epidemiological Calculators were used to compute the beta distribution of different parameters (Sergeant, 2018). The literature was searched to define informative prior distributions for these parameters. A complete list of the priors used, with references, is presented in Table 10. For accuracy parameters for which no prior information was available in the peer-reviewed literature, we used vague priors of the form beta (1.0, 1.0). Note that, for all scenarios and thresholds of SCC, the same prior distributions were used for SCC Se and Sp of first lactation and older cows since this information was never reported as function of parity. We used the method described by Hanson et al. (2003) to define priors for the mean herd-prevalence (Mu) and its precision (Psi). Using data from previous studies (Cameron et al., 2013, Rowe et al., 2020a), the mean herd-prevalence was estimated at 0.18 with its 95th percentile at 0.25. Therefore, a Beta distribution (20.3, 88.8) was chosen to represent the prior Mu distribution. The prior distribution for Psi was computed as it was described by Hanson et al. (2003). There were no studies reporting the data needed to define the prior distribution for Psi. However, using the data of 53 herds with bulk milk SCC < 250,000 cells/mL (similarly to our 9 herds) from the National Cohort of Dairy Farms of the Mastitis Network followed in 2007-2008 (Reyher et al., 2011), we were able to estimate the 50th and 95th percentiles for the 90th percentile of the herd-prevalence distribution of quarter to treat at dry off. Based on these data, the 50th percentile of the 90th herd was 0.50 and its 95th percentile was 0.83, and, thus, a prior gamma (3.2, 0.31) was chosen as prior distribution for Psi.

Predictive values

Negative and positive predictive values (NPV and PPV, respectively) were estimated for each test by using its Se and Sp estimates and the estimated mean herd-prevalence (Mu). The NPV for a given test was estimated as (Dohoo et al., 2009):

$$NPV = \frac{(1 - Mu\ ) * Sp}{Mu * (1 - Se) + (1 - Mu) * Sp}$$

The PPV was estimated as (Dohoo et al., 2009):

$$PPV = \frac{Mu * Se}{Mu * Se + (1 - Mu) * (1 - Sp)}$$

Combining SCC and Petrifilm®

The impact of combining a cow-level SCC-based approach with a subsequent quarter-level Petrifilm® confirmation (series interpretation) was then investigated. With this approach, when SCC was greater than the threshold, then Petrifilm® milk culture of each quarter would be conducted and only quarters with a positive result on Petrifilm® would be treated. To estimate the Se and Sp of this approach, we simply used the accuracy parameters of both tests as followed (Dohoo et al., 2009):

$$Se = Se_{SCC} * Se_{Petrifilm}$$

$$Sp = Sp_{SCC} + Sp_{Petrifilm} - (Sp_{SCC} * Sp_{Petrifilm})$$

Estimation

The models were estimated using OpenBUGS version 3.2.3 (The GNU General Public License). Convergence of the model was evaluated by running 3 chains starting from different initial values with visual inspection of the time series plots and Brooks-Gelman-Rubin diagnostic plots (Brooks and Gelman, 1998, Toft et al., 2007b). In addition, the values underlying the Brooks-Gelman-Rubin plots were examined for each estimated parameter. Each chain was run for 20,000 iterations and a burn in of 5000 iterations was applied. An example of the OpenBUGS code is presented as Appendix 3.

Sensitivity Analysis

The influence of the informative priors used for accuracy parameters was investigated by running alternative models using priors where 0.05 was subtracted from the elicited mode and 0.15 from its 5th percentile as suggested by Johnson et al. (2019). This sensitivity analysis was conducted only for a few of the most relevant testing approaches and thresholds (i.e., approaches that resulted in good tests accuracy (Se and Sp) and the best NPV without too much compromise on PPV) and were judged to be practical and likely to be implemented by producers). Also, models considering covariance between Petrifilm® and standard bacteriology, SCC and Petrifilm® and SCC

and standard bacteriology were compared, using the deviance information criterion (DIC, (Spiegelhalter et al., 2002)).

Estimating reduction in use of antimicrobials

For this analysis, the primary unit for statistical analyses was the quarter. To estimate the reduction in antimicrobial use associated with a given selection strategy, we computed the probability of a quarter being treated with antimicrobials, for each cow-level SCC-based selection approach, interpretation, and threshold. We then computed the probability of a quarter being treated with antimicrobials for the Petrifilm® (used alone) and lab-based bacteriology. Finally, we also computed the probability of a quarter being treated with antimicrobials when using a combined cow-level SCC followed by quarter-level Petrifilm® approach. For all these analyses, we applied a logistic model using SAS Proc GENMOD procedure (version 9.4, SAS Institute Inc., Cary, NC) to the data describing our cows' tests results. In these models, the outcome was whether the quarter was qualified as eligible for a dry off without antimicrobials using a given testing approach. For diagnostic strategies involving quarter-milk Petrifilm® (alone) or standard bacteriology, the model contained only one intercept. Robust variance was used to account for clustering of observations by cow and herd. The same model was used for cow-level SCC approaches or for the approach involving the use of cow-level SCC followed by quarter-milk Petrifilm®, but parity (first vs. ≥ 2nd parity cows) was added in the model as a predictor. Again, robust variance was used. Marginal predictions were then transformed into probabilities of being left untreated using the invert logit function.

Results

Study population

A total of 282 Holstein cows from 9 dairy herds were included in the analysis. On average, 31 cows were recruited per herd (range: 13 – 86 cows). Parity of studied cows varied from 1 to 7 (median=2). Among the 282 cows, 113 were at the end of their first lactation and 169 were older cows.

Of the 1128 potential quarters, Petrifilm® results were available for 1114 quarters. Fourteen quarters (5 for first parity cows and 9 for older cows) were not functional at dry off. Among the 1114 quarters with Petrifilm® culture results, 1083 (437 quarters of first parity cows and 646 quarters of older cows) had standard bacteriology culture results and 31 (10 for first parity cows and 21 for older cows) were contaminated.

SCC data of 236 cows (97 for first parity cows and 139 for ≥2 parity cows) were available for the last DHI test, 230 cows (96 for first parity cows and 134 for ≥2 parity cows) were available when considering the three last DHI tests, and 229 cows (96 for first parity cows and 133 for ≥2 parity cows) were available for all DHI tests of the lactation. SCC data of 46 cows (16 for first parity cows and 30 for ≥2 parity cows) were not considered for any DHI tests-based approaches, because the duration between the last test and the dry off date was > 50 days. Forty two out of these 46 cows were from the herd which discontinued DHI testing during the course of the project. Thus, for this herd, only 44 out of 86 cows were included in the analyses. After all exclusions, an average of 26 cows per herd was included in the analyses (range: 13 – 47 cows).

In addition, SCC data for six cows (one for first parity cows and five for ≥2 parity cows) were considered only for SCC-based approaches using the last DHI test and were not considered for approaches based on the last three tests or all DHI tests of the lactation, because only two tests were available. Finally, SCC data of one cow (≥2 parity cows) were considered for SCC-based approaches using the last or the last three tests, but were considered as missing for statuses based on all DHI tests of the lactation, because only three DHI tests were available. The interval between the latest and the earliest of the last three DHI tests ranged from 60 to 160 days.

Accuracy and predictive values results

Cross-tabulated results of the three diagnostic tests for the approach using only the last DHI test with a SCC threshold of 200,000 cells/mL are presented in Table 11, as an example of the approach. Cross-tabulated tables for all other SCC scenarios are presented as supplementary materials (Kabera et al., 2021b).

Sensitivity and Specificity posterior distributions for the different SCC-based approaches are presented in Figure 16A for first parity cows and in Figure 16B for older cows. Regardless of parity, in general, Sp was increased when using just the last test versus the last three or all tests, but with a small decrease of Se. Further, regardless of parity, when multiple DHI tests were considered, using the mean of the tests, rather than each individual values, led to higher Sp, but with a decreased Se. Finally, as expected, we observed increasing Sp and decreasing Se as we moved from the lowest to the highest SCC threshold.

Posterior distributions of predictive values are presented in Figure 17A for first parity cows and in Figure 17B for older cows. Regardless of parity, PPV was increased when using just the last test (compared to last 3 or all tests), with only small differences in NPV. Also, regardless of parity, when multiple DHI tests were considered, using the mean of the tests, rather than each individual values, led to slightly higher PPV, with little changes in NPV. Finally, in general, increasing the SCC threshold led to higher PPV and lower NPV.

Both for first parity cows and for older cows, using simply the last DHI test, often yielded the highest NPV with PPV that were comparable to the other approaches. The highest NPV was obtained for first parity cows using the last DHI test with a SCC threshold of 100,000 cells/mL. Median estimates of the Se, Sp, NPV, and PPV were 85.6% (95%CI: 69.6 – 95.6), 86.0% (95%CI: 80.0 – 91.7), 96.4 (95%CI: 91.3 – 99.0) and 58.0 (95%CI: 42.3 – 74.2), respectively. However, for first parity cows, the last DHI test with a SCC threshold of 200,000 cells/mL also appeared as an interesting option with a very similar NPV. For this threshold, median estimates of 77.7% (95%CI: 64.7 – 88.0), 91.3% (95%CI: 87.0 – 95.1), 95.5% (95%CI: 91.7 – 98.0) and 63.3% (95%CI: 46.5 – 79.0) were obtained for the Se, Sp, NPV, and the PPV, respectively. For older cows, using the last DHI test with a SCC threshold of 200,000 cells/mL yielded the highest NPV. Median estimates of 75.3% (95%CI: 55.8– 87.3), 84.0% (95%CI: 78.8 – 89.3), 94.7% (95%CI: 89.0 – 97.6) and 47.2% (95%CI: 32.0 – 63.7) were obtained for Se, Sp, NPV, and PPV, respectively. Complete results for the LCM when only the last DHI test was considered and with a SCC threshold of 200,000 cells/mL are presented in Table 12. The median estimates of Se, Sp, NPV and PPV of quarter-level Petrifilm® and of quarter-level laboratory-based culture obtained using the model where they were compared with last DHI test SCC with a threshold of 200,000 cells/mL are also presented in Table

12. The estimates were 82.2% (95%CI: 74.0 – 89.5) and 62.0% (95%CI: 58.6 – 65.6) for Se and Sp of quarter-level Petrifilm® and 67.4% (95%CI: 55.8 – 81.2) and 79.6% (95%CI: 76.4 – 83.0) for Se and Sp of quarter-level laboratory-based culture. The NPV of these tests were comparable to that of cow-level SCC, but with lower PPV. The covariance term for conditional dependence between quarter-level Petrifilm® and quarter-level laboratory-based culture and the mean prevalence of quarter that should be treated with antimicrobials at dry off across the studied 9 herds and its precision are also presented in this table. The conditional dependence estimates from the LCM comparing the last SCC test with a threshold of 200,000 cells/mL, a quarter-level Petrifilm® and quarter-level laboratory-based culture resulted in a positive covariance term of 8.3 (95% CI: 1.4 – 13.1) and in a negative covariance term of 8.1 (95% CI: 6.3 – 9.8), suggesting that both Petrifilm® and standard bacteriology could miss a quarter which should be treated or allow a quarter to be treated while it should not (i.e., they are dependent conditionally on the identification of quarters that should be treated and on quarters that should not).

Combining cow-level SCC with subsequent quarter-level Petrifilm® results

Figures 18A and 18B present, respectively, the posterior distributions of the Se, Sp, and predictive values of the thresholds of 100,000 and 200,000 cells/mL for first parity cows, and of 200,000 cells/mL for older cows, when the last DHI test is used alone vs. in combination with a subsequent quarter-level Petrifilm® result. Confirming antimicrobial treatment of a quarter with a Petrifilm® result increased the Sp and reduced the Se. As a consequence, this serial test strategy increased the PPV, but with very little change of the NPV (a reduction of around 2%), for both SCC thresholds and for cows of all parities. The PPV was improved from 58.0 to 77.4% and from 63.3 to 79.0% for first parity cows at SCC thresholds of 100,000 and 200,000 cells/mL, respectively, and from 47.2 to 65.9% for older cows at SCC threshold of 200,000 cells/mL.

Sensitivity analysis

For all cows, the sensitivity analysis was conducted only for the LCM comparing the last DHI test SCC with a threshold of 200,000 cells/mL with Petrifilm® on-farm culture and standard bacteriology and for the same model, but using the last DHI test SCC with a threshold of 100,000 cells/mL. For most parameters, perturbing the priors yielded difference of less than 5 percentage-

points (Table 13 and Table 14). Some parameters, however, were more strongly affected by the perturbed priors (a change of more than 10 percentage points). More specifically, the Se of Petrifilm® changed from 85.3 to 69.5% and the Se of standard bacteriology from 69.4 to 48.8%, in the model comparing these with a SCC threshold of 100,000 cells/mL. In the model comparing the culture-based tests with the SCC-based selection with a threshold of 200,000 cells/mL, the Se of Petrifilm® changed from 82.2 to 57.3% and the Se of standard bacteriology went from 67.4 to 39.7% when using the perturbed priors. Moreover, in that model, the Se of the last DHI test SCC changed from 77.7 to 60.5% for first parity cows, and the PPV changed from 47.2 to 77.8% for older cows when using the perturbed priors. DIC statistics imply that the model considering covariance between Petrifilm® and standard bacteriology is superior to models with covariance between SCC and Petrifilm® and SCC and standard bacteriology.

Antimicrobial reduction

The proportion of untreated quarters (i.e., of antimicrobial use reduction) was estimated for all DHI SCC-based approaches and for the combination of the last DHI test of the cow with a subsequent quarter-level milk culture using Petrifilm® and are presented in Figure 19 and in Table 15. In general, more quarters were left untreated with antimicrobials when using simply the last DHI test, compared to using all DHI tests available or only the last three DHI tests. Moreover, the estimated reduction was greater when the mean of the DHI tests was considered instead of each individual value and when using higher SCC thresholds to trigger treatment. Proportion of untreated quarters was estimated at 76.3% (95%CI: 73.5 – 78.9) when using last DHI test SCC with a threshold of 100,000 cells/mL for first parity cows and of 200,000 cells/mL for older cows. When using 200,000 cells/mL for all cows, the proportion of untreated quarters was 80.1% (95%CI: 77.4 – 82.5). Finally, adding subsequent quarter-level Petrifilm® results to the last DHI test with a SCC threshold of 200,000 cells/mL for all cows resulted in an estimated proportion of untreated quarters of 89.9% (95%CI: 87.8 – 91.7).

Discussion

This study assessed the diagnostic accuracy of different cow-level SCC based approaches, of a quarter-level on-farm milk culture using Petrifilm®, and of a quarter-level standard bacteriological

culture in the laboratory for the identification of quarters/cows requiring an antimicrobial treatment at dry off. Our most important findings were that: 1) using only the last DHI SCC test appeared sufficient for selecting cows to treat with an antimicrobial at dry off; 2) a SCC threshold of 200,000 cells/mL for all cows or, alternatively, of 100,000 cells/mL for first parity cows and 200,000 cells/mL for older cows could be used; 3) adding to cow-level SCC a quarter-milk Petrifilm® culture to confirm quarters to be treated would increase (but only slightly) the PPV and further reduce proportion of treated quarters; and 4) a reduction of antimicrobial treatments of around 90% can be achieved using the last DHI test SCC followed with a quarter-milk Petrifilm® for SCC-positive cows testing scenario.

Accuracy of cow-level SCC-based approaches

The estimated tests characteristics indicated that SCC data could be used to decide whether a cow should be treated with antimicrobials at dry off. Among the different evaluated scenarios, the use of the last DHI test seems to be very favorable with relatively good test accuracy, high NPV, and an important reduction of antimicrobial use at dry off. In addition, it is the easiest approach to implement on-farm for deciding which cows should be treated at dry off. Thresholds of > 100,000 cells/mL or of > 200,000 cells/mL of milk could be used for first parity cows, while a threshold of >200,000 cells/mL resulted in good test accuracy for older cows. These thresholds maximized the NPV when using SCC-based approaches. In fact, when implementing selective dry cow treatment, achieving a high NPV is essential since, in most settings, a false negative case (i.e., an infection that should be treated, but is not) would be costlier than a false positive case (i.e., an antimicrobial is given to a quarter or cow that does not need it). Since there were very little differences in NPV and PPV for 1st parity cows when using the 100,000 vs. 200,000 cells/mL threshold, a very simple approach could be to use a SCC threshold of 200,000 cells/mL for all cows, regardless of age. Previously, this threshold of > 200,000 cells/mL has been reported to be optimal for detecting IMI (Dohoo and Leslie, 1991, Schepers et al., 1997, Vasquez et al., 2018).

Other studies (Addis et al., 2016, Jaeger et al., 2017, Vissio et al., 2014) reported on the accuracy of SCC to detect intramammary infections using LCM. Addis et al. (2016) were interested in all identified bacterial species, regardless of their udder-health importance and did not investigate

whether SCC accuracy varied between first parity and older cows. Vissio et al. (2014) and Jaeger et al. (2017) considered only major pathogens. Thus, milk samples from quarters yielding non-*aureus staphylococci* or *Corynebacterium bovis* were not considered as relevant, while they were considered as such in our study. These differences in the identification of quarters or cows that should be treated explain differences with these previous studies, regarding our estimates for the test accuracy of DHI SCC or standard bacteriology culture in the laboratory which was used as a reference test.

In our study, most of the isolated pathogens from quarters of cows that were identified as healthy using the last DHI test with a SCC threshold of > 200,000 cells/mL were non-*aureus staphylococci* (NAS; 46/62 isolates for first parity and 88/113 isolates for ≥ 2nd parity). This number was 33/49 isolates for the threshold of > 100,000 cells/mL for first parity cows. This is in agreement with previous studies that reported that IMI caused by minor pathogens (mostly NAS) induced relatively small or no SCC increase (Barkema et al., 1999, Schukken et al., 2003). As, these are the most common pathogens in our sample population, it is understandable to find a certain number of cows misclassified as uninfected using solely a SCC-based approach.

Accuracy of on-farm milk culture using Petrifilm®

No previous study has reported on the test accuracy of Petrifilm® on-farm culture to identify quarters that should be treated with antimicrobials at dry off, using a latent class approach. With its test accuracy, Petrifilm® on-farm culture provided less information compared with what could be obtained by using DHI tests SCC. In addition, quarter-level Petrifilm® on-farm culture require extra labor and cost, in comparison with a selective treatment relying on DHI tests SCC. Moreover, the reduction of antimicrobial use obtained using quarter-level Petrifilm® on-farm culture was lower, in comparison with the one obtained using the last DHI SCC. Hence, Petrifilm® on-farm culture may not be the first option, when we need to decide which quarter or cow should be treated with antimicrobials at dry off. However, it may be a valuable addition to the cow-level SCC data to differentiate quarters of infected cows based on SCC threshold that should be treated with antimicrobials at dry off.

Combination of cow-level SCC data with subsequent quarter-level Petrifilm® results

A cow with a high SCC may have only one or two infected quarters. Therefore, we investigated adding an on-farm quarter-milk culture using Petrifilm® to the cow-level SCC data. With this approach, in the case of a SCC greater than the threshold, results of Petrifilm® culture could be considered to decide which quarters must be treated with an antimicrobial. With this combination, the Se was reduced, but the Sp and PPV were improved, while the NPV was nearly unchanged. The PPV improved from 58.0 to 77.4% and from 47.2 to 65.9%, when Petrifilm® was added to the last DHI test with a threshold of 100,000 for the first parity cows or 200,000 cells/mL for older cows, respectively. Consequently, if Petrifilm® is added to the last DHI test, fewer quarters or cows would be treated with antimicrobials unnecessarily. This approach, however, would be considered quite cumbersome for many producers. Nevertheless, in situations where use of antimicrobials has to be greatly reduced, this selection strategy could help achieve very substantial reductions (i.e., approximately 90%).

Some authors suggested using CM historical data to determine whether a cow has to be treated at dry off (Rowe et al., 2020a, Torres et al., 2008, Vasquez et al., 2018). However, in our data, CM history provided little information beyond what was obtained using SCC data alone. For instance, only one cow had ≥ two CM cases during the lactation (a case-definition used by Rowe et al. (2020a) and Vasquez et al. (2018)), but this cow already had a last DHI test SCC of 690,000 cells/mL, well above all the thresholds investigated. Considering cows with a CM during the last three months of lactation (a case-definition used by Cameron et al. (2014)) would have led to a change of status for only two cows. This situation could be explained not only by the low frequency of CM during the last three months of lactation, but also by the fact that a cow with a CM case during this period would likely have a SCC > 100,000 or even >200,000 cells/mL at the last DHI test. In farm conditions, however, it would be very difficult to recommend not using antimicrobials when there was a CM case some days before dry off, especially if the CM occurred after the last DHI test. Therefore, it should probably be suggested to treat cows with CM during this period, even though, in our study, occurrence of CM during the last three months did not differ much from the last DHI test SCC-based status.

Sensitivity analysis

The sensitivity analysis showed that the posterior distributions were not very sensitive to perturbing our priors. This is possibly explained by the substantive amount of information available from our data, compared to what was provided by prior information. There was large variation for some parameters but, in most instances, there were also a large overlap in the posterior distributions from the main and alternative models. One exception was observed when a threshold of 200,000 cells/mL was considered in the model for the last SCC test. In this case, decreases for the Se estimates for Petrifilm® and standard bacteriology were observed (reduction of 25.0 and 27.7 percentage-points, respectively), but with little overlap this time between posterior distributions. The LCM was, thus, sensitive to the prior information used for these specific parameters. The initial prior distributions were generated using results from peer-reviewed articles and we are, therefore, confident in their validity (as compared, for instance with priors generated using expert opinions). However, none of those articles used LCM. In addition, those studies used a different disease definition than the current study (detecting all quarters with an intramammary infection vs. detecting quarters needing an antimicrobial treatment at dry off). So, we can hypothesize that this latter situation would explain the sensitivity of our models to the prior information used, in some scenarios of comparisons.

Strength and limitations of the study

One farm discontinued DHI testing over the course of the project and as a result, 42 cows from this herd were excluded from the analyses because of the interval between the last available test and dry off was ≥ 50 days. This herd was the second largest in terms of number of included cows in the analyses. In fact, this herd represented 30% (86 cows) of the 282 cows of the study.

In our study, an IMI was defined by the presence of ≥1 cfu/0.01mL of milk of any bacteria, as the objective was to identify as many infections as possible (Dohoo et al., 2011). This IMI definition was suggested to have a high Se and almost perfect Sp for most pathogens (Dohoo et al., 2011). Using ≥1 cfu/0.01mL as case definition, however, would have a lower Sp than a ≥2 cfu/0.01mL definition for some pathogens, such as the NAS, but would yield a substantially higher Se (Dohoo

et al., 2011). The liberal IMI definition used in our study explains, in part, the high proportion of NAS identified in quarters that were otherwise considered healthy based on SCC.

Also, in our study, we considered that not all bacterial species should be treated with antimicrobials at dry off. We defined quarters that should be treated with antimicrobial at dry off, based on bacterial species that are suspected to have a sufficient cure rate or recognized as significant for udder health. This definition differs from previous studies and, therefore, it is not possible to make a direct comparison with older studies. In fact, a previous study considered an antimicrobial treatment at dry off, regardless of the type of isolated pathogen (Addis et al., 2016). Others (Jaeger et al., 2017, Vissio et al., 2014) considered only major pathogens and therefore non-*aureus staphylococci* or *Corynebacterium bovis* were not considered as relevant for an antimicrobial treatment.

Selective dry-cow therapy may require more time and a certain expertise to identify cows or quarters to be treated compared to blanket dry-cow therapy. However, selective dry-cow therapy based on the last DHI test SCC is simple, cheap, and easy to implement by producers. This study highlights the test accuracy of using the last DHI test cow-level SCC in selecting cows for an antimicrobial treatment at dry off. This study included herds with an average bulk tank SCC below 250,000 cells/mL over the last 12 months (12 month mean herd bulk tank SCC range: 125,000 to 242,000 cells/mL). Hence, these results would be generalizable to herds with the same characteristics. Other investigation would be necessary for herds with an average bulk tank SCC ≥ 250,000 cells/mL.

Latent class models and assumptions

Diagnostic test accuracy is commonly evaluated through its comparison with a gold-standard test (i.e., a test that has perfect Se and Sp). When no gold-standard test are available, latent class models allow for estimating Se and Sp of the evaluated tests, as compared to another imperfect reference test, in a population where the underlying true infection status is unknown (Hui and Walter, 1980, Toft et al., 2005). When an imperfect reference test is wrongly used as a gold-standard test to evaluate the test accuracy of a new test, the new test would be penalized for being better than the imperfect reference test. This would result in an underestimation of the

new test's Se and Sp (Toft et al., 2007a). The approach used in our study did take into consideration this potential issue, and was, therefore, an important strength of this study.

Conclusion

These results suggest that SCC data could be used alone to decide whether an antimicrobial treatment is required at dry off. This would result in an important reduction of antimicrobial use. Moreover, using only the last DHI test seems to be a viable option since it was accurate, easy and simple to implement for dairy producers. The last SCC test with a threshold of 100,000 cells/mL for first parity cows and of 200,000 cells/mL for older cows would represent an optimal diagnostic strategy from an accuracy point of view. However, the last SCC test with a threshold of 200,000 cells/mL for all cows would represent an appropriate diagnostic strategy from the standpoint of simplicity and reduction of antimicrobial use and with relatively little loss of accuracy. Finally, testing all quarters of a cow identified as infected using SCC data with Petrifilm® could be used to further reduce use of antimicrobial at dry off.

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References

Addis, M. F., Tedde, V., Puggioni, G. M. G., Pisanu, S., Casula, A., Locatelli, C., Rota, N., Bronzo, V., Moroni, P. & Uzzau, S. (2016). Evaluation of milk cathelicidin for detection of bovine mastitis. *J. Dairy Sci.*, 99, 8250-8258.

Aghamohammadi, M., Haine, D., Kelton, D. F., Barkema, H. W., Hogeveen, H., Keefe, G. P. & Dufour, S. (2018). Herd-Level Mastitis-Associated Costs on Canadian Dairy Farms. *Front. Vet. Sci.*, 5.

Barkema, H. W., Deluyker, H. A., Schukken, Y. H. & Lam, T. J. (1999). Quarter-milk somatic cell count at calving and at the first six milkings after calving. *Prev. Vet. Med.*, 38, 1-9.

Brooks, S. P. & Gelman, A. (1998). General methods for monitoring convergence of iterative simulations. *J COMPUT GRAPH STAT.*, 7, 434-455.

Browning, J. W., Mein, G. A., Barton, M., Nicholls, T. J. & Brightling, P. (1990). Effects of antibiotic therapy at drying off on mastitis in the dry period and early lactation. *Aust. Vet. J.*, 67, 440-2.

Browning, J. W., Mein, G. A., Brightling, P., Nicholls, T. J. & Barton, M. (1994). Strategies for mastitis control: dry cow therapy and culling. *Aust. Vet. J.*, 71, 179-81.

Cameron, M., Barkema, H. W., De Buck, J., De Vliegher, S., Chaffer, M., Lewis, J. & Keefe, G. P. (2017). Identification of bovine-associated coagulase-negative staphylococci by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a direct transfer protocol. *J. Dairy Sci.*, 100, 2137-2147.

Cameron, M., Keefe, G. P., Roy, J. P., Dohoo, I. R., Macdonald, K. A. & Mckenna, S. L. (2013). Evaluation of a 3M Petrifilm on-farm culture system for the detection of intramammary infection at the end of lactation. *Prev. Vet. Med.*, 111, 1-9.

Cameron, M., Mckenna, S. L., Macdonald, K. A., Dohoo, I. R., Roy, J. P. & Keefe, G. P. (2014). Evaluation of selective dry cow treatment following on-farm culture: risk of postcalving intramammary infection and clinical mastitis in the subsequent lactation. *J. Dairy Sci.*, 97, 270-84.

Cameron, M., Perry, J., Middleton, J. R., Chaffer, M., Lewis, J. & Keefe, G. P. (2018). Short communication: Evaluation of MALDI-TOF mass spectrometry and a custom reference spectra expanded database for the identification of bovine-associated coagulase-negative staphylococci. *J. Dairy Sci.*, 101, 590-595.

Dendukuri, N. & Joseph, L. (2001). Bayesian approaches to modeling the conditional dependence between multiple diagnostic tests. *Biometrics*, 57, 158-67.

Dohoo, I. R. & Leslie, K. E. (1991). Evaluation of changes in somatic cell counts as indicators of new intramammary infections. *Prev. Vet. Med.*, 10, 225-237.

Dohoo, I. R., Martin, S. W. & Stryhn, H. (2009). Screening and diagnostic tests. Veterinary Epidemiologic Research. 2nd ed. Charlottetown, P.E.I., Canada: VER Inc., p. 91-133.

Dohoo, I. R., Smith, J., Andersen, S., Kelton, D. F. & Godden, S. (2011). Diagnosing intramammary infections: evaluation of definitions based on a single milk sample. *J. Dairy Sci.*, 94, 250-61.

Dufour, S., Dohoo, I. R., Barkema, H. W., Descôteaux, L., Devries, T. J., Reyher, K. K., Roy, J. P. & Scholl, D. T. (2012). Manageable risk factors associated with the lactational incidence, elimination, and prevalence of Staphylococcus aureus intramammary infections in dairy cows. *J. Dairy Sci.*, 95, 1283-300.

Dufour, S., Wellemans, V., Roy, J. P., Lacasse, P., Ordonez-Iturriaga, A. & Francoz, D. (2019). Non-antimicrobial approaches at drying-off for treating and preventing intramammary infections in dairy cows. Part 1. Meta-analyses of efficacy of using an internal teat sealant without a concomitant antimicrobial treatment. *Anim. Health Res. Rev.*, 20, 86-97.

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

Hanson, T., Johnson, W. O. & Gardner, I. A. (2003). Hierarchical models for estimating herd prevalence and test accuracy in the absence of a gold standard. *J Agric Biol Environ Stat.* , 8, 223.

Hassan, Z., Daniel, R. C., O'boyle, D. & Frost, A. J. (1999). Effects of dry cow intramammary therapy on quarter infections in the dry period. *Vet. Rec.*, 145, 635-9.

Hui, S. L. & Walter, S. D. (1980). Estimating the error rates of diagnostic tests. *Biometrics*, 36, 167-71.

Huxley, J. N., Greent, M. J., Green, L. E. & Bradley, A. J. (2002). Evaluation of the efficacy of an internal teat sealer during the dry period. *J. Dairy Sci.*, 85, 551-61.

Jaeger, S., Virchow, F., Torgerson, P. R., Bischoff, M., Biner, B., Hartnack, S. & Rüegg, S. R. (2017). Test characteristics of milk amyloid A ELISA, somatic cell count, and bacteriological culture for detection of intramammary pathogens that cause subclinical mastitis. *J. Dairy Sci.*, 100, 7419-7426.

Johnson, W. O., Jones, G. & Gardner, I. A. (2019). Gold standards are out and Bayes is in: Implementing the cure for imperfect reference tests in diagnostic accuracy studies. *Prev. Vet. Med.*, 167, 113-127.

Kabera, F., Dufour, S., Keefe, G., Cameron, M. & Roy, J. P. (2020). Evaluation of quarter-based selective dry cow therapy using Petrifilm on-farm milk culture: A randomized controlled trial. *J. Dairy Sci.*, 103, 7276-7287.

Kostoulas, P., Nielsen, S. S., Branscum, A. J., Johnson, W. O., Dendukuri, N., Dhand, N. K., Toft, N. & Gardner, I. A. (2017). STARD-BLCM: Standards for the Reporting of Diagnostic accuracy studies that use Bayesian Latent Class Models. *Prev. Vet. Med.*, 138, 37-47.

Lindstrom, U. B., Kenttamies, H., Arstila, J. & Tuovila, R. (1981). USEFULNESS OF CELL COUNTS IN PREDICTING BOVINE MASTITIS. *Acta agric. Scand.*, 31, 199-203.

Mahmmod, Y. S., Nonnemann, B., Svennesen, L., Pedersen, K. & Klaas, I. C. (2018). Typeability of MALDI-TOF assay for identification of non-aureus staphylococci associated with bovine intramammary infections and teat apex colonization. *J. Dairy Sci.*, 101, 9430-9438.

Mccarron, J. L., Keefe, G. P., Mckenna, S. L., Dohoo, I. R. & Poole, D. E. (2009). Laboratory evaluation of 3M Petrifilms and University of Minnesota Bi-plates as potential on-farm tests for clinical mastitis. *J. Dairy Sci.*, 92, 2297-305.

Mcdermott, M. P., Erb, H. N. & Natzke, R. P. (1982). Predictability by Somatic Cell Counts Related to Prevalence of Intrammary Infection Within Herds. *J. Dairy Sci.*, 65, 1535-1539.

National Mastitis Council 2016. Current concepts of bovine mastitis, Fifth Ed., New Prague, MN.

National Mastitis Council (2017a). Chapter one: Sample collection and handling. Laboratory Handbook on Bovine Mastitis. Third ed. New Prague, MN: National Mastitis Council Inc.

National Mastitis Council 2017b. Laboratory Handbook on Bovine Mastitis, New Prague, MN, National Mastitis Council Inc.

Pantoja, J. C., Hulland, C. & Ruegg, P. L. (2009). Dynamics of somatic cell counts and intramammary infections across the dry period. *Prev. Vet. Med.*, 90, 43-54.

Reyher, K. K., Dufour, S., Barkema, H. W., Des Côteaux, L., Devries, T. J., Dohoo, I. R., Keefe, G. P., Roy, J. P. & Scholl, D. T. (2011). The National Cohort of Dairy Farms—A data collection platform for mastitis research in Canada. *J. Dairy Sci.*, 94, 1616-1626.

Rindsig, R. B., Rodewald, R. G., Smith, A. R. & Spahr, S. L. (1978). Complete versus selective dry cow therapy for mastitis control. *J. Dairy Sci.*, 61, 1483-97.

Robert, A., Roussel, P., Bareille, N., Ribaud, D., Sérieys, F., Heuchel, V. & Seegers, H. (2008). Risk factors for new intramammary infections during the dry period in untreated dairy cows from herds using selective dry cow therapy. *Animal*, 2, 247-54.

Robinson, T. C., Jackson, E. R. & Marr, A. (1988). Mastitis incidence in quarters with different infections status at drying off and calving in two treatment groups. *Br. Vet. J.*, 144, 166-73.

Rowe, S. M., Godden, S. M., Nydam, D. V., Gorden, P. J., Lago, A., Vasquez, A. K., Royster, E., Timmerman, J. & Thomas, M. J. (2020). Randomized controlled non-inferiority trial investigating the effect of 2 selective dry-cow therapy protocols on antibiotic use at dry-off and dry period intramammary infection dynamics. *J. Dairy Sci.*, 103, 6473-6492.

Saini, V., Mcclure, J. T., Léger, D., Dufour, S., Sheldon, A. G., Scholl, D. T. & Barkema, H. W. (2012). Antimicrobial use on Canadian dairy farms. *J. Dairy Sci.*, 95, 1209-21.

Sanford, C. J., Keefe, G. P., Dohoo, I. R., Leslie, K. E., Dingwell, R. T., Descôteaux, L. & Barkema, H. W. (2006a). Efficacy of using an internal teat sealer to prevent new intramammary infections in nonlactating dairy cattle. *J. Am. Vet. Med. Assoc.*, 228, 1565-73.

Sanford, C. J., Keefe, G. P., Sanchez, J., Dingwell, R. T., Barkema, H. W., Leslie, K. E. & Dohoo, I. R. (2006b). Test characteristics from latent-class models of the California Mastitis Test. *Prev. Vet. Med.*, 77, 96-108.

Schepers, A. J., Lam, T. J. G. M., Schukken, Y. H., Wilmink, J. B. M. & Hanekamp, W. J. A. (1997). Estimation of Variance Components for Somatic Cell Counts to Determine Thresholds for Uninfected Quarters. *J. Dairy Sci.*, 80, 1833-1840.

Schukken, Y. H., Wilson, D. J., Welcome, F., Garrison-Tikofsky, L. & Gonzalez, R. N. (2003). Monitoring udder health and milk quality using somatic cell counts. *Vet. Res.*, 34, 579-96.

Sergeant, E. S. G. (2018). Epitools Epidemiological Calculators. Ausvet. Available at: http://epitools.ausvet.com.au.

Spiegelhalter, D. J., Best, N. G., Carlin, B. P. & Van Der Linde, A. (2002). Bayesian measures of model complexity and fit. *J R Stat Soc Series B Stat Methodol*, 64, 583-639.

Thomson, K., Rantala, M., Hautala, M., Pyörälä, S. & Kaartinen, L. (2008). Cross-sectional prospective survey to study indication-based usage of antimicrobials in animals: results of use in cattle. *BMC Vet. Res.*, 4, 15.

Toft, N., Akerstedt, J., Tharaldsen, J. & Hopp, P. (2007a). Evaluation of three serological tests for diagnosis of Maedi-Visna virus infection using latent class analysis. *Vet Microbiol*, 120, 77-86.

Toft, N., Innocent, G. T., Gettinby, G. & Reid, S. W. (2007b). Assessing the convergence of Markov Chain Monte Carlo methods: an example from evaluation of diagnostic tests in absence of a gold standard. *Prev. Vet. Med.*, 79, 244-56.

Toft, N., Jørgensen, E. & Højsgaard, S. (2005). Diagnosing diagnostic tests: evaluating the assumptions underlying the estimation of sensitivity and specificity in the absence of a gold standard. *Prev. Vet. Med.*, 68, 19-33.

Torres, A. H., Rajala-Schultz, P. J., Degraves, F. J. & Hoblet, K. H. (2008). Using dairy herd improvement records and clinical mastitis history to identify subclinical mastitis infections at dry-off. *J. Dairy Res.*, 75, 240-247.

Vasquez, A. K., Nydam, D. V., Foditsch, C., Wieland, M., Lynch, R., Eicker, S. & Virkler, P. D. (2018). Use of a culture-independent on-farm algorithm to guide the use of selective dry-cow antibiotic therapy. *J. Dairy Sci.*, 101, 5345-5361.

Vissio, C., Dieser, S. A., Agnelli, H. L., Odierno, L. M. & Larriestra, A. J. (2014). Accuracy of the composite somatic cell count to detect intra-mammary infection in dairy cows using latent class analysis. *Prev. Vet. Med.*, 113, 547-555.

Woolford, M. W., Williamson, J. H., Day, A. M. & Copeman, P. J. (1998). The prophylactic effect of a teat sealer on bovine mastitis during the dry period and the following lactation. *N Z Vet J*, 46, 12-9.

Tableau 9. – List of bacterial species retrieved in the study on selective dry cow treatment in 9 dairy herds from Québec, Canada and their categorization (should or should not be treated with antimicrobials at dry off in dairy cows; based on current NMC's recommendations), along with number of samples from which they were retrieved.

Should be treated	**Should not be treated**	
	Low cure rate	**Not significant for treatment**
Aerococcus viridans (n=18)	*Pseudomonas* species (n=1)	Unspeciated Aerococcus (n=11)
Enterococcus faecalis (n=1)	*Trueperella pyogenes* (n=1)	Acinetobacter species (n=2)
Escherichia coli (n=3)		Bacillus species (n=2)
Lactococcus garvieae (n=1)		*Brachybacterium faecium* (n=1)
Staphylococcus aureus (n=21)		*Brevibacillus agri* (n=1)
Non-*aureus staphylococci* (n=189)		*Corynebacterium ammoniagenes* (n=1)
Corynebacterium bovis (n=31)		*Corynebacterium amycolatum* (n=3)
Unspeciated Corynebacterium (n=14)		*Corynebacterium casei* (n=1)
Streptococcus dysgalactiae (n=4)		*Corynebacterium glutamicum* (n=1)
Streptococcus uberis (n=3)		*Corynebacterium stationis* (n=4)
		Corynebacterium xerosis (n=8)
		Kocuria species (n=7)
		Other Gram positive (n=9)
		Streptococcus parauberis (n=1)

Tableau 10. – Prior distributions used in a latent class model comparing SCC obtained from DHI data, Petrifilm® on-farm milk culture, and standard milk bacteriology for identifying quarters to treat with antimicrobials in dairy cows at dry off.

Test	References	Parameter	Mode	5th or 95th percentile	Corresponding distribution
Petrifilm®	Cameron et al. (2013)	Se	0.852	0.785	Beta (91.1, 16.6)
		Sp	0.732	0.664	Beta (98.5, 36.7)
Standard bacteriology	Sanford et al. (2006b), Dohoo et al. (2011)	Se	0.904	0.810	Beta (6.4, 5.8)
		Sp	0.726	0.610	Beta (37.1, 14.6)
Last DHI test SCC with threshold of 200,000 cells/mL	McDermott et al. (1982), Dohoo and Leslie (1991), Schepers et al. (1997), Pantoja et al. (2009)	Se	0.770	0.640	Beta (30.6, 9.8)
		Sp	0.805	0.589	Beta (13.1, 3.9)
Last test DHI SCC with threshold of 150,000 cells/mL	Pantoja et al. (2009)	Se	0.510	0.760	Beta (4.9, 4.7)
		Sp	0.690	0.600	Beta (57.4, 26.4)
		Se	0.846	0.650	Beta (15.6, 3.7)

Last test DHI SCC with threshold of 100,000 cells/mL	Lindstrom et al. (1981), Pantoja et al. (2009)	Sp	0.490	0.830	Beta (2.5, 2.5)
Last test DHI SCC with threshold of 50,000 cells/mL	Pantoja et al. (2009)	Se Sp	0.860 0.400	0.940 0.370	Beta (16.5, 3.5) Beta (280.1, 419.6)
All other SCC approaches and thresholds	Not available	Se Sp	--- ---	--- ---	Beta (1.0, 1.0) Beta (1.0, 1.0)
---	Dendukuri and Joseph (2001)	Covp	NA	NA	Uniform (a, b)[a]
---	Dendukuri and Joseph (2001)	Covn	NA	NA	Uniform (c, d)[a]
---	Cameron et al. (2013), Rowe et al. (2020a)	Mu	0.180	0.250	Beta (20.3, 88.8)
---	Reyher et al. (2011)	Psi	NA	NA	Gamma (3.2, 0.31)

Sensitivity (Se); specificity (Sp); covariance between Se of Petrifilm® and standard milk bacteriology (Covp); covariance between Sp of Petrifilm® and standard milk bacteriology (Covn); mean herd-prevalence of quarters to treat at dry off (Mu); spread of the distribution of herd-prevalence of quarters to treat at dry off based on Hanson et al. 2003 (Psi).

[a] Where a is (1-Petrifilm® Se)*(Se standard bacteriology-1); b is the smallest Se between Petrifilm® and standard bacteriology minus the product of these Se; c is (Petrifilm® Sp-1)*(1- standard bacteriology Sp); and d is the smallest Sp between Petrifilm® and standard bacteriology minus the product of these Sp.

Tableau 11. – Cross-tabulated results of last DHI test SCC at a threshold of 200,000 cells/mL of milk (SCC), of quarter-level Petrifilm® on-farm culture (Petri) and of quarter-level standard bacteriology in laboratory (Lab) for the detection of quarters that should be treated with antimicrobial at dry off, using a sample of 905 quarters from 282 dry cows from 9 dairy herds from Québec, Canada.

	Parity = 1									Parity ≥ 2								
Herd	Petri+ Lab+ SCC+	Petri+ Lab- SCC+	Petri- Lab+ SCC+	Petri- Lab- SCC+	Petri+ Lab+ SCC-	Petri+ Lab- SCC-	Petri- Lab+ SCC-	Petri- Lab- SCC-	**Total**	Petri+ Lab+ SCC+	Petri+ Lab- SCC+	Petri- Lab+ SCC+	Petri- Lab- SCC+	Petri+ Lab+ SCC-	Petri+ Lab- SCC-	Petri- Lab+ SCC-	Petri- Lab- SCC-	**Total**
1	4	5	0	3	3	6	0	8	**29**	0	1	0	3	5	2	1	3	**15**
2	3	2	0	3	6	9	1	20	**44**	1	1	1	0	10	21	0	40	**74**
3	2	1	0	1	4	4	0	8	**20**	0	0	0	0	26	9	1	15	**51**
4	1	0	0	3	8	7	1	7	**27**	2	4	0	2	11	18	0	14	**51**
5	1	0	1	2	1	4	0	14	**23**	4	6	2	7	3	12	3	20	**57**
6	4	5	0	5	2	10	0	11	**37**	11	9	1	20	7	8	3	14	**73**
7	2	0	1	1	7	2	1	49	**63**	11	1	0	26	11	5	7	41	**102**
8	2	1	0	1	6	6	3	17	**36**	1	0	0	3	3	7	0	2	**16**
9	2	1	0	1	11	21	5	54	**95**	0	3	0	1	9	20	5	54	**92**
Total	**21**	**15**	**2**	**20**	**48**	**69**	**11**	**188**	**374**	**30**	**25**	**4**	**62**	**85**	**102**	**20**	**203**	**531**

Tableau 12. – Median estimates (95% credibility interval) for sensitivity (Se), specificity (Sp) and positive (PPV) and negative predictive values (NPV) of the last DHI test SCC with a threshold of 200,000 cells/mL, of quarter-level Petrifilm® on-farm culture, and of quarter-level standard bacteriology in laboratory for the identification of quarters that should be treated with antimicrobial in 905 quarters from 282 dry cows from 9 dairy herds Québec, Canada. Estimates were obtained using a latent class model allowing for conditional dependence between Petrifilm® on-farm culture and standard bacteriology in laboratory and allowing for different accuracy of SCC in first lactation (first parity cows) vs. older cows (parity≥2).

Parameter	**Median estimate**	**95%CI**
Lab Se	67.4	55.8 – 81.2
Petri Se	82.2	74.0 – 89.5
SCC Se - First parity cows	77.7	64.7 – 88.0
SCC Se - Parity ≥2	75.3	55.8– 87.3
Lab Sp	79.6	76.4 – 83.0
Petri Sp	62.0	58.6 – 65.6
SCC Sp - First parity cows	91.3	87.0 – 95.1
SCC Sp - Parity ≥2	84.0	78.8 – 89.3
Lab NPV	92.8	87.8 – 96.4
Petri NPV	94.8	90.8 – 97.4
SCC NPV - First parity cows	95.5	91.7 – 98.0
SCC NPV - Parity ≥2	94.7	89.0 – 97.6
Lab PPV	39.0	27.8 – 51.3

Petri PPV	29.4	20.7 – 39.4
SCC PPV - First parity cows	63.3	46.5 – 79.0
SCC PPV - Parity ≥2	47.2	32.0 – 63.7
Mu	16.2	11.0 – 22.7
Psi	6.1	2.2 – 14.7
CovN	8.1	6.3 – 9.8
CovP	8.3	1.4 –13.1

Covariance between Se of Petrifilm® and standard milk bacteriology (Covp); covariance between Sp of Petrifilm® and standard milk bacteriology (Covn); mean herd-prevalence of quarters to treat at dry off (Mu); spread of the distribution of herd-prevalence of quarters to treat at dry off based on Hanson et al. 2003 (Psi)

Tableau 13. – Posterior median and 95% credible intervals of the initial Bayesian latent class model and the model with perturbed priors used for estimating test accuracy of a cow-level SCC-based approach with a threshold of 200,000 cells/mL on last DHI test, of quarter-level Petrifilm® on-farm culture, and of quarter-level standard bacteriology in nine dairy herds from Québec, Canada. All parameters are presented in percentage.

	Initial model				Model with perturbed priors			
	Last 200		Petrifilm®	Standard bacteriology	Last 200		Petrifilm®	Standard bacteriology
Parameter	First parity cows	≥2 parity cows			First parity cows	≥2 parity cows		
Se	77.7 (64.7 – 88.0)	75.3 (55.8 – 87.3)	82.2 (74.0 – 89.5)	67.4 (55.8 – 81.2)	60.5 (42.2 - 80.0)	83.3 (69.3 - 93.7)	57.3 (48.8 - 66.4)	39.7 (31.6 - 49.0)
Sp	91.3 (87.0 – 95.1)	84.0 (78.8 – 89.3)	62.0 (58.6 – 65.6)	79.6 (76.4 – 83.0)	92.9 (88.3 - 96.9)	94.5 (89.2 - 98.1)	58.6 (54.8 - 62.5)	77.7 (74.4 - 80.9)
NPV	95.5 (91.7 – 98.0)	94.7 (89.0 – 97.6)	94.8 (90.8 – 97.4)	92.8 (87.8 – 96.4)	91.2 (84.8 - 96.0)	96.1 (91.7 - 98.7)	85.6 (79.0 - 91.0)	84.8 (78.4 - 90.1)
PPV	63.3 (46.5 – 79.0)	47.2 (32.0 – 63.7)	29.4 (20.7 – 39.4)	39.0 (27.8 – 51.3)	66.4 (45.9 - 84.9)	77.8 (58.8 - 91.9)	24.3 (16.5 - 33.3)	29.2 (19.8 - 40.0)

Tableau 14. – Posterior median and 95% credible intervals of the initial Bayesian latent class model and the model with perturbed priors used for estimating test accuracy of a cow-level SCC-based approach with a threshold of 100,000 cells/mL on last DHI test, of quarter-level Petrifilm® on-farm culture, and of quarter-level standard bacteriology in nine dairy herds from Québec, Canada. All parameters are presented in percentage.

	Initial latent class model				Latent class model with perturbed priors			
	Last 100		Petrifilm®	Standard	Last 100		Petrifilm®	Standard
Parameter	First parity cows	≥2 parity cows		bacteriology	First parity cows	≥2 parity cows		bacteriology
Se	85.6 (69.6 – 95.6)	77.9 (62.9 - 91.9)	85.3 (77.5 - 91.2)	69.4 (57.7 - 82.7)	86.4 (69.3 - 96.8)	85.7 (67.9 - 96.4)	69.5 (59.3 - 82.6)	48.8 (39.2 - 61.4)
Sp	86.0 (80.0 – 91.7)	58.9 (53.2 - 64.9)	65.6 (61.8 - 69.6)	83.1 (79.6 - 86.8)	89.2 (83.0 - 94.2)	63.2 (56.2 - 70.2)	62.8 (58.6 - 67.4)	81.0 (77.3 - 84.8)
NPV	96.4 (91.3 – 99.0)	92.2 (85.0 - 97.4)	95.2 (91.3 - 97.5)	92.3 (87.1 - 96.2)	96.4 (90.9 - 99.2)	94.8 (87.0 - 98.8)	89.4 (83.0 - 94.6)	86.5 (80.4 - 91.6)
PPV	58.0 (42.3 – 74.2)	30.2 (20.8 - 41.3)	36.2 (26.5 - 46.7)	48.5 (36.2 - 61.2)	66.3 (49.1 - 81.4)	36.4 (24.7 - 48.8)	31.7 (22.3 - 42.3)	39.0 (27.2 - 52.2)

Tableau 15. – Proportion of untreated quarters, when using quarter-level Petrifilm®, quarter-level laboratory-based milk culture, or different approaches using cow-level SCC from the last DHI test alone or in combination with a subsequent quarter-level milk culture using Petrifilm® in nine dairy herds from Québec, Canada.

Parameter	**Parity**	**SCC threshold (in 1000 cells/mL)**	**% untreated**	**95% CI**
Petrifilm®	All	-	58.0	55.1 -60.9
Standard bacteriology	All	-	75.3	72.6 – 77.7
Last test SCC	First	100	75.3	70.7 – 79.3
Last test SCC	First	200	84.5	80.6 – 87.8
Last test SCC	≥ 2nd	200	77.0	73.3 – 80.3
Last test SCC	All	200	80.1	77.4 – 82.5
Last test SCC	All	100 for 1st/200 for ≥ 2nd	76.3	73.5 - 78.9
Last test SCC+ Petrifilm®	First	100	85.8	81.9 – 88.9
Last test SCC+ Petrifilm®	First	200	90.4	87.1 – 93.0
Last test SCC+ Petrifilm®	≥ 2nd	200	89.5	86.7 – 91.8
Last test SCC+ Petrifilm®	All	200	89.9	87.8 – 91.7
Last test SCC+ Petrifilm®	All	100 for 1st/200 for ≥ 2nd	88.0	85.7 – 89.9

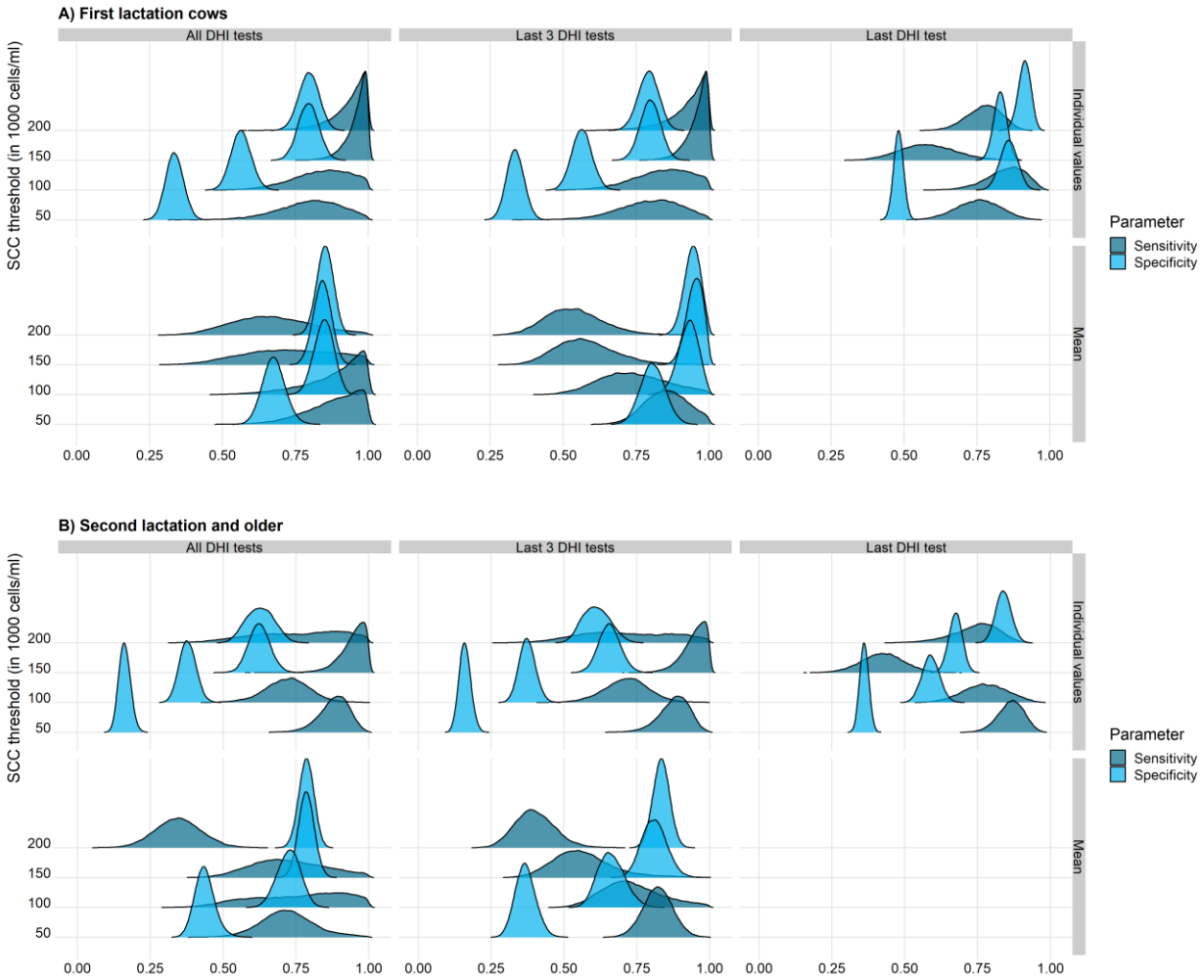

Figure 16. – Posterior distributions of accuracy parameters for different cow-level SCC-based approaches (last DHI test, last three DHI tests, all DHI test of the lactation) interpreted using the mean or each individual test values, and using different somatic cell count thresholds to determine quarters that should be treated with antimicrobials at dry off in A) first lactation cow; and B) older cows.

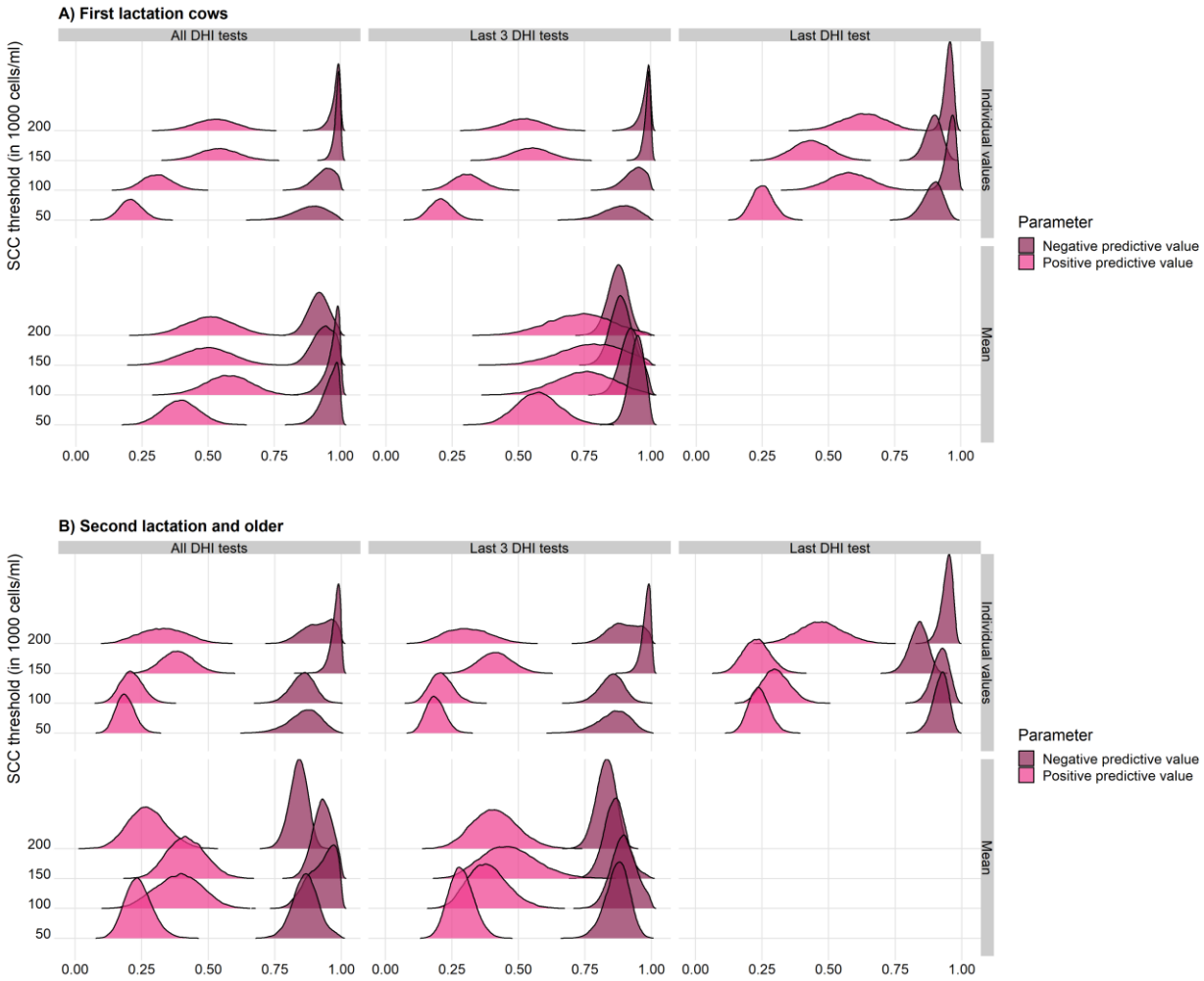

Figure 17. – Posterior distributions of the positive and negative predictive values for different cow-level SCC-based approaches (last DHI test, last three DHI tests, all DHI tests of the lactation) interpreted using the mean or each individual values, and using different somatic cell count thresholds to determine quarters that should be treated with antimicrobials at dry off in A) first lactation cow; and B) older cows.

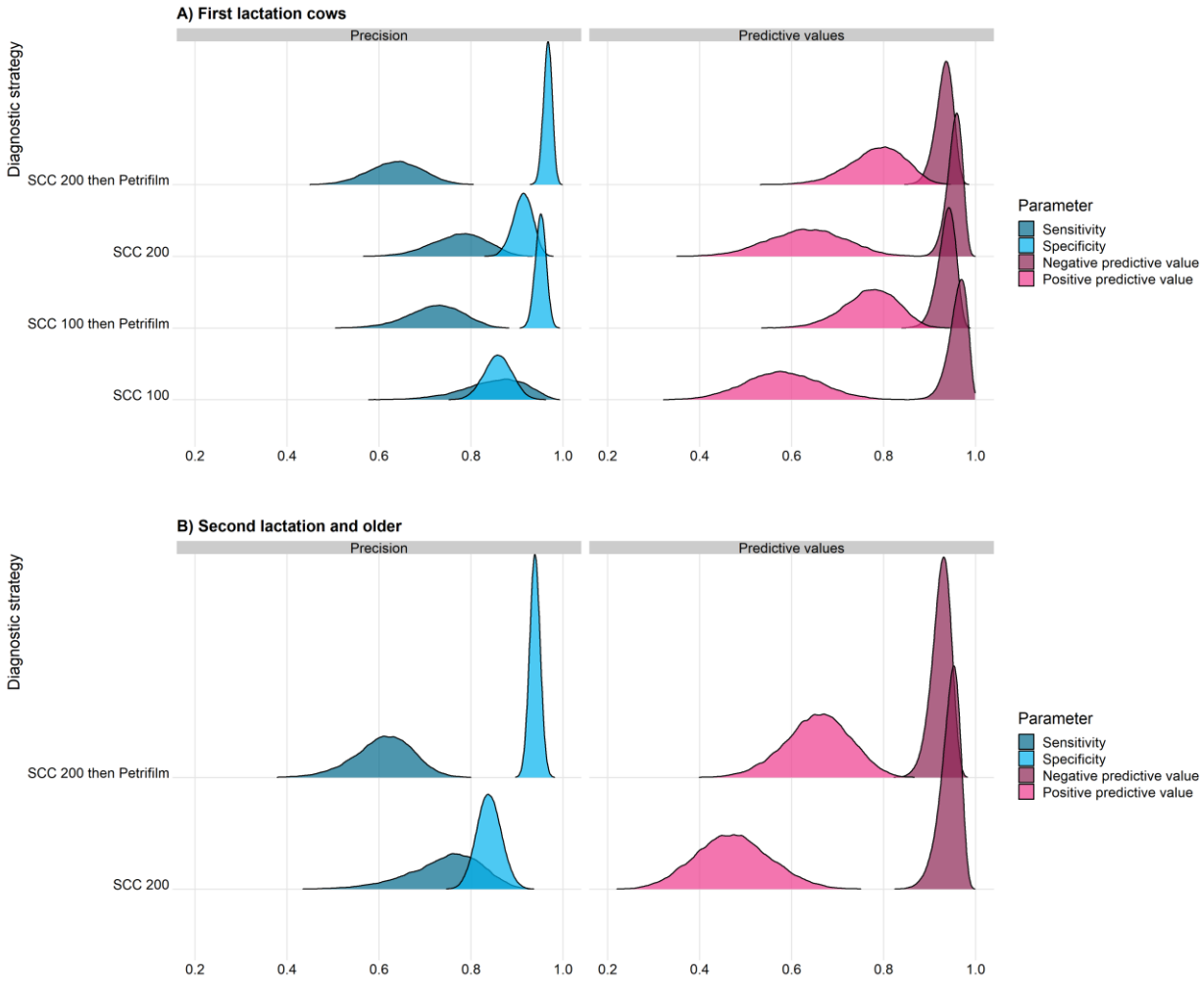

Figure 18. – Posterior distributions of accuracy parameters and predictive values when using last cow-level DHI test somatic cell count followed or not by quarter-milk bacteriology analyses using Petrifilm® to determine quarters or cows eligible for an antimicrobial treatment at dry off in A) first lactation cow; and B) older cows.

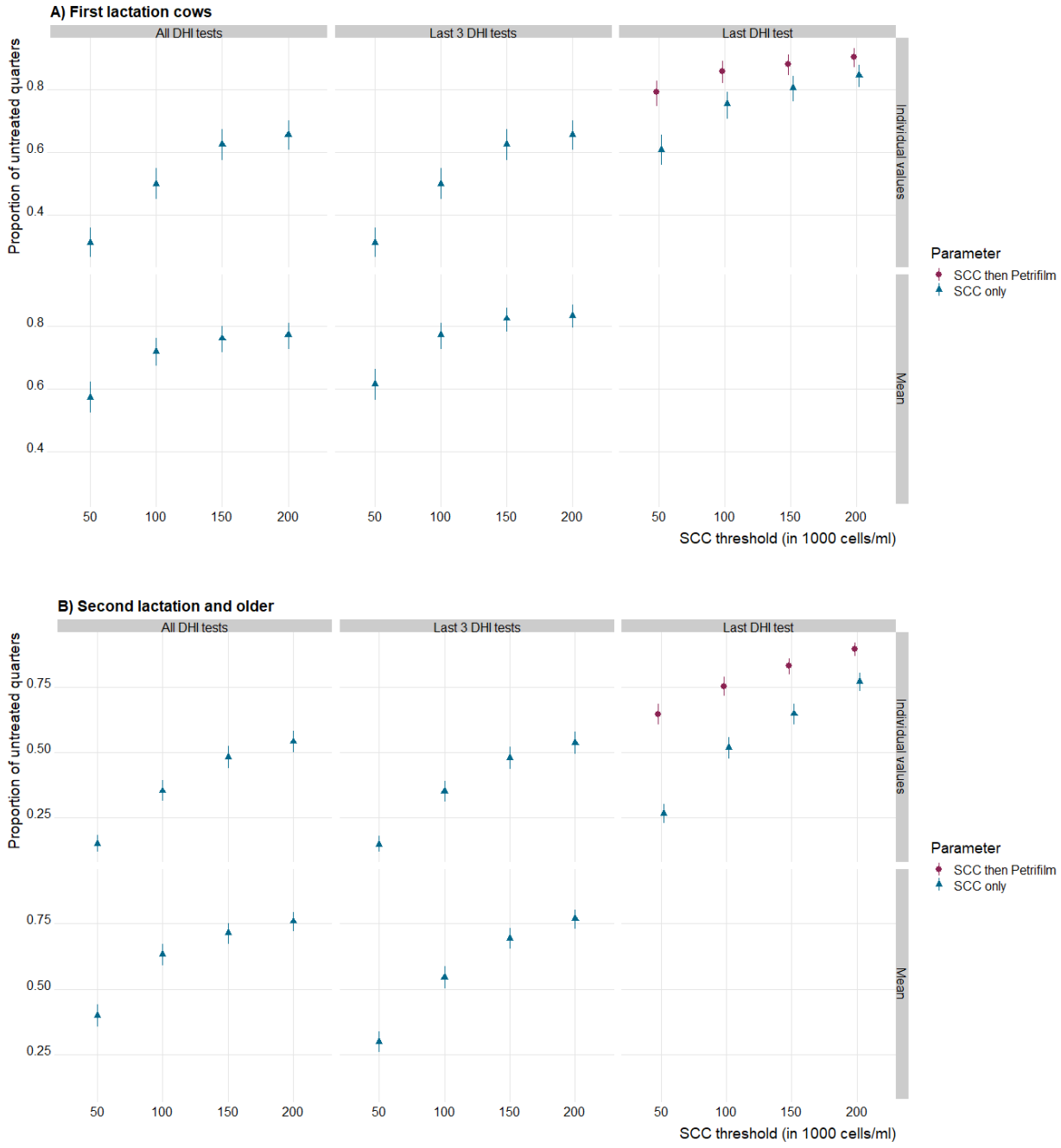

Figure 19. – Estimated reduction in use of antimicrobial when using different cow-level SCC-based approaches (last DHI test, last three DHI tests, all DHI tests of the lactation) interpreted using the mean or each individual values, and using different somatic cell count thresholds and, for some scenarios, when combined with a subsequent quarter-level Petrifilm® result to determine quarters that should be treated with antimicrobials at dry off in A) first lactation cows; and B) older cows.

Chapitre 7 – An observational cohort study on persistency of an internal teat sealant residues in milk after calving in dairy cows

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Abstract

Our objectives were to evaluate the prevalence of quarters with an observable internal teat sealant (ITS) plug at first milking following calving and investigate persistency of ITS residues in milk after calving. An observational cohort study was carried out on 557 quarters of 156 cows treated with ITS in 6 farms in Quebec, Canada. The presence of an ITS plug at first milking and ITS residues in milk at each milking were observed by producers. The effects of various factors on the odds of observing an ITS plug and persistency of ITS residues in milk were studied using generalized logistic mixed and generalized negative binomial mixed models, respectively. Milk samples were taken on the day before dry-off and on 2 occasions after calving for bacterial

identification to detect intramammary infection (IMI) using bacteriological culture followed by MALDI-TOF identification. The association between the absence of an ITS plug and the presence of new IMI was assessed using a mixed logistic regression model. Internal teat sealant plugs after calving were more often observed in rear quarters and in quarters receiving ITS alone at drying-off versus antimicrobial and ITS. We observed an average (standard deviation) persistency of 4.0 d (2.3 d). When an ITS plug was still present at first milking (83% of quarters), the elimination of ITS residues in milk after calving was significantly longer (4.5 d, on average) compared with 1.2 d when an ITS plug was absent. In cows with an ITS plug at calving, we observed a higher number of days of excretion in older cows. When a plug could not be observed, rear quarters, older cows, and cows with a long dry period duration excreted ITS residues for a significantly longer period. The lack of a significant association between the absence of a plug and the odds of new IMI at calving suggests that despite the loss of the plug, cows were still protected against new IMI. Although we were able to highlight some statistically significant risk factors explaining persistency of ITS residues following calving, observed differences were often relatively small and, perhaps, not clinically relevant. In conclusion, an ITS plug was present until first milking after calving for 83% quarters, quarters without an ITS plug at first milking appeared to have been protected from new IMI, and ITS residues could be observed in milk up to 12 d in milk.

Key words: internal teat sealant, residue, intramammary infection, calving, dry period

Introduction

Dairy cows are at high risk of developing new IMI (NIMI) during the dry period, which often remain undetected until calving or even a long time after calving in some cases (Bradley, 2002, Smith et al., 1985b). These dry-period-acquired NIMI combined with IMI that persist from the previous lactation are important determinants of the IMI prevalence in the subsequent lactation (Green et al., 2002). Factors that influence the susceptibility to NIMI at the beginning of the dry period include the functional transition associated with mammary involution, the delay in the complete formation of keratin plug in the teat canal, and the cessation of teat sanitization (Dingwell et al., 2003, Halasa et al., 2009b, Smith et al., 1985b). In fact, a previous study in New Zealand observed that 50% of teats did not develop a keratin plug during the first 10 d of the dry period (Williamson

et al., 1995). Furthermore, other studies reported that 23% of teats were still open up to 6 wk after drying off and, for 3 to 5% of teats, a keratin plug was never observed (Dingwell et al., 2004, Williamson et al., 1995).

To prevent NIMI during the dry period, the application of an internal teat sealant (ITS) alone or combined with the administration of antimicrobial is now widely used. An ITS forms a physical barrier to the entry of bacteria responsible for mastitis and thus reduces the risk of NIMI occurring during the dry period (Berry and Hillerton, 2002a, Godden et al., 2003, Sanford et al., 2006a). For example, Orbeseal (Zoetis Canada, Kirkland, Quebec, Canada) is an ITS consisting of bismuth subnitrate formulated into an inert viscous malleable paste. It is a sterile and non-antimicrobial intramammary infusion. Use of ITS can complement or provide an alternative to antimicrobial dry cow therapy to protect quarters during the dry period (Berry and Hillerton, 2002b, Huxley et al., 2002, Woolford et al., 1998). Such strategy, which does not involve antimicrobials, is of considerable importance because of public health concerns on antimicrobial resistance and antimicrobial residues in milk. The ITS is not absorbed systemically from the mammary gland and can persist in the teat for at least 100 d during the dry period (Woolford et al., 1998). It remains in the teat cistern over the dry period until it is physically removed manually at first milking, or by suckling by the calf.

Currently, few studies have evaluated the proportion of quarters at first milking after calving that still have an ITS plug. The proportion of quarters truly protected through the entire dry-off period is, therefore, not well described. Furthermore, it is not clear whether quarters, having lost the sealant plug before first milking, were still substantially protected from NIMI during the dry period. Finally, the risk factors that may influence the persistence of sealant plug until the first milking are not well described.

Moreover, few data are currently available on the persistency of ITS residues in milk after calving and on factors affecting ITS excretion following calving have not been reported. Some authors reported presence of sealant residues in milk up to 3 wk after calving Berry and Hillerton (2002a). Bhutto et al. (2011) reported that most of the product was eliminated at the first milking, but that

some residues may be observed over the subsequent milkings. However, these authors did not investigate the average duration of residue excretion.

Consequently, the primary objectives of the current study were to (1) quantify prevalence of quarters with an observable sealant plug at first milking following calving, (2) investigate persistency of ITS residues in milk after calving, and (3) identify risk factors that could affect presence of an ITS plug at calving and number of days of ITS excretion after calving. A secondary objective of the study was to investigate whether quarters without an observable sealant plug at the first milking after calving were equally protected from NIMI acquisition during the dry period compared with those with an observable sealant plug.

Materials and methods

Participants

The current study was an observational cohort study performed on 557 quarters from 156 cows treated with ITS in 6 dairy farms in Quebec (Canada) between October 2015 and July 2016. This cohort of 6 farms was a convenience sample from a larger randomized controlled trial (RCT) on quarter-based selective dry cow therapy conducted on 9 farms. From that larger sample, only farms where the milking staff agreed to record the presence of residues in milk following calving were selected. For the RCT, herd inclusion criteria were (1) a bulk tank SCC mean <250,000 cells/mL over the last year, (2) a targeted dry period of 35 to 75 d, (3) participation in a DHI program, and (4) willingness to commit to the project protocol. In these herds, all pregnant dairy cows ready for drying-off, having at least 3 functional quarters, and not treated with antimicrobials during the 14 d before dry-off were enrolled. Cows that failed to meet the inclusion criteria were treated as per routine farm procedures for dry-off.

In the RCT, a total of 574 cows were recruited and allocated, using a random number generator, to 4 groups: (1) intramammary (IMM) infusion of dry cow antimicrobial therapy alone; (2) IMM infusion of dry cow antimicrobial therapy and ITS; (3) on-farm culture using a Petrifilm® Aerobic Count Plate (3M, London, Ontario, Canada) with positive quarters (defined as ≥5 cfu/mL on the Petrifilm® Aerobic Count Plate) treated with IMM infusion of dry cow antimicrobial therapy alone

and negative quarters treated with ITS alone; and (4) on-farm culture using Petrifilm® with positive quarters treated with IMM infusion of dry cow antimicrobial therapy and ITS and negative quarters treated with ITS alone. Farm staff were blinded to treatment allocation, and therefore, they could not choose a group for a cow or keep her out of the study.

For the current cohort study, conducted on 6 of these farms, only quarters dry treated with ITS alone, or with an antimicrobial and ITS were selected. These quarters received, based on group allocation and dryoff IMI status, 4 g of an ITS containing 65% wt/wt of bismuth subnitrate (Orbeseal) with or without an IMM infusion of dry cow antimicrobial (200,000 IU of Penicillin G Procaine and 400 mg of Novobiocin; Novodry Plus, Zoetis Canada, Kirkland, Quebec, Canada). All treatments were applied by farm personnel immediately after the last milking before dry-off, following the procedures recommended on the Canadian Bovine Mastitis and Milk Quality Research Network's factsheet on administration technique for IMM treatment in dairy cows (CBMQRN, 2010). Treatments were recorded to verify compliance with the study protocol.

Data Collection

Before the start of the study, each participant was trained in aseptic IMM infusion techniques when using ITS, the stripping out of the ITS after calving, and the observation of ITS residues before milking. In addition, a practical illustrated sheet on administration technique for IMM treatment in dairy cattle was provided, explained, and left on farms. The research team visited participating farms every other week throughout the course of the study for enrollment of cows at dry-off and for monitoring the progress and respect of the study protocol. In each farm, 1 (the owner, in general) or 2 employees (the owner and 1 employee) were allocated to the project and collected all the observations.

The presence of an observable ITS plug at first milking following calving and persistency of ITS residues in milk after calving were monitored visually by dairy producers.

An ITS plug was considered effective if the pressure on the teat required to remove the plug was greater than required for normal milk removal, and significant ITS was observed following stripping. Residues were observed directly on the floor or using a filter-cup at each milking, depending on milkers' preferences. The monitoring of residues in milk was pursued until

observation of at least 4 successive milkings without residues. The last day that residues were observed in each quarter was used to calculate the number of days with residues for statistical analyses.

Putative Risk Factors

Data on parity, milk production at drying off, and milk production in the early lactation were extracted from farm milking system software (1 herd) and from monthly DHI data (5 herds) and investigated as potential predictors of ITS excretion. For milk production at drying off, the measurement collected on last DHI test before drying-off was used. For milk production in the early lactation, we hypothesized that milk production in the first few DIM may affect the presence of an ITS plug or days of ITS excretion or both. For many cows, however, the first milk production measurement was that of DHI control and this measurement could be collected sometimes very early in the lactation, but sometimes up to 40 to 45 DIM (depending on concordance between calving date and next scheduled DHI control). Therefore, to get a more stable, homogeneous, and reliable measure, instead of using only the first milk production measurement reported, we used the average milk production of the first 15 wk in milk for the farm from which we could get weekly milk production data, and the average milk production of the first 3 DHI tests following calving for the other farms. Our hypothesis was that high producing cows in the first 15 wk in milk were possibly also high-producing cows around calving. Parity was categorized based on its distribution into 2nd parity, 3rd parity, and ≥4th parity. Information regarding the residue visualization method used (filter-cup vs. floor) was provided by the participating producers.

Samples for Bacteriological Analyses

Using aseptic sample technique (National Mastitis Council, 2017a), single quarter milk samples of all enrolled cows were collected on the day before dry-off (S1), and after calving at 3 to 4 DIM (S2), and 5 to 18 DIM (S3) to detect IMI using bacteriological culture followed by MALDI-TOF MS identification. Sample S2 was collected by the producer or farm personnel, whereas S1 and S3 samples were collected by the research team. Milk samples were frozen at −20°C before a monthly shipment to the Maritime Quality Milk research laboratory at the University of Prince

Edward Island where culture and bacterial identification were conducted. Laboratory personnel were blinded to treatment allocation when conducting bacteriological analyses.

Laboratory Analyses

Milk Bacteriological Culture

Milk samples were thawed and cultured in the Maritime Quality Milk research laboratory using standardized methods outlined in the Laboratory Handbook on Bovine Mastitis (National Mastitis Council, 2017b). Briefly, disposable plastic loops were used to streak 0.01 mL of milk on bi-plates containing half Columbia agar + 5% sheep blood and half MacConkey agar. Plates were incubated at 35°C and examined for bacterial growth after 24 and 48 h. Colonies were tentatively identified as staphylococci, streptococci, coliforms, or other pathogens based on colony growth characteristics, morphology, pattern of hemolysis, catalase reaction, and Gram stain. For each positive sample, the number of colony-forming units per 0.01 mL of milk was enumerated up to a maximum of 10 colonies. The identification of all microorganisms recovered on the plate was attempted, regardless of the number of colonies. Samples with 3 or more phenotypically different colony types were classified as contaminated. However, if a contaminated sample had one or more hemolytic colonies (suspected to be *Staphylococcus aureus*), the hemolytic colonies were enumerated and analyzed further. Yeast and Prototheca spp. were recorded based on Gram stain results. Colonies of bacteria were subcultured onto blood agar plates to obtain pure culture for further classification using MALDI-TOF MS.

MALDI-TOF MS

Final isolates identification was carried out using the direct transfer method (MALDI Biotyper 3.1 User Manual, Bruker Daltonics Inc., Billerica, MA). Briefly, a single-use, 15-cm sterile wooden applicator stick was used to lift material from a well isolated bacterial colony followed by smearing a thin film of colony material onto a ground steel MSP 96-spot target (Bruker Daltonics Inc.). The spots were allowed to air dry at room temperature. Subsequently, the spots were overlaid with 1.0 µL of a saturated solution of α-cyano-4-hydroxycinnamic acid matrix in 50% acetonitrile, 47.5% water, and 2.5% trifluoroacetic acid (Sigma-Aldrich Canada Inc., Oakville, ON, Canada) using single-use pipette tips and air-dried at room temperature.

All targets were calibrated using Bacterial Test Standard (Bruker Daltonics Inc.) and included *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 control classification samples in duplicate. To confirm that target cleaning was effective and no residual bacterial material from a previous run remained, one spot on each target contained only matrix with no bacterial sample. All bacterial spectral captures and classifications were carried out using Bruker Daltonics Research Use Only microbial classification platform that included a Microflex LT mass spectrometer, flex-Control software (version 3.4), MALDI Biotyper Real Time Classification, Offline Classification (version 3.1) with a 5,627 Main Spectrum reference database library (MBT-BDAL-5627) and a custom CNS library developed by Cameron et al. (2017). All spectra reviews were carried out using flexAnalysis software (version 3.4, Bruker Daltonics Inc.). The MALDI Biotyper RTC was carried out according to the MALDI Biotyper 3.1 User Manual. FlexControl settings were medium mass range (1,960–21,200 m/z), detector gain set to 8.6× (3,227 V), sample and digitizer settings at 0.50 GS/s, and ion source 1 and 2 values of 20.12 and 18.25 kV, respectively. Each sample spectrum was summed from a maximum of 240 shots accumulated from a minimum of 6 raster points using a spiral small laser movement pattern.

After acquisition of a sample's mass spectrum, the Biotyper software compares the spectrum to the reference spectra contained in the database. The software then displays matching identifications and computes a score ranging from 0.0 to 3.0, indicating the degree of similarity between the sample's spectrum and the reference spectrum. Identification scores were interpreted as per manufacturer's recommendations as follows: a score of 2.0 to 3.0 was deemed as acceptable for species-level identification, a score of 1.7 to <2.0 indicated confident identification to the genus level, and a score of <1.7 was considered a nonreliable identification.

Definition of NIMI

To determine whether a NIMI by a specific pathogen was acquired during the dry period, the pre-dry sample (S1) and first sample after calving (S2) were used. If the first sample after calving (S2) was missing or contaminated, then the second sample (S3) was used. The information on NIMI was, therefore, missing, if sample at drying off (S1) was contaminated or if both samples after calving (S2 and S3) were missing or contaminated. Presence of ≥1 cfu/0.01 mL of milk was considered sufficient to qualify a quarter as having an IMI.

If an IMI by a specific pathogen-species was present at calving and if that same pathogen species was not found in the drying off sample, then the quarter was considered as having experienced a NIMI. In our study, groups of bacteria that can be reported by MALDITOF analyses, but are not relevant for bovine mastitis were not considered when defining NIMI. In the current study, these were *Corynebacterium amycolatum*, other gram-positive, *Curtobacterium flaccumfaciens, Brachybacterium* species, *Brevibacillus parabrevis, Kocuria* species, *Weissella confusa, Bacillus pumilus, Micrococcus luteus,* and *Neisseria flavescens*. Because ITS are used mainly to prevent NIMI by environmental bacteria, we also investigated specifically NIMI by these type of pathogens, by creating a second NIMI definition (NIMIenv), which further excluded NIMI by contagious mastitis pathogens (*Corynebacterium bovis* and other nonspeciated *Corynebacterium* species, and *Staphylococcus aureus*). Note that *Streptococcus agalactiae, Mycoplasma bovis*, and other *Mycoplasma* species were not recovered in the current study.

Statistical Analyses

The primary experimental unit was the quarter. Four outcomes of interest were investigated: (1) presence of an observable ITS plug at first milking after calving; (2) number of days of persistency of ITS residues in milk after calving; (3) development of a NIMI over the dry period; and (4) development of NIMIenv during the dry period.

Descriptive statistics were first computed, then the unconditional effect of the different expositions was tested in generalized linear mixed models using SAS Proc GLIMMIX procedure (version 9.4, SAS Institute Inc., Cary, NC). As observations were clustered within cows (4 quarters per cow) and within herds, random intercepts for cow and herd were included in all statistical models. Models used for the different outcomes are described in the following sections. Assumption of linearity between quantitative predictors and outcomes was evaluated using polynomial terms.

Conditional associations were then computed by introducing the putative confounders in the model; conditional and unconditional associations were then compared and confounders were retained only if measure of association (i.e., odds ratio or incidence ratio) differed by more than 10%, as suggested by Mickey and Greenland (1989). For all models, the significance threshold was

set at 0.05. A posteriori adjustments for multiple comparisons were used for predictors >2 categories using the Tukey-Kramer method. Only subjects with complete information on all variables in the final model were included in the analyses (i.e., complete case analysis).

Risk Factors Affecting Odds of an Observable ITS Plug at the First Milking After Calving

A generalized mixed model with a logit link was used to investigate the effect of the different predictors on odds of observing an ITS plug at first milking following calving. Predictors evaluated were parity, milk production at drying off, milk production in the early lactation, treatment received at drying off (antimicrobial and ITS vs. ITS alone), duration of dry period, and quarter position (front vs. rear). Parity was investigated as a putative confounder for the association between milk production at drying off and odds of observing an ITS plug at calving and for the association between early lactation milk production and odds of a plug. Also, milk production at drying off was investigated as a potential confounder for the association between the duration of the dry period and odds of observing an ITS plug at calving.

Risk Factors for Number of Days of Persistency of ITS Residues in Milk After Calving

For the number of days of persistency of ITS residues in milk after calving, 2 strikingly different distributions were observed for quarters for which a plug was or was not observed at calving. Therefore, for this outcome, separate analyses were carried out for quarters with an observable ITS plug and quarters without a plug. For these analyses, generalized mixed models using a log link were used to investigate the effect of the different predictors on number of days of ITS residues following calving. For quarters with an observable ITS plug at calving, we investigated whether a Poisson or negative binomial regression model would best fit the data using a t-test investigating whether the dispersion parameter was different from 0. The Poisson regression model offered the best fit and was retained for these analyses. For quarters without an observable ITS plug, we first investigated whether a Poisson or negative binomial would best fit the data, and then, based on the observed distribution, we evaluated whether a zero-inflated or conventional model would provide the best fit using the Vuong test (Vuong, 1989). The conventional negative binomial offered the best fit and was retained for these analyses.

Parity, milk production at drying off, milk production in the early lactation, treatment received at drying off (antimicrobial and ITS vs. ITS alone), duration of dry period, quarter position (front vs. rear), and visualization method used by the participating producer were evaluated as potential predictors of number of days of excretion. Again, parity was investigated as putative confounder for the association between milk production at drying off and persistence of ITS residues in milk after calving and for the association between early lactation milk production and persistence of ITS residues in milk after calving. Also, milk production at drying off was investigated as potential confounder for the association between duration of the dry period and persistence of ITS residues in milk after calving.

Effect of ITS Plug Retention on Odds of NIMI Over the Dry Period

Two separate generalized mixed models with a logit link were used for modeling the effect of the presence or not of an observable ITS plug at the first milking on odds of NIMI and on odds of NIMIenv. The only exposure considered in this model was presence or not of the ITS plug at calving. The following variables were investigated as potential confounder of these relationships: parity, milk production at drying off, milk production in the early lactation, treatment received at drying off (antimicrobial and ITS vs. ITS alone), quarter position (front vs. rear), and dry period duration.

Power Calculation

Because the sample size was already predetermined by the number of available quarters treated with teat sealant from the RCT, we instead evaluated the minimum differences that could be observed given the actual sample size, using an α of 0.05, and with a power of 0.90. For these calculations, we used a power of 0.90 (vs. 0.80) because clustering of quarters by cow and by herd was not accounted for. Thus, true power was probably less than 0.90. For instance, considering a standard deviation for mean excretion of 3 d, we would have been able to detect differences ≥0.83 and ≥0.94 d between levels of a dichotomic predictor present in 50 or 75% of quarters, respectively. When evaluating the effect of a dichotomic predictor on odds of having an observable ITS plug at calving, assuming that 85% of quarters will have an observable plug, and that the predictor would be present in either 50% or 75% of quarters, we computed that we could

detect, with a power ≥90%, difference in odds corresponding to an odds ratio of ≥2.2 (for the predictor with a 50% distribution) and of ≥2.7 (for the predictor with a 75% distribution).

When evaluating the effect of a dichotomic predictor (absence of the plug) on the odds of acquiring a NIMI during the dry period, assuming that 10% of quarters will have a NIMI, and that the predictor would be present in 15% of quarters, we computed that we could detect, with a power ≥90%, difference in odds corresponding to an odds ratio of ≥2.9.

Results

Descriptive Statistics

Participants

The 6 participating dairy farms with primarily Holstein cows are described in Table 16. In total, 377 cows from the 6 participating farms calved during the cohort study. Two hundred fifty-six of those 377 cows received ITS at least in one quarter. One hundred (100/256) cows were excluded from the study for different reasons: missing data on ITS residues (n=73), cows died before or at calving (n=9), cows aborted (n=4), cows were culled just after calving (n=4), or dry period was <35 or >75 d (n=10). One hundred fifty-six cows (557 quarters) were monitored during this cohort study with an average of 26 (range 11–67) monitored cows per farm. Sixty-seven quarters could not be included in the study because 60 quarters did not receive ITS at drying off (dry cow antimicrobial therapy alone), and 7 quarters were not functional at drying off.

Predictors

Internal teat sealant residues were observed using a filter-cup for 110/557 (20%) of quarters and forestripped milk was observed directly on the floor for 447 quarters (80%). Two hundred eighty-four front quarters (51%) and 273 rear quarters (49%) were observed during this study. Three hundred twenty-one quarters (57.6%) received a combination of antimicrobial and ITS, whereas 236 quarters (42.4%) received ITS alone at drying off. Two hundred fourteen quarters (38.4%) were from 2nd parity cows, 140 quarters (25.1%) from 3rd parity cows, and 203 quarters (36.5%) from cows with parity ≥4. Mean (SD) daily milk production at drying off was 24.7 kg/d (7.3 kg)

with minimum and maximum of 8.6 and 47.4 kg/d, respectively. Mean (SD) daily milk production after calving was 45.2 kg/d (6.6 kg) with a minimum and maximum of 27.1 and 62.1 kg/d, respectively. The mean (SD) dry period duration was 51.6 d (10.3 d) with a minimum and maximum of 35 and 73 d, respectively.

Internal Teat Sealant

An ITS plug was still present at the first milking after calving for 441/531 (83%) of quarters (range by herd: 45–100%), absent in 90 quarters (17%), and 26 observations were missing [3 cows (6 quarters) from farm 4 and 5 cows (20 quarters) from farm 1]. The duration of ITS residues varied between 0 and 12 d, with a mean (SD) of 4.0 d (2.3 d; Figure 20). When considering separately quarters for which a plug could be visualized after calving and those for which the plug was already gone, 2 strikingly different distributions were observed (Figure 20). For quarters for which an ITS plug was still present at the first milking after calving, persistency of ITS residues varied between 1 and 12 d with a mean (SD) of 4.5 d (1.9 d) and appeared to follow an almost normal distribution, or a Poisson or binomial negative distribution. For quarters without an ITS plug at the first milking after calving, persistency of ITS residues varied between 0 and 8 d with a mean (SD) of 1.2 d (1.7 d) and followed a typical count data distribution (i.e., Poisson or binomial negative with lower mean).

NIMI at Calving

New intramammary infections were observed in 87/530 of quarters (16.4%) and absent in 443 quarters (83.6%), according to the first definition excluding only bacteria not relevant for mastitis. There were 27 missing observations because (1) samples at drying off were contaminated (n = 21), or (2) both samples after calving were missing or contaminated (n= 6). When considering environmental mastitis pathogens only, NIMIenv were present in 60 of 530 quarters (11.3%) and absent in 470 quarters (88.7%). Table 17 presents the information on pathogen species isolated in cases of NIMI or NIMIenv.

Risk Factors Affecting Odds of an Observable ITS Plug at the First Milking After Calving

Associations between predictors and odds of having an observable ITS plug at the first milking after calving are presented in Table 18. Only type of treatment administered at drying-off and

quarter position were significantly associated with odds of having an observable ITS plug at calving. Quarters that received ITS alone had 2.6 times greater odds (95% CI: 1.1 – 6.1) of having an ITS plug after calving than those that received a combination of antimicrobial and ITS. Rear quarters had 2.1 times greater odds (95% CI: 1.1 – 3.9) of having a plug after calving than front quarters.

Risk Factors for Number of Days of Persistency of ITS Residues in Milk After Calving

Quarters with an ITS Plug Present at Calving

Associations between predictors and number of days of persistency of ITS residues in milk after calving in quarters with an observable ITS plug at calving are presented in Table 19. When an ITS plug was still present at the first milking after calving, Poisson regression with random effects offered the best fit and was used to evaluate the effects of different predictors on the number of days of persistence of ITS residues in milk. Only cow's parity influenced significantly the number of days of excretion of ITS residues in milk after calving. After adjusting for multiple comparisons, quarters of cows ≥4th parity had 1.3 times more days with residues (95% CI: 1.0 – 1.5) than quarters from 3rd parity cows. Quarters of cows ≥4th parity also had 1.2 times more days with residues (95% CI: 1.0 – 1.4) than quarters from 2nd parity cows. Number of days of residues were not statistically different between quarters from 3rd and 2nd parity cows. Predicted number of days of residues were 3.9, 3.7, and 4.9 d for 2nd, 3rd, and ≥4th parity cows, respectively.

Quarters Without an ITS Plug at Calving

Associations between predictors and number of days of persistency of ITS residues in milk after calving in quarters without an observable ITS plug at calving are presented in Table 20. When an ITS plug was not present at the first milking after calving, negative binomial regression with random effects offered the best fit and was used to evaluate the effect of different predictors on the number of days of persistence of ITS residues in milk. Parity, duration of the dry period, and quarter position all significantly affected number of days of ITS residues after calving in quarter without an observable ITS plug at calving.

After adjusting for multiple comparisons, quarters of cows ≥4th parity had 7.9 times more days with residues (95% CI: 1 .4 – 44.0) than quarters from 3rd parity cows. Quarters of cows ≥4th parity also had 6.1 times more days with residues (95% CI: 1.3 – 27.7) than quarters from 2nd parity cows. We did not observe a significant difference between quarters from 2nd and 3rd parity cows. Predicted number of days of residues were 0.4, 0.3, and 2.6 d for 2nd, 3rd, and ≥4th parity cows, respectively.

The number of days of persistence of ITS residues in milk after calving were multiplied by a factor of 1.7 (95% CI: 1.1 – 2.5) for every 1-wk increase of dry period length. For instance, number of days of residues were 0.1, 0.2, and 0.6 for quarters with a dry period of 6, 8, and 10 wk, respectively.

Finally, rear quarters had 1.6 times more days with residues (95% CI: 1.1 – 2.4) than front quarters. Predicted number of days of residues were 0.5, and 0.7 d for front and rear quarters, respectively.

Effect of ITS Plug Retention on Odds of NIMI Over the Dry Period

Associations between presence of an ITS plug at calving and odds of acquiring a NIMI or a NIMIenv are presented in Table 21. Treatment at drying-off (antimicrobials and ITS vs. ITS alone) was retained as a significant confounder of the association between presence of a plug at calving and NIMI and NIMIenv. After controlling for this confounder, the fact that ITS plug was present or not at calving was not significantly associated with odds of NIMI at calving (P=0.33, odds=1.4, 95% CI: 0.7 – 3.0) nor with odds of NIMIenv (P=0.54, odds=1.3, 95% CI: 0.6 – 3.1). When all relevant mastitis pathogens were considered, NIMI rates were 16.2, and 16.7% of quarters when plug was present or absent, respectively. When environmental mastitis pathogens only were considered, NIMIenv rates were 11.4, and 10% of quarters when a plug was present or absent, respectively.

Discussion

Participants

A total of 156 cows from 6 participating farms were monitored during this cohort study. Seventy-three cows were not included in the study because producers did not observe and complete the

information on the presence of ITS plug and ITS residues in milk. On some farms, when the employee allocated to the project was away, data collection was suspended. Moreover, a significant proportion (45.2%) of these missing data (33 of 73 cows) was registered between April and July 2016 when farmers were busy with field work and compliance with the research protocol was difficult. If the outcomes or exposures studied are strongly influenced by season, then it is possible that our results may apply well to cows calving in the August–March period, but perhaps not as much to cows calving during April–July where most of the missing data were observed. Moreover, because different observers were used to monitor presence of an effective plug and days of residues, some variation in these measures could be expected between observers. Accuracy of these measures related to individual observer was not evaluated in our study. We could expect, however, that the farm random effect would have captured some of these variations (e.g., for farms with just one observer; n=4). Moreover, the fact that method of residue visualization (i.e., filter-cup versus the floor) was not significantly associated with days of residues seems to suggest a certain homogeneity between observers. Still, possible variations in measurement between observers is one drawback from our study.

Persistency of Internal Teat Sealant Plug and NIMI over the Dry Period

The prevalence of quarters with an observable ITS plug at calving was comparable to previous observations made by Meaney (1977). The number of cows and quarters, however, was quite small in that study and there was no mention whether the study was conducted in one or many farms. According to his observations, a plug was observable around 3 d before calving by xray in 32/38 (84%) of quarters. In another experiment, they reported a proportion of 93% of quarters (13 out of 14 quarters x-rayed once a week during the dry period) that kept the sealant in place for the complete dry period duration. Note that the remaining quarter kept the sealant for 11 wk out of 14 wk. Moreover, none of those 14 quarters experienced NIMI during the dry period, despite quarters being dipped into a bacteriological culture of *Staphylococcus aureus* once a week during the dry period.

In our study, in 4 out of the 6 studied dairy farms, a proportion of or all cows calved in a maternity pen (vs. in a tie stall). Therefore, cows may have been in contact with calves after calving for some

time. We can hypothesize that suckling could have played a role on the probability of observing an ITS plug at first milking. In fact, our producers confirmed that suckling by the calf could occur on some occasions, especially for those cows that calved during the night. Unfortunately, the information on suckling or on calving time were not recorded. However, one of the 2 herds with the lowest proportion of ITS plugs present at calving was a free stall herd where cows calved in a tie stall barn. Considering these findings, factors other than suckling by the calf needs to be identified. These may include genetic characteristics of cows, the size and conformation of the teat, and so on. The relatively small associations (odds ratio of 1.4 for NIMI and 1.3 for NIMIenv) observed between presence or not of an observable ITS plug and odds of NIMI suggest that cows were possibly still protected against NIMI during most of the dry period. Therefore, we can hypothesize that loss of the plug occurred closely around calving because of suckling or another reason (e.g., milk hydrostatic pressure).

In our study, we hypothesized that the milk production level at drying-off would possibly affect the persistence of ITS plug, as the hydrostatic pressure due to milk production could possibly lead to the expulsion of the sealant. However, we found no evidence of association between cow-level milk production at drying off and the presence of an ITS plug after calving. This observation was in accordance with Williamson (2001) who reported that the leaking of milk was not associated with the expulsion of the teat sealant at drying off. Moreover, we did observe a higher retention rate in rear quarters, which typically produce more milk than front quarters. In fact, ITS plug was present for 86% of rear quarters and for 80% of front quarters.

Another possibility for explaining absence of the ITS plug at first milking is post infusion dispersion of the product in the gland cistern. This possibility was observed by Meaney (1977) and Bradley et al. (2010). Such an event could explain quarters without ITS plug at first milking and could possibly also increase persistency of ITS residues in milk in early lactation. However, we observed a reverse scenario. Quarters without ITS plug at calving had lower persistence of observable residues. Thus, our study would suggest that postinfusion dispersion, if it happens, would not increase number of days of residues after calving. In the current study, administration of ITS in conjunction with an IMM antimicrobial resulted in lower retention rates. Whether, the administration of these antimicrobials could hamper formation or retention of the plug, or

promote dispersion of the product in the gland is possibly worth investigating because these products are often used together. The same question was raised by Bradley et al. (2010). The later authors hypothesized that the miscibility of an ITS and an oil-based antimicrobial may modify the viscosity of the ITS and consequently affect the ability of plug formation. Thus, they come up with a proposition for using a water based antimicrobial when an antimicrobial is used in combination with an ITS.

We also considered that the duration of the dry period may influence the prevalence of quarters without an ITS plug at calving. However, no significant association was observed between dry period length and odds of having an observable ITS plug at first milking. Woolford et al. (1998) reported that the sealant material was still present at the base of the teat in 19 quarters x-rayed after 100 d of the dry period. In the current study, all cows had dry period length <75 d, thus possibly not long enough to affect ITS retention.

Persistency of ITS Residues in Milk After Calving

Meaney (1977) reported on presence of ITS residues in milk after calving during the first 5 d of lactation. In the Meaney (1977) study, ITS residues were observed with a level <2.5 µg of residues/mL of composite milk at 10 DIM. On d 5, no residues were present in 26/40 (60%) of samples, and ≤5 µg of residues/mL of bulk milk in 13 samples (32.5%), and 5.1 to 16.7 µg of residues/mL in 1 sample (2.5%). Similarly, in our study, 75% of quarters had expelled the last ITS residues by 5 DIM. For some quarters, however, residues were observed up to 12 DIM. Our results on persistency of ITS residues are also similar to those of Bhutto et al. (2011). These authors report that the main part of the sealant is removed at first milking and that the remaining part is observed in milk during the subsequent milkings. However, in this later study, the exact excretion duration was not reported. These results differ from another study (Berry and Hillerton, 2002a) that reported ITS residues in milk up to 3 wk in some quarters. However, these authors noted that none of dairy producers involved in the study reported that residue persistency was a serious concern.

Although we were able to highlight some statistically significant risk factors explaining persistency of residues following calving, observed differences were often relatively small and, perhaps, not

very relevant from a clinical standpoint. For instance, 2 of the risk factors (dry period duration and quarter position) affecting ITS persistency in quarters for which the ITS plug could not be observed at first milking yielded differences in residues excretion <1 d. Parity, however, appeared to affect residue persistency more substantially, with >1 additional day of excretion for quarters of cows ≥4th lactation. Data from this study indicate a higher probability of postinfusion dispersion of the product in the gland cistern for ≥4th lactation. The reasons for this are unclear, but it may be due to the teat conformation in older cows, the higher hydrostatic milk pressure, or other unknown factors.

In the current study, due to the relatively large sample size, x-ray images illustrating location and persistence of ITS plug during the dry period and after calving could not be collected as was done in other studies (Meaney, 1977, Woolford et al., 1998). Further studies will be needed to confirm our results on the presence of an observable ITS plug at first milking, on ITS residues excretion during the early lactation, and on the effect of cow parity on ITS plug retention and residue excretion. For instance, in future studies, repeated ultrasound exams during the dry period and the early lactation could possibly be used to help understand ITS plug formation, retention, and excretion dynamic during these time periods.

Conclusions

Results from our study revealed that an ITS plug was present until first milking after calving for 83% of quarters and ITS residues could be observed in milk up to 12 DIM. There was no evidence that quarters without ITS plug at first milking after calving were at higher risk of NIMI or NIMIenv during the dry period.

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References

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

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

Bhutto, A. L., R. D. Murray, and Z. Woldehiwet. 2011. The effect of dry cow therapy and internal teat-sealant on intra-mammary infections during subsequent lactation. *Res. Vet. Sci.* 90:316–320.

Bradley, A. 2002. Bovine mastitis: An evolving disease. *Vet. J.* 164:116–128.

Bradley, A. J., J. E. Breen, B. Payne, P. Williams, and M. J. Green. 2010. The use of a cephalonium containing dry cow therapy and an internal teat sealant, both alone and in combination. *J. Dairy Sci.* 93:1566–1577.

Cameron, M., H. W. Barkema, J. De Buck, S. De Vliegher, M. Chaffer, J. Lewis, and G. P. Keefe. 2017. Identification of bovine-associated coagulase-negative staphylococci by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a direct transfer protocol. *J. Dairy Sci.* 100:2137–2147.

Mastitis Network. 2010. Administration technique of intramammary treatment in dairy cows [Online]. Accessed Dec. 14, 2017. http://www.reseaumammite.org/tactic/wp-content/uploads/2020/02/FR-Fiche-Infusion-intramammaire.pdf

Dingwell, R. T., D. F. Kelton, and K. E. Leslie. 2003. Management of the dry cow in control of peripartum disease and mastitis. *Vet. Clin. North Am. Food Anim. Pract.* 19:235–265.

Dingwell, R. T., K. E. Leslie, Y. H. Schukken, J. M. Sargeant, L. L. Timms, T. F. Duffield, G. P. Keefe, D. F. Kelton, K. D. Lissemore, and J. Conklin. 2004. Association of cow and quarter-level factors at drying-off with new intramammary infections during the dry period. *Prev. Vet. Med.* 63:75–89.

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

Green, M. J., L. E. Green, G. F. Medley, Y. H. Schukken, and A. J. Bradley. 2002. Influence of dry period bacterial intramammary infection on clinical mastitis in dairy cows. *J. Dairy Sci.* 85:2589–2599.

Halasa, T., O. Østerås, H. Hogeveen, T. van Werven, and M. Nielen. 2009. Meta-analysis of dry cow management for dairy cattle. Part 1. Protection against new intramammary infections. *J. Dairy Sci.* 92:3134–3149.

Huxley, J. N., M. J. Greent, L. E. Green, and A. J. Bradley. 2002. Evaluation of the efficacy of an internal teat sealer during the dry period. *J. Dairy Sci.* 85:551–561.

Meaney, W. J. 1977. Effect of a dry period teat seal on bovine udder infection. *Isr. J. Agric. Res.* 16:293–299.

Mickey, R. M., and S. Greenland. 1989. The impact of confounder selection criteria on effect estimation. *Am. J. Epidemiol.* 129:125–137.

National Mastitis Council. 2017a. Sample collection and handling. Chapter 1 in Laboratory Handbook on Bovine Mastitis. 3rd ed. National Mastitis Council, New Prague, MN.

National Mastitis Council. 2017b. Laboratory Handbook on Bovine Mastitis. National Mastitis Council Inc., New Prague, MN.

Sanford, C. J., G. P. Keefe, I. R. Dohoo, K. E. Leslie, R. T. Dingwell, L. DesCoteaux, and H. W. Barkema. 2006. Efficacy of using an internal teat sealer to prevent new intramammary infections in nonlactating dairy cattle. *J. Am. Vet. Med. Assoc.* 228:1565–1573.

Smith, K. L., D. A. Todhunter, and P. S. Schoenberger. 1985. Environmental pathogens and intramammary infection during the dry period. *J. Dairy Sci.* 68:402–417.

Vuong, Q. H. 1989. Likelihood ratio tests for model selection and non nested hypotheses. *Econometrica* 57:307–333.

Williamson, J. 2001. Strategies for protecting the teat at dry-off. The National Mastitis Council 40th Annual Meeting, February 11–14, 2001 2001 Reno, Nevada. National Mastitis Council, Verona, WI., 88 - 94.

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

Woolford, M. W., J. H. Williamson, A. M. Day, and P. J. A. Copeman. 1998. The prophylactic effect of a teat sealer on bovine mastitis during the dry period and the following lactation. *N. Z. Vet. J.* 46:12–19.

Tableau 16. – Description of 6 dairy farms selected in Québec (Canada) for the study on persistency of internal teat sealant (ITS) residues in milk after calving.

Farm	Number of lactating cows	Housing of dairy cows	305 day mean milk production (kg)	Number of cows with missing data	% of quarters with ITS plug at calving
1	80	tie-stall	9,820	7	88
2	200	free-stall	9,687	21	95
3	100	free-stall	9,050	7	45
4	80	free-stall	9,679	7	81
5	140	free-stall	10,149	24	59
6	50	tie-stall	9,614	7	100

Tableau 17. – Pathogens isolated in 87 quarters with a new IMI in a cohort of 557 quarters from 156 cows from 6 commercial dairies in Quebec, Canada[1] .

Microorganism	**Number of isolates**
Aerococcus species	13
Aerococcus viridans	1
Bacillus species	1
Corynebacterium species	19
Enterococcus faecalis	2
Other NAS	8
Pseudomonas species	1
Staphylococcus aureus	10
Staphylococcus chromogenes	2
Staphylococcus epidermidis	1
Staphylococcus equorum	5
Staphylococcus haemolyticus	3
Staphylococcus hominis	2
Staphylococcus sciuri	5
Staphylococcus simulans	2
Staphylococcus xylosus	10
Streptococcus dysgalactiae	1
Streptococcus species	2
Trueperella pyogenes	1
Yeast	2
Total	**91[2]**
Contagious Mastitis Pathogens	29
Environmental Mastitis Pathogens	62

[1]Pathogens were identified by bacteriological culture followed by MALDI-TOF identification and, when MALDI-TOF species identification was not conclusive, by routine bacteriological methods.

[2]Among the 87 quarters with new IMI, 4 quarters had new IMI by 2 different species.

Tableau 18. – Results from the generalized logistic mixed models used to model effect of various predictors on odds of having an observable internal teat sealant (ITS) plug at calving in a cohort of 557 quarters, from 156 cows from 6 commercial dairies.

Parameter	**Estimate**	**SE**	**Odds ratio**	**95% CI**	**P-value[1]**
(1) Parity					
Intercept	1.981	0.772			
Parity					0.08
2	Reference	Reference	Reference	Reference	
3	-0.563	0.555	0.57	0.19, 1.7	
≥4	0.836	0.593	2.3	0.72, 7.4	
Herd variance	2.591	2.059			
Cow variance	2.891	0.692			
(2) Milk production pre-dry					
Intercept[2]	1.157	0.880			
Milk production pre-dry[3]	0.330	0.188	1.4	0.96, 2.0	0.08
Herd variance	2.737	2.183			
Cow variance	2.808	0.676			
(3) Milk production early lactation					
Intercept[2]	1.193	1.421			
Milk production early lactation[3]	0.118	0.182	1.1	0.79, 1.6	0.52
Herd variance	2.413	1.957			
Cow variance	2.894	0.691			
(4) Treatment at dry-off					
Intercept	2.683	0.815			
Treatment					0.04
Antimicrobials + ITS	Reference	Reference	Reference	Reference	
ITS only	0.936	0.444	2.6	1.1, 6.1	
Herd variance	2.939	2.343			
Cow variance	3.119	0.734			

(5) Dry period duration					
Intercept[4]	0.517	1.223			
Dry period duration[5]	0.161	0.196	1.2	0.8, 1.7	0.41
Milk production pre-dry[6]	0.407	0.214			0.06
Herd variance	3.204	2.553			
Cow variance	2.853	0.688			
(6) Quarter position					
Intercept	2.479	0.763			
Quarter position					0.02
Front	Reference	Reference	Reference	Reference	
Rear	0.741	0.318	2.1	1.1, 3.9	
Herd variance	2.776	2.197			
Cow variance	3.039	0.707			

[1]Joint P-value for the group of predictors (type III test).

[2]Intercept for a cow producing 10 kg of milk per day.

[3]Effect for a 5 kg/d increase in milk production.

[4]Intercept for a cow with a 35-d dry period and producing 10 kg of milk per day pre-dry.

[5]Effect for a 7-d increase in dry period duration.

[6]Milk production pre-dry was retained as a confounder of the relationship between dry period duration and odds of having an observable ITS plug at calving (change in odds ratio: 18%).

Tableau 19. – Results from the generalized negative Poisson mixed models used to model effect of various predictors on number of days of persistency of internal teat sealant (ITS) residues in milk after calving in 441 quarters with an observable ITS plug at calving.

Parameter	**Estimate**	**SE**	**Incidence ratio**	**95% CI**	**P-value[1]**
(1) Parity					
Intercept	1.367	0.105			
Parity					< 0.01
2	Reference	Reference	Reference	Reference	
3	-0.066	0.081	0.94	0.80, 1.1	
≥4	0.167	0.069	1.2	1.0, 1.4	
Herd variance	0.048	0.035			
Cow variance	0.046	0.014			
(2) Milk production pre-dry					
Intercept[2]	1.395	0.118			
Milk production pre-dry[3]	0.008	0.021	1.0	0.97, 1.1	0.71
Herd variance	0.051	0.038			
Cow variance	0.054	0.015			
(3) Milk production early lactation					
Intercept[2]	1.448	0.200			
Milk production early lactation[3]	-0.005	0.025	1.0	0.95, 1.0	0.85
Herd variance	0.051	0.038			
Cow variance	0.057	0.016			
(4) Treatment at dry-off					
Intercept	1.375	0.107			
Treatment					0.26
Antimicrobials + ITS	Reference	Reference	Reference	Reference	
ITS only	-0.068	0.061	0.093	0.83, 1.1	
Herd variance	0.050	0.037			
Cow variance	0.052	0.015			

(5) Dry period duration					
Intercept[4]	1.422	0.120			
Dry period duration[5]	-0.002	0.023	1.0	0.95, 1.0	0.94
Herd variance	0.051	0.038			
Cow variance	0.054	0.015			
(6) Quarter position					
Intercept	1.435	0.103			
Quarter position					0.43
Front	Reference	Reference	Reference	Reference	
Rear	0.036	0.046	1.0	0.95, 1.1	
Herd variance	0.051	0.038			
Cow variance	0.053	0.014			
(7) ITS residues visualisation method					
Intercept	1.325	0.114			
Method					0.16
On floor	Reference	Reference	Reference	Reference	
Filter-cup	0.280	0.197	1.3	0.90, 2.0	
Herd variance	0.042	0.034			
Cow variance	0.053	0.015			

[1]Joint P-value for the group of predictors (type III test).

[2]Intercept for a cow producing 10 kg/d of milk.

[3]Effect for a 5 kg/d increase in milk production.

[4]Intercept for a cow with a 35-d dry period.

[5]Effect for a 7-d increase in dry period duration.

Tableau 20. – Results from the generalized negative binomial mixed models used to model effect of various predictors on number of days of persistency of internal teat sealant (ITS) residues in milk after calving in 90 quarters without an observable ITS plug at calving.

Parameter	**Estimate**	**SE**	**Incidence ratio**	**95% CI**	**P-value[1]**
(1) Parity					
Intercept	-0.850	0.545			
Parity					< 0.01
2	Reference	Reference	Reference	Reference	
3	-0.259	0.615	0.77	0.2, 3.4	
≥4	1.808	0.628	6.1	1.3, 27.7	
Herd variance	0.609	0.816			
Cow variance	1.223	0.522			
(2) Milk production pre-dry					
Intercept[2]	0.687	0.710			
Milk production pre-dry[3]	-0.490	0.275	0.61	0.35,1.1	0.08
Herd variance	0.003	0.543			
Cow variance	1.741	0.716			
(3) Milk production early lactation					
Intercept[2]	-2.754	1.662			
Milk production early lactation[3]	0.317	0.230	1.4	0.87, 2.2	0.17
Herd variance	0.915	1.271			
Cow variance	1.490	0.611			
(4) Treatment at dry-off					
Intercept	-0.404	0.522			
Treatment					0.48
Antimicrobials + ITS	Reference	Reference	Reference	Reference	
ITS only	0.265	0.370	1.3	0.62, 2.7	
Herd variance	0.597	1.018			
Cow variance	1.638	0.637			

(5) Dry period duration					
Intercept[4]	-2.126	0.821			
Dry period duration[5]	0.520	0.200	1.7	1.1, 2.5	0.01
Herd variance	0.955	1.082			
Cow variance	1.214	0.518			
(6) Quarter position					
Intercept	-0.307	0.492			
Quarter position					0.03
Front	Reference	Reference	Reference	Reference	
Rear	0.465	0.202	1.6	1.1, 2.4	
Herd variance	0.709	1.077			
Cow variance	1.527	0.601			
(7) ITS residues visualisation method					
Intercept	-0.711	0.637			
Method					0.78
On floor	Reference	Reference	Reference	Reference	
Filter-cup	0.433	1.574	1.5	0.07, 36.2	
Herd variance	1.196	1.842			
Cow variance	1.594	0.627			

[1]Joint P-value for the group of predictors (type III test).

[2]Intercept for a cow producing 10 kg of milk per day.

[3]Effect for a 5 kg/d increase in milk production.

[4]Intercept for a cow with a 35-d dry period.

[5]Effect for a 7-d increase in dry period duration.

Tableau 21. – Results from the generalized logistic mixed models used to model effect of having an observable internal teat sealant (ITS) plug at calving on odds of acquiring a new intramammary infection (NIMI) or a NIMI caused specifically by an environmental pathogen (NIMIenv) during the dry period.

Parameter	**Estimate**	**SE**	**Odds ratio**	**95% CI**	**P-value[1]**
(1) NIMI					
Intercept	-1.256	0.344			
ITS plug at calving					0.33
Present	Reference	Reference	Reference	Reference	
Absent	0.362	0.373	1.4	0.7, 3.0	
Treatment[2]					< 0.01
Antimicrobials + ITS	Reference	Reference	Reference	Reference	
ITS only	0.885	0.276	2.4	1.4, 4.2	
Herd variance	0.405	0.369			
Cow variance	0.273	0.285			
(2) $NIMI_{env}$					
Intercept	-1.655	0.408			
ITS plug at calving					0.54
Present	Reference	Reference	Reference	Reference	
Absent	0.268	0.435	1.3	0.56, 3.1	
Treatment[3]					< 0.01
Antimicrobials + ITS	Reference	Reference	Reference	Reference	
ITS only	0.853	0.313	2.4	1.3, 4.3	
Herd variance	0.619	0.532			
Cow variance	0.073	0.339			

[1]Joint P-value for the group of predictors (type III test).

[2]Treatment at dry-off was retained as a confounder of the relationship between presence of an observable ITS plug at calving and odds of acquiring a NIMI over the dry period (change in odds ratio: 15%).

[3]Treatment at dry-off was retained as a confounder of the relationship between presence of an observable ITS plug at calving and odds of acquiring a NIMIenv over the dry period (change in odds ratio: 11%).

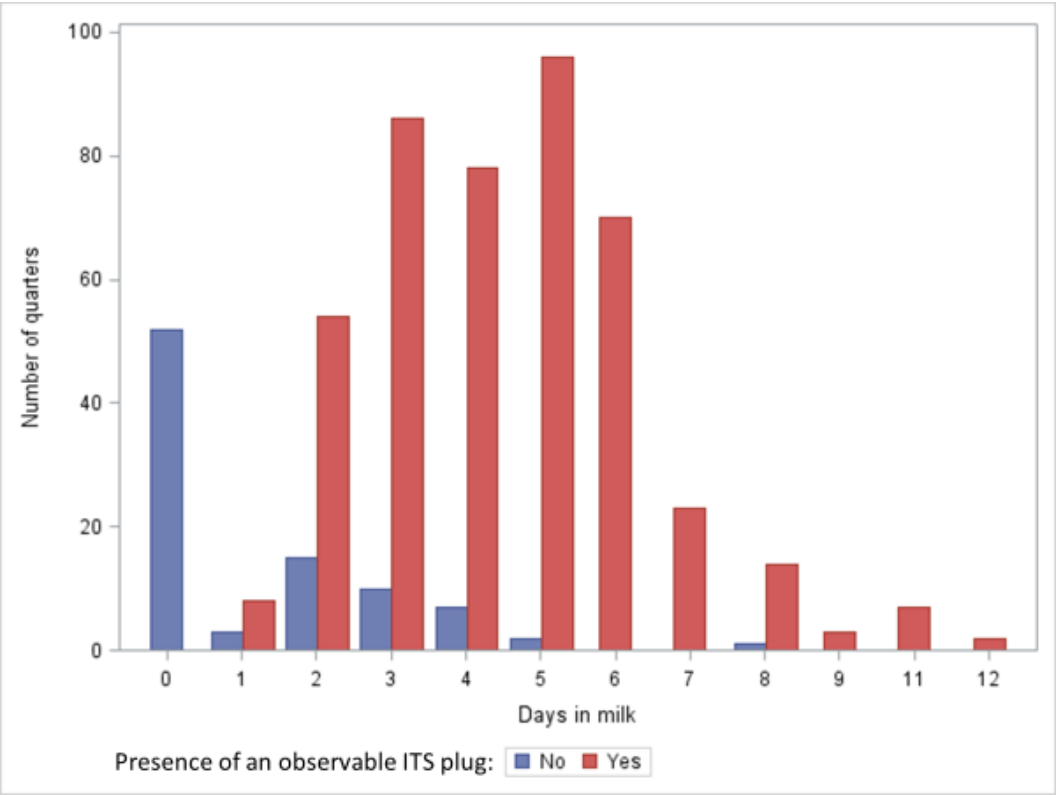

Figure 20. – Distribution of number of days with internal teat sealant (ITS) residues in milk after calving as a function of presence or not of an observable ITS plug at first milking.

Chapitre 8 – Discussion générale

Dans cette section, nous allons d'abord discuter les résultats liés aux objectifs de cette thèse qui visait à apporter plus de lumière sur le TS au tarissement des vaches laitières. L'essai clinique réalisé, en comparant le TU et le TS basé sur le système de culture de lait à l'aide de Petrifilm®, a montré qu'une réduction importante (> 50%) de l'utilisation des antimicrobiens peut être obtenue, sans effet négatif sur la santé du pis ou la production de lait au cours de la lactation subséquente. Les autres chapitres ont concerné la revue systématique et méta-analyse comparant le TS et le TU, les différentes approches de sélection des quartiers ou vaches éligibles à un traitement antimicrobien au tarissement et les conséquences d'utilisation de scellant interne à trayon sur la qualité du lait dans la lactation subséquente. À la fin de cette section, les limites de cette thèse et les perspectives seront présentées. Les résultats de cette thèse apportent de l'information pour aider les producteurs laitiers et médecins vétérinaires à réduire l'utilisation des antimicrobiens chez les vaches laitières au tarissement, sans toutefois mettre en péril non seulement la santé financière de la ferme, mais également la santé et le bien-être des vaches.

À la suite des préoccupations croissantes concernant l'utilisation massive d'antimicrobiens, le TS au tarissement a prouvé qu'il constitue une alternative potentielle à une approche de TU, avec l'avantage de réduire la quantité d'antimicrobiens utilisés en production laitière (Cameron et al., 2014, Rowe et al., 2020a). Selon ce nouveau protocole de TS, le traitement antimicrobien est réservé aux vaches ayant une IIM au moment du tarissement. De plus, en accord avec Rowe et al. (2020a), cette thèse a montré qu'il est possible de faire la sélection au niveau du quartier, pour réduire davantage l'utilisation des antimicrobiens au tarissement des vaches laitières.

En identifiant les quartiers infectés par les IIM à l'aide d'un système de culture de lait Petrifilm® et en appliquant un TS par quartier au tarissement des vaches en fin de lactation, une réduction de l'utilisation d'antimicrobien de 52% (IC à 95%: 39 – 64) a été réalisée. Cette réduction est plus importante que celle de 22% rapportée par Cameron et al. (2013) lorsqu'ils ont appliqué un TS par vache en se basant sur les résultats de culture de lait composite sur Petrifilm®. Pour les troupeaux ayant un CCS < 250,000 cellules/mL, grâce à une bonne identification des IIM au

moment du tarissement et par conséquent à l'application d'un traitement approprié, le TS par quartier permettrait de conserver les mêmes niveaux de santé du pis et de production et de qualité du lait lors de la lactation suivante, en comparaison avec le TU.

Les résultats de cette thèse montrent que les quartiers qui n'ont pas reçu d'antimicrobiens au tarissement étaient protégés contre les NIIM durant la période de tarissement. Ce qui montre l'efficacité du scellant interne à trayon dans leur prévention (Dufour et al., 2019, Rabiee and Lean, 2013, Woolford et al., 1998). Ainsi, il mérite sa place dans les protocoles de TS au tarissement, comme alternative aux antimicrobiens à titre prophylactique. Il devrait être obligatoire pour les quartiers non traités avec des antimicrobiens au tarissement (Kabera et al., 2021a, Winder et al., 2019). Il reste en place dans le trayon, et protège contre les NIIM jusqu'au vêlage et est expulsé lors de la première traite ou tétée du veau (Laven et al., 2014, Woolford et al., 1998).

Cette thèse n'a pas pu démontrer un avantage de combiner un antimicrobien et un scellant interne à trayon vs. un antimicrobien seul, pour le traitement des vaches laitières ou des quartiers identifiés comme non-infectés au tarissement. De même, certaines études antérieures (Bradley et al., 2010, Laven and Lawrence, 2008, Woolford et al., 1998) n'ont indiqué aucune différence significative entre l'antimicrobien seul et la combinaison d'antimicrobien et de scellant interne à trayon, chez les vaches avec un CCS faible. Toutefois, plusieurs études ont montré l'avantage de combiner un antimicrobien et un scellant interne à trayon (Bates et al., 2016, Berry and Hillerton, 2007, Bradley et al., 2010, Cook et al., 2005, Godden et al., 2003, Golder et al., 2016, Mütze et al., 2012, Newton et al., 2008). Par exemple, cette combinaison a été recommandée dans les troupeaux ayant un CCS élevé où les vaches présentent un risque élevé de NIIM pendant la période de tarissement (Bradley et al., 2010, Cook et al., 2005). On peut s'attendre, aussi, à détecter un plus grand bénéfice de la combinaison d'un antimicrobien et d'un scellant interne à trayon avec une période de tarissement plus longue. Berry and Hillerton (2007) ont signalé une incidence significativement plus faible de NIIM pendant la période de tarissement pour les vaches recevant le traitement combiné par rapport à l'antimicrobien seul, mais cette différence n'était pas significative lorsque la période de tarissement était <10 semaines. Cependant, d'autres études ont montré l'effet positif de la combinaison du scellant interne à trayon et de

l’antimicrobien au tarissement, indépendamment de la durée de la période de tarissement (Laven et al., 2014, Runciman et al., 2010).

Dans leur étude, Berry and Hillerton (2007) n’ont pas inclus des vaches qui étaient infectées par les principaux agents pathogènes de la mammite au moment du tarissement, ce qui n’est pas le cas pour notre étude et celle de Laven et al. (2014), car les vaches ont été sélectionnées indépendamment de leur statut infectieux au moment du tarissement. Il est possible que ceci pourrait avoir affecté la réponse du scellant interne à trayon (Runciman et al., 2010) et possiblement l'effet de la durée de la période de tarissement sur cette réponse.

Le suivi des quartiers qui ont été traité avec un scellant interne à trayon au tarissement a montré que 83% de quartiers ont gardé le bouchon de scellant jusqu’à la première traite. Cependant, il n’y avait pas d'association significative entre l'absence de bouchon et la probabilité de NIIM au moment du vêlage. Ce qui suggère que, malgré la perte du bouchon, les vaches étaient protégées contre les NIIM pendant la majeure partie de la période de tarissement. Par conséquent, nous pouvons émettre l'hypothèse que la perte du bouchon s'est produite à proximité du vêlage à cause de la tétée des veaux ou pour une autre raison (par exemple, la pression hydrostatique du lait). L’importance du scellant interne à trayon a été prouvée également par la revue systématique et méta-analyses, lorsque nous avons comparé le TS et le TU au tarissement.

Les résultats de la revue systématique et méta-analyses montrent clairement que le TS est aussi efficace que le TU pour éliminer les IIM existantes au moment du tarissement, prévenir les NIIM pendant la période de tarissement et prévenir les MC au début de la lactation suivante, si les scellants internes à trayons sont utilisés pour des quartiers sains et non traités avec des antimicrobiens. Aussi, la production laitière et le CCS durant les premiers mois de la lactation suivante ne diffèrent pas entre les quartiers traités selon une approche de TS ou de TU. Ces résultats sont en accord avec les revues précédentes (Halasa et al., 2009a, Halasa et al., 2009b, Winder et al., 2019), sur la comparaison du TS et du TU.

Winder et al. (2019) ont rapporté un risque similaire entre le TS et le TU, en ce qui concerne la prévalence d’IIM au vêlage, lorsque le scellant interne à trayon est utilisé pour les quartiers qui n’ont pas reçu d’antimicrobiens au tarissement. En revanche, ce risque était plus élevé pour le

TS, lorsque les quartiers qui n'ont pas reçu d'antimicrobiens n'étaient pas protégés par un scellant interne à trayon. Notons que ces derniers auteurs n'ont pas rapportés sur les autres indicateurs que nous avons mesurés. Halasa et al. (2009b) ont rapporté que le TU protégeait davantage des NIIM que le TS lorsque la sélection était basée au niveau du quartier (RR=2,0 ; IC à 95%: 1,3 – 3,0), mais qu'il n'y avait pas de différence significative lorsque la sélection était basée au niveau de la vache (RR=0,52 ; IC à 95%: 0,12 – 2,31). Pour les études incluses dans cette dernière revue, cependant, le scellant interne à trayon n'était pas utilisé pour les quartiers sains qui n'ont pas été traités avec des antimicrobiens au tarissement. Notre essai clinique et notre revue systématique ont montré que le fait de traiter seulement les quartiers infectés d'une vache n'augmente pas le risque de NIIM, lorsque le scellant interne à trayon est utilisé pour les quartiers qui ne reçoivent pas d'antimicrobiens. Ce qui prouve aussi l'efficacité du scellant interne à trayon dans la réduction de l'impact d'interdépendance des quartiers de la vache, à travers la prévention de NIIM. Halasa et al. (2009a) ont rapporté, également, un effet similaire pour le TS et le TU, en ce qui concerne l'élimination des IIM existantes au moment du tarissement. Ce qui suppose une bonne identification des quartiers qui méritent d'être traités avec des antimicrobiens au tarissement.

L'évaluation de différentes approches diagnostiques, pour déterminer les quartiers ou les vaches à traiter avec des antimicrobiens au tarissement, a montré que l'utilisation des données de CCS d'une vache est suffisamment précise pour guider les décisions de TS au tarissement au niveau de vache. En plus, l'utilisation de ces données représente un avantage économique plus important pour les producteurs laitiers, vu qu'elle n'implique pas un coût diagnostic supplémentaire. En effet, ces données sont déjà disponibles dans les fermes enrôlées dans le programme de contrôle laitier.

Au niveau vache, il est aussi facile et simple pour les producteurs d'utiliser les données du dernier contrôle laitier, plutôt que les trois derniers contrôles ou tous les contrôles de la lactation (en utilisant la moyenne des résultats ou chaque valeur individuelle). L'évaluation du dernier contrôle laitier a montré que l'utilisation d'un seuil de CCS > 100,000 cellules/mL pour les vaches primipares et > 200,000 cellules/mL pour les pluripares, donne une précision supérieure à celle du Petrifilm® seul (c'est-à-dire, de plus grandes valeurs prédictives positive et négative). Également, elle serait à l'origine d'une plus importante réduction d'utilisation d'antimicrobiens

par rapport à celle obtenue avec le Petrifilm®. En revanche, l'évaluation montre que le Petrifilm® constituerait un complément intéressant à l'usage des données de CCS. En effet, si on fait la culture de lait par quartier sur Petrifilm® pour les vaches dont le CCS est supérieur au seuil fixé, on parviendrait à réduire davantage l'usage d'antimicrobiens, en comparaison avec la considération des données du CCS seul.

La sélection des quartiers ou vaches à traiter avec des antimicrobiens qui se base sur les systèmes de culture à la ferme représente aussi un avantage important par rapport à la bactériologie standard du lait au laboratoire et a été rapportée par différentes études (Cameron et al., 2013, Kabera et al., 2020, Rowe et al., 2020a). Ces types de diagnostic présentent des avantages comme un délai d'exécution plus rapide et une réduction des dépenses, en comparaison avec la bactériologie standard au laboratoire. Toutefois, ils peuvent présenter aussi des désavantages, pour certains producteurs, comme une demande en personnel qualifié et un besoin de gestion élevé à la ferme. Un producteur peut décider d'utiliser les données de CCS ou les systèmes de diagnostic à la ferme seuls ou en les combinant, dépendamment de ses objectifs en termes de contrôle et prévention des agents de la mammite, d'utilisation judicieuse d'antimicrobiens, de bénéfice économique, de disponibilité de main d'œuvre qualifiée, etc.

L'évaluation de différentes approches diagnostiques a été réalisée à l'aide des modèles bayésiens. Ces modèles incorporent des informations préalables pour générer des estimations postérieures des paramètres de précision du test. Une recherche documentaire nous a permis d'obtenir des informations préalables provenant des publications scientifiques (versus des avis d'experts). Cependant, aucune de ces publications n'avait utilisé les modèles à classe latente pour, eux-mêmes, estimer les différents paramètres. Pour les approches où aucune donnée antérieure n'était disponible, nous avons utilisé des informations préalables vagues (c'est-à-dire en considérant que l'estimé se situe dans l'intervalle de 0 – 100%). Une analyse de sensibilité utilisant des informations préalables perturbées a donné, en général, des résultats similaires aux modèles originaux, ce qui suggère que les modèles étaient peu sensibles aux choix des *a priori*.

Dans cette étude, nous avons considéré que toutes les espèces bactériennes ne devaient pas être traitées avec des antimicrobiens au moment du tarissement. Nous avons défini les quartiers qui

devraient être traités avec des antimicrobiens au tarissement, en nous basant sur les espèces bactériennes isolées qui sont suspectées d'avoir un taux de guérison élevé ou qui sont reconnues comme importantes pour la santé du pis. Cette définition diffère des études précédentes et il n'est donc pas possible de faire une comparaison directe entre nos estimés des paramètres de précision et ceux de ces études. En effet, une étude précédente a considéré un traitement antimicrobien au tarissement, quel que soit le type d'agent pathogène isolé (Addis et al., 2016). D'autres (Jaeger et al., 2017, Vissio et al., 2014) n'ont considéré que les principaux agents pathogènes et, par conséquent, les NAS ou *Corynebacterium bovis*, qui sont considérées comme agents pathogènes mineurs (Bradley et al., 2012), n'ont pas été considérés comme pertinents pour un traitement antimicrobien. Cependant, nous les avons considérés pour le traitement antimicrobien, étant donné qu'il est rapporté dans la littérature qu'ils peuvent être à l'origine d'IIM avec des répercussions importantes sur la santé du pis et la production laitière (National Mastitis Council, 2016).

La plupart des agents pathogènes isolés des quartiers des vaches identifiées comme saines à l'aide du dernier test avec un seuil de CCS > 200,000 cellules/mL ou celui de 100,000 cellules /mL étaient des NAS. Ceci est en accord avec les études précédentes qui ont rapporté que les IIM causées par des agents pathogènes mineurs (principalement les NAS) induisaient une augmentation relativement faible ou nulle du CCS (Barkema et al., 1999, Schukken et al., 2003). Ainsi, on peut s'attendre à un nombre important de vaches classées à tort comme non infectées (faux négatifs) par une approche basée sur le CCS dans les troupeaux où ces agents pathogènes sont très répandus.

Certains auteurs (Cameron et al., 2014, Torres et al., 2008, Vasquez et al., 2018) ont préconisé la combinaison de données du CCS et l'historique des MC, pour décider du traitement antimicrobien au tarissement des vaches laitières. Cette thèse n'a pas pu démontrer la nécessité de cette combinaison. Cette situation pourrait s'expliquer non seulement par la faible fréquence des cas de MC pendant les trois derniers mois de la lactation, mais aussi par le fait qu'une vache présentant un cas de MC pendant cette période aurait probablement un CCS > 100 000 ou même > 200 000 cellules/mL au dernier contrôle. Cependant, il est difficile de recommander à un producteur de ne pas traiter une vache avec des antimicrobiens, alors que cette même vache a

un historique de MC durant les trois derniers mois avant le tarissement. Ainsi, même si les données de CCS indiqueraient qu'il n'est pas nécessaire de traiter avec des antimicrobiens, le producteur pourrait décider, quand même, de traiter une vache qui a un historique de MC.

Évolution de la pensée de la thèse. L'idée de départ, pour notre projet, était que la culture est indispensable pour une réussite du TS, étant donné qu'il est important d'avoir une méthode de sélection qui a une précision satisfaisante dans l'identification de quartiers ou vaches infectés au tarissement. En effet, il faut minimiser les risques de ne pas traiter des quartiers ou des vaches alors qu'ils sont réellement infectés au tarissement. Durant l'étude de Cameron et al. (2013), les vaches étaient retenues si leur CCS était inférieur ou égal à 200,000 cellules/mL. Or, les résultats de culture sur Petrifilm® ont montré que 53% de vaches du groupe de TS étaient positives et ont reçues par conséquent un traitement antimicrobien. Ceci a suscité l'idée qu'une culture était indispensable pour identifier les quartiers ou vaches éligibles au traitement antimicrobien au tarissement. Durant leur étude dont la sélection était basée sur la culture de lait composite de la vache sur le Petrifilm®, Cameron et al. (2013) ont rapporté une réduction d'utilisation d'antimicrobiens de 22%. Mais, dans la même étude, ils ont rapporté une proportion de 83% de quartiers de vaches qui n'étaient pas infectés au tarissement, selon les résultats de microbiologie de laboratoire. D'où est venu l'idée de faire la culture et prendre des décisions de traitement antimicrobien ciblées par quartier. Durant notre projet, un essai clinique randomisé a montré une réduction d'utilisation d'antimicrobiens de 58% lorsque le TS est basé sur la culture de lait de quartier sur Petrifilm®.

Cependant, comme la culture de lait sur Petrifilm® implique un temps de travail supplémentaire et une certaine expertise, plusieurs producteurs n'étaient pas ouverts à l'idée de s'embarquer, à long terme, à faire des cultures à la ferme. Nous avons donc décidé de réaliser une évaluation de différents protocoles d'identification des quartiers ou vaches à traiter avec des antimicrobiens, à l'aide des modèles Bayésiens. Ainsi, différentes approches utilisant les données de CCS de la vache, la culture de lait par quartier sur Petrifilm® et la bactériologie standard au laboratoire ont été comparées. À notre grande surprise, les résultats de la comparaison de précision de ces différentes approches de sélection des vaches éligibles au traitement antimicrobien ont montré que les données de CCS du dernier contrôle laitier étaient suffisamment précises pour permettre

des décisions sur le TS au tarissement au niveau de la vache. À la suite de ces résultats, notre vision a changé après avoir déterminé la précision de test considérant seulement le CCS.

Dans le cas de notre étude, un quartier était traité avec un antimicrobien, à chaque fois qu'il y avait une croissance bactérienne sur Petrifilm®. Or, lors de l'évaluation des tests diagnostiques, les différentes approches de sélection de quartiers ou vaches éligibles au traitement antimicrobiens ont été comparées sur base des IIM qui devraient être traitées avec des antimicrobiens. Possiblement que la majeure partie des résultats positifs au Petrifilm®, pour les vaches dont le CCS ≤ 200,000 cellules/mL, comme dans l'étude de Cameron et al. (2013) est constituée par des agents pathogènes de moindre importance pour la mammite bovine et qui ne devraient pas ainsi être traités avec des antimicrobiens. Ce qui pourrait expliquer la précision des données du CCS lorsqu'on considère seulement les agents pathogènes qui doivent être traités avec des antimicrobiens au tarissement.

Au début de notre projet, nous avons constaté qu'il y avait des réserves des producteurs pour utiliser les scellants internes à trayons, en ce qui concerne leurs résidus dans le lait après le vêlage. Ainsi, nous avons ajouté à notre projet un chapitre supplémentaire, pour apporter des réponses aux préoccupations des producteurs. C'est dans ce sens que nous avons déterminé la durée de présence de résidus de scellant observables dans le lait après vêlage. En plus de cela, nous étions intéressés par l'observation de présence de bouchon de scellant dans le trayon avant la première traite après vêlage. Les résultats de ce chapitre renseignent sur la durée de présence de résidus dans le lait, avec une moyenne de 4 jours et un maximum de 12 jours. Durant cette période, les premiers jets de lait devraient être jetés, pour éviter d'envoyer des résidus de scellant dans le réservoir. En plus, ces résultats renforcent l'idée de l'efficacité du scellant interne à trayon dans la prévention d'entrée de bactéries dans le canal du trayon durant la période de tarissement. Ce chapitre apporte donc une information importante quant à l'importance du scellant interne à trayon et à sa sécurité d'emploi. Ces résultats ont ensuite servi à produire une fiche à l'usage des producteurs sur les bonnes pratiques quant à l'application des scellants internes à trayons et à leur gestion suite au vêlage (Mastitis Network, 2020).

À quelle ferme et quelle vache s'adresse le tarissement sélectif ? Les décisions concernant l'utilisation des programmes de TS doivent être prises d'abord au niveau du troupeau, puis au niveau de la vache et, finalement, au niveau du quartier. Au niveau du troupeau, des facteurs tels que la prévalence d'IIM, le type d'agents pathogènes prédominants (agents pathogènes contagieux versus agents pathogènes d'origine environnementale) et la minutie du producteur pour, par exemple, les analyses à la ferme (si elles font partie du protocole), les traitements au tarissement, la tenue de dossiers ainsi que la gestion des résidus de scellant dans le lait après le vêlage, doivent être pris en compte lors de la décision d'appliquer le TS au tarissement. Chaque troupeau doit accorder une attention particulière à sa propre situation spécifique. Par exemple, concernant le type d'agents pathogènes prédominants, la proportion de vaches avec infection contagieuse < 5% a été utilisée par Swinkels et al. (2021) pour sélectionner les troupeaux de leur étude sur l'approche de TS. Dans cette dernière étude, comme dans le cadre de notre étude, seuls les troupeaux ayant un CCS ≤ 250,000 cellules/mL de lait du réservoir était sélectionnés pour une approche de TS. Pour une prise de décision optimale, le producteur a besoin d'une méthode précise, simple et rapide, facile à utiliser et peu coûteuse, pour déterminer les quartiers ou vaches éligibles à un traitement antimicrobien au moment du tarissement. Pour cela, il peut recourir à l'usage des données de CCS ou à la culture de lait à la ferme ou encore à la combinaison des deux méthodes, selon les objectifs à atteindre. Dans le cas d'une combinaison de ces méthodes, une culture serait nécessaire pour les quartiers des vaches ayant un CCS supérieur au seuil fixé, ce qui permettra de seulement traiter avec antimicrobiens les quartiers positifs à la culture.

Cette étude a montré qu'une réduction substantielle de l'utilisation des antimicrobiens pourrait être atteinte, en plus d'une précision satisfaisante de l'utilisation des données de CCS du dernier contrôle laitier avant le tarissement. Un seuil de 100,00 cellules/mL chez les primipares et celui de 200,000 cellules/mL pour les pluripares peuvent être utilisés. Toutefois, le seuil de 200,000 cellules/mL peut être utilisé pour toutes les vaches dans un soucis de simplicité, indépendamment de leur parité, selon les objectifs du producteur en termes de réduction d'utilisation d'antimicrobiens. En effet, notre étude a montré que la considération du seuil de 200,000 versus celui de 100,000 cellules/mL pour les primipares permet de réduire davantage l'utilisation d'antimicrobiens, mais avec une très faible modification sur la précision du test.

L’utilisation des données de CCS peut être plus pratique dans les pays où ces données sont disponibles, pour la sélection des vaches éligibles au traitement antimicrobien au tarissement. En revanche, dans les pays où ces données ne sont pas disponibles, il est important de réévaluer avec d’autres méthodes disponibles, pour la précision, la facilité d’utilisation et le coût. Les troupeaux peuvent différer dans la gestion de la santé pis et la prévalence des agents pathogènes responsable d’IIM. Ainsi, les effets du TS peuvent être très différents entre les troupeaux (Hassan et al., 1999, Rajala-Schultz et al., 2011). Ainsi, il convient de décider en fonction des paramètres et de l'historique du troupeau.

Le TS par quartier au tarissement constitue une façon d'optimiser davantage la réduction d’utilisation d’antimicrobiens, par rapport au TS par vache. Une préoccupation qui pourrait théoriquement compromettre le succès d'une telle approche est l'interdépendance des quartiers pour l'acquisition de NIIM pendant la période de tarissement (Berry et al., 2003, Robert et al., 2006a). Notre essai clinique et notre revue systématique ont montré que le fait de traiter seulement les quartiers infectés d’une vache n’augmente pas le risque de NIIM, lorsque le scellant interne à trayon est utilisé pour les quartiers qui ne reçoivent pas d’antimicrobiens. Ce qui prouve aussi l’efficacité du scellant interne à trayon dans la réduction de l’impact d’interdépendance des quartiers de la vache, à travers la prévention de NIIM.

Dans les fermes où la prévalence d’IIM est élevée, la majorité de vaches peuvent avoir besoin d'antimicrobiens et la possibilité de réduire l'utilisation d’antimicrobiens est donc limitée, dans ce cas. Dans ces fermes, il convient d'examiner en premier lieu la gestion globale du troupeau et de cibler les éléments à améliorer pour réduire la prévalence d’IIM. Une fois que cette situation est résolue et que la prévalence est réduite, l'utilisation d'antimicrobiens pour toutes les vaches au moment du tarissement devrait être moins nécessaire et un protocole de TS pourrait être envisagé. Ce n’est pas une bonne idée de faire un TS, alors que la prévalence des agents pathogènes contagieux (exemples : *Streptococcus agalactiae*, *Staphylococcus aureus, Mycoplasma* spp) est élevée. Également, avec un TS, il faut que le producteur soit prêt à utiliser le scellant interne à trayon pour prévenir les NIIM dans les quartiers de vaches non traitées avec des antimicrobiens au tarissement. Dans un troupeau, certaines vaches sont connues avec une historique de MC récurrentes ou d’infections chroniques. Ce qui veut dire probablement que,

dans un troupeau, il peut y avoir des vaches éligibles au protocole de TS et d'autres pour lesquelles il est nécessaire de faire un traitement antibiotique ou plutôt une réforme.

Au Pays-Bas et dans les pays scandinaves, le TU au tarissement est interdit depuis plusieurs années, dans le but de diminuer l'utilisation des antimicrobiens en production animale (Santman-Berends et al., 2016). D'autres pays sont en voie d'emboîter le pas, pour l'application de cette règlementation. Ainsi, pour les producteurs et les professionnels de la santé animale, des changements dans la gestion des troupeaux sont nécessaires, en ce qui concerne la prévention des maladies animales. Par exemple, aux Pays-Bas, l'utilisation préventive d'antimicrobiens en production animale est interdite depuis 2013, et depuis lors, les producteurs laitiers ont adopté progressivement le TS. Le principal critère utilisé pour sélectionner les vaches éligibles au traitement antimicrobien est principalement l'historique du CCS et de MC pendant la lactation précédente (Scherpenzeel et al., 2016b). Krattley-Roodenburg et al. (2021) ont montré que la santé du pis ne semble pas affectée par la réduction de l'utilisation d'antimicrobiens. Ces derniers auteurs indiquent, toutefois, que les producteurs doivent équilibrer la réduction d'utilisation d'antimicrobiens au tarissement avec des mesures d'hygiène optimales pour maintenir une bonne santé du pis pendant la période de tarissement.

L'impact de l'utilisation des scellants internes à trayon sur la qualité du lait après vêlage. Les résidus de scellants peuvent être difficile à gérer pour certains producteurs. Ainsi, il y a un risque de se préoccuper du problème de réduction d'utilisation d'antimicrobiens mais en créant un autre problème en encourageant l'utilisation des scellants internes à trayons. En effet, la qualité du lait pourrait être affectée par la présence de ces résidus dans le lait de réservoir. Ce qui se répercuterait, par conséquent, à l'industrie de transformation plus particulièrement au niveau de la fabrication des fromages (e.g., production de fromages de longue maturation, par exemple, les cheddar). Il a été rapporté que la présence de résidus de scellants dans le lait pourrait potentiellement être associée à l'apparition des taches noires dans certains fromages à maturation longue (Lay et al., 2007).

Il est important que les producteurs puissent extraire le bouchon de scellant à la première traite et éliminer les premiers jets contenant les résidus de scellant avant d'envoyer le lait dans le

réservoir. La surveillance de présence de résidus doit être poursuivie jusqu'à ce qu'ils ne soient plus observables dans les premiers jets pour au moins deux traites successives. Notre étude a montré que les résidus peuvent être présents jusqu'à 12 jours après vêlage et qu'il n'y a plus de résidus observables dans plus de 75% de quartiers au bout de 5 jours après vêlage.

Limites de la thèse et perspectives de recherche

Le TS au tarissement peut nécessiter plus de temps et une certaine expertise pour identifier les vaches ou les quartiers à traiter par rapport au TU. Cependant, la sélection basée sur les données de CCS du dernier contrôle laitier est simple, peu coûteuse et plus facile à mettre en œuvre par les producteurs. Cette étude met en évidence la précision de l'utilisation des données de CCS du dernier contrôle laitier pour sélectionner les vaches éligibles au traitement antimicrobien au tarissement. Cependant, en plus des paramètres de précision du test rapportés dans cette étude, il serait important de l'évaluer dans le cadre d'un essai clinique comparant le TS et le TU, afin de déterminer les impacts de cette méthode sur la santé du pis ou la production laitière, pendant la période de tarissement et durant les premiers jours de la lactation suivante ou même durant toute la durée de la lactation suivante. Une étude clinique sur un plus grand nombre de troupeaux et de vaches est nécessaire pour valider ce qui a été dégagé par le modèle bayésien sur les tests diagnostiques pour sélectionner les quartiers ou vaches éligibles au traitement antimicrobien au tarissement.

Cette étude a porté sur des troupeaux laitiers dont le CCS moyen était inférieur à 250,000 cellules/mL au cours des 12 derniers mois. Par conséquent, ces résultats pourraient être généralisés aux troupeaux présentant les mêmes caractéristiques. Plus d'investigations devraient être réalisées pour décider de l'approche de sélection des quartiers ou vaches à traiter avec des antimicrobiens dans les troupeaux avec un CCS moyen ≥ 250 000 cellules/mL. Précédemment, la pertinence du TS au tarissement a été questionnée, pour ce genre de troupeaux (Rajala-Schultz et al., 2011). En effet, les valeurs prédictives positives et négatives qui reflètent la capacité d'un test à prédire une maladie (infection) dans une population donnée, sont affectées par la prévalence de la maladie (Dohoo et al., 2009). Or, dans les troupeaux ayant un CCS élevé, la prévalence d'IIM est élevée aussi. Ce qui affectera considérablement les valeurs prédictives du

test. Plus la prévalence augmente, plus la VPN diminue. Ainsi, les troupeaux à forte prévalence d'IIM doivent être prudents quant au choix de la méthode de sélection de quartiers ou vaches éligibles à un traitement antimicrobien au tarissement. Peut-être que les troupeaux présentant une prévalence très élevée d'IIM ne devraient pas envisager de faire un TS et devraient continuer à traiter toutes les vaches et envisager une investigation du problème présent dans le troupeau. Des études sont nécessaires pour identifier les caractéristiques des troupeaux au-delà desquelles le TU au tarissement peut être nécessaire pour maintenir une bonne santé du pis et en deçà desquelles un TS pourrait être envisagé et donner de bons résultats.

Une autre limite pourrait être liée au fait qu'un seul des neuf troupeaux participants représente 30% des vaches de l'étude. Ce poids important d'une des fermes de l'étude pourrait influencer (positivement ou négativement) les résultats si elle a une meilleure ou moins bonne gestion que les autres fermes de l'étude. Cependant, les analyses statistiques ont été réalisées avec des modèles mixtes qui permettent d'ajuster le poids donné à chacun des troupeaux. En plus, il faut noter que le protocole de tarissement sélectif durant cette étude a été réalisé et suivi de près par l'équipe de recherche dans toute les fermes de l'étude.

Aussi, une ferme a arrêté de faire des tests de contrôle laitier au cours du projet et, par conséquent, nous n'avons pas pu obtenir de données du CCS pour la période restante de l'étude. Cela a conduit à la réduction de la taille de l'échantillon, pour la mesure de l'effet du TS basé sur la culture de lait de quartier sur le Petrifilm® sur le CCS et la production de la lactation subséquente. Cependant, les analyses statistiques de l'effet du groupe de traitement sur le CCS ont montré que, sans les données de ce troupeau, les résultats et la conclusion n'ont pas changé de façon substantielle. De cette ferme, 165 vaches étaient incluses dans l'étude, soit 30% de toutes les vaches retenues pour le projet. Les données de CCS étaient disponibles seulement pour 27 vaches, alors que les données de production de lait étaient disponibles pour 130 vaches. Pour l'évaluation de l'effet du TS sur le CCS dans la lactation subséquente, à la suite des données manquantes, cette ferme est passée du plus gros au deuxième plus petit troupeau de l'étude. Pour rappel, l'intervalle de vaches sélectionnées/ferme était de 26 à 165.

Malgré cette réduction de l'échantillon et par conséquent de la puissance statistique, pour la mesure de l'effet du TS sur le CCS, la conclusion reste valide, étant donné que l'échantillon initial n'était pas modifié pour quatre autres éléments étudiés (élimination des IIM présentes au tarissement, les NIIM durant la période de tarissement, les MC et production laitière durant les 120 premiers jours de la lactation suivante).

Réduction d'utilisation d'antimicrobiens et précision des tests diagnostiques pour sélectionner les quartiers ou vaches éligibles au traitement antimicrobien. Du point de vue santé publique, la réduction d'utilisation d'antimicrobiens est très importante, considérant que cette réduction contribuera, possiblement, à la réduction de l'ampleur d'apparition des résistances bactériennes aux antimicrobiens. Dans les pays où le TU de toutes les vaches au moment du tarissement n'est pas proscrit, l'adoption de l'approche de TS dépendrait non seulement de l'idée de réduire l'usage des antimicrobiens mais principalement de ses avantages économiques. Ainsi, les producteurs seront motivés non seulement par cette volonté de contribuer à la préservation de la santé publique, mais également par la santé financière de leurs fermes laitières.

Cependant, il est très important qu'il n'y ait pas d'impact négatifs du TS sur la santé du pis et la production laitière durant la lactation suivante, en comparaison avec le TU. Un risque élevé aurait, comme conséquences, des répercutions économiques plus importantes, en plus du risque de subséquemment utiliser, pour des traitements curatifs, une quantité d'antimicrobiens supérieure à celle qu'on a sauvée au tarissement. Ainsi, il faut aller chercher le maximum de réduction d'utilisation d'antimicrobiens, mais sans toutefois mettre en péril la santé du pis et la production laitière durant la lactation suivante.

Selon la méthode utilisée pour sélectionner les quartiers ou vaches à traiter avec des antimicrobiens au tarissement, il serait important de déterminer quel pourcentage de réduction de l'utilisation d'antimicrobiens serait acceptable pour garantir une marge de profit positive du protocole de TS. Toutefois, un producteur pourrait être motivé par sa contribution à la santé publique, peu importe le résultat économique du protocole de TS, pourvu que sa ferme reste économiquement viable. Ainsi, les producteurs pourront prendre leur décision en tenant compte

non seulement des résultats mais également de leur propres objectifs et motivation par rapport au TS et à la réduction d'utilisation des antimicrobiens au tarissement.

Les futures orientations de recherche devraient inclure l'évaluation économique de l'approche de TS, selon la méthode choisie pour identifier les quartiers ou vaches éligibles au traitement antimicrobiens au moment du tarissement, le pourcentage de réduction d'antimicrobiens et l'association ou non du scellant à l'antimicrobien pour les quartiers éligibles au traitement antimicrobien. Pour une santé financière des fermes laitières, il importe d'analyser les implications économiques du TS par quartier au tarissement des vaches laitières. En effet, la décision de TS doit être basée non seulement sur son impact sur la santé du pis et la production laitière durant la lactation subséquente, mais aussi sur les implications financières de ses différentes stratégies. Selon que la méthode choisie est plus précise et que les quartiers non traités avec des antimicrobiens reçoivent des scellants internes à trayons, des réductions plus importantes de l'utilisation d'antimicrobiens pourraient être atteintes et sans effets néfastes sur la santé du pis ou la production de lait durant la lactation suivante. Ce qui occasionnera des économies potentiellement plus importantes pour les producteurs laitiers. Les coûts supplémentaires du TS, par rapport au TU, pourraient essentiellement provenir du temps de travail supplémentaire et du coût lié au diagnostic, alors que les avantages supplémentaires proviendraient de la quantité de produits épargnée à la suite des quartiers ou vaches non traités avec des antimicrobiens.

Protocole de traitement sélectif basé sur la culture de lait de quartier sur Petrifilm®. Durant le projet, le prélèvement de lait de quartier ainsi que la culture sur Petrifilm® ont été réalisés par l'équipe de recherche. En effet, certains producteurs n'avaient pas l'expertise pour faire la culture de lait sur Petrifilm®, étant donné qu'ils ne les avaient jamais réalisées dans leurs fermes. Ça aurait été intéressant si les prélèvements, les cultures sur Petrifilm® ainsi que l'application des traitements étaient réalisés par les producteurs. Ainsi, on aurait eu des résultats d'une activité 100% des producteurs. Comme, vers la fin du projet, plusieurs producteurs étaient intéressés à continuer à appliquer eux-mêmes le TS des vaches au tarissement, nous les avons initiés à faire des cultures de lait sur Petrifilm®.

Cependant, aucun suivi à long terme n'a été effectué pour toutes les fermes sélectionnées pour cette étude. Parmi cinq troupeaux clients de la clinique ambulatoire bovine de la faculté de médecine vétérinaire de l'Université de Montréal, pour lesquels nous pourrions avoir des nouvelles, 4 ont continué le protocole de TS après la fin du projet. Mais, trois ans après la fin du projet, un seul troupeau pratiquait encore ce protocole. En effet, le TS basé sur la culture de lait sur Petrifilm® nécessite plus de temps et une certaine expertise par rapport au TU au tarissement. En revanche, le TS basé sur les données de CCS serait une possibilité qui ne nécessite pas un temps supplémentaire ou une autre expertise.

On pourrait se demander quel aurait été l'impact sur les données et les résultats de l'essai clinique, si les producteurs s'étaient occupés du prélèvement d'échantillons de lait et de l'ensemencement sur le Petrifilm®. Si c'était le cas, probablement que les résultats de l'essai seraient les mêmes, en ce qui concerne la santé du pis et la production de lait, mais le pourcentage de réduction d'utilisation d'antimicrobiens serait réduit. En effet, plusieurs contaminations de Petrifilm® pourraient avoir lieu lorsque l'ensemencement est réalisé par du personnel moins spécialisé, avec comme conséquence le traitement de plusieurs quartiers qui ne sont pas réellement infectées.

Durant notre projet, la culture de lait de quartier était réalisée pour toutes les vaches, sans considération du CCS de la vache. Peut-être qu'on aurait dû réaliser un test de CMT par quartier, pour décider de la culture sur Petrifilm®. Ainsi, pour un quartier avec un score du test CMT de 2 ou plus (seuil appliqué pour sélectionner les vaches par Cameron et al., 2013), on aurait dû donner un antimicrobien sans la nécessité de faire la culture de lait sur Petrifilm®. Ce qui réduirait le coût lié à la culture sur Petrifilm®. Cameron et al. (2013) a montré que, au niveau vache, l'ajout du test de CMT au protocole de TS basé sur la culture sur Petrifilm® n'apporte presque rien de plus. Cependant, cela pourrait possiblement apporter une information supplémentaire au niveau quartier afin d'affiner la décision de traitement antimicrobien du niveau vache jusqu'au niveau quartier.

Le traitement sélectif au tarissement et l'antibiorésistance. La promotion de l'approche de TS a été surtout lancée pour limiter le développement de la résistance aux antimicrobiens par les

bactéries. On pourrait s'intéresser à la question de savoir si le TS au tarissement constitue vraiment la bonne cible pour limiter ce développement. Peut-être qu'il s'agit d'une cible facile, puisque ce sont des traitements antimicrobiens inutiles qu'on évite. Mais, ce ne sont que des traitements locaux, avec une petite quantité d'antimicrobiens (le plus souvent des pénicillines) et dans un compartiment biologique (la glande mammaire) où il y a peu de microbes et peu de contacts avec les microbes du tractus digestif. Est-ce qu'on n'aurait pas plus d'impact en visant des utilisations qui affectent, par exemple, la flore intestinale (qui, elle, est abondante) et avec des antibiotiques d'importance critique? Probablement que ça devrait être une première étape dans la contribution au frein à l'apparition des résistances aux antimicrobiens.

Après l'adoption du TS au tarissement, l'industrie laitière pourrait considérer le TS des mammites cliniques durant la lactation. En plus, il serait intéressant de trouver une solution d'anticiper les problèmes de santé qui affecte la production, la reproduction ou la réforme des vaches laitières ou les problèmes de maladies néonatales et qui pourraient nécessiter l'usage des antimicrobiens à visée générale. Les veaux près de la période de sevrage sont les animaux laitiers où il y a une plus grande proportion de bactéries résistantes. Les conditions qui les affectent (diarrhée, pneumonie) sont aussi souvent traitées par voie parentérale ou entérale. Comme les antimicrobiens sont souvent non-nécessaires (exemple, cas de diarrhée virale), il y aurait donc là une belle "cible". Par contre, sachant que quelques grammes de fèces de veaux sont rapidement dilués par les kg de fèces des vaches adultes, ceci sera une cible moins intéressante pour la réduction de transfert d'antibiorésistance vers l'environnement. Aussi, ces veaux ne produisent pas encore d'aliments et ainsi, représentent moins de risque pour le transfert de l'antibiorésistance aux humains par l'alimentation.

Les recherches sur les mécanismes de prévention à travers la gestion technique des élevages de façon à minimiser l'apparition des problèmes de santé seraient les bienvenues. Par exemples, certaines maladies néonatales peuvent être évitées à travers la préparation de la vache au vêlage, l'hygiène autour de la mise-bas, la prise contrôlée du colostrum par le veau, etc. Pour certaines maladies bactériennes entériques, les mesures de biosécurité stricte pourraient être proposées, pour la prévention et/ou le contrôle. Le TS doit faire partie d'un ensemble d'actions qui peuvent

être posées en production laitière pour limiter les risques de développement de l'antibiorésistance.

Chapitre 9 – Conclusion générale

Cette thèse a permis de déterminer que le système de culture lait à la ferme sur Petrifilm® était un outil diagnostique intéressant pour la détection d'IIM au tarissement des vaches laitières (Se=82%, Sp=62%, VPP=29%, VPN=95%). Lorsqu'il a été appliqué dans les troupeaux à faible CCS (< 250,000 cellules/mL), le TS par quartier basé sur la culture lait sur Petrifilm® a entraîné une diminution de l'utilisation d'antimicrobiens de 58% par rapport au TU et il n'y avait pas d'effets néfastes sur la santé du pis ou la production laitière durant les 120 premiers jours de la lactation suivante.

Cependant, l'usage des données de CCS a montré sa supériorité en termes de précision. Cet usage serait à l'origine d'une plus importante réduction d'utilisation d'antimicrobiens par rapport à celle obtenue avec le Petrifilm®. Plus encore, les données de CCS sont disponibles dans les fermes qui sont enrôlées sur le programme de contrôle laitier et, ainsi, leur usage n'implique pas un coût supplémentaire lié au diagnostic. Il faut, toutefois, noter qu'il est plus facile et simple pour les producteurs d'utiliser les données de CCS du dernier contrôle laitier pré-tarissement, en comparaison avec la considération des trois derniers contrôles ou tous les contrôles de la lactation. Pour les vaches de première parité, le dernier test de CCS pré-tarissement avec un seuil de 100,000 cellules/mL s'est avéré assez précis, avec des Se, Sp, VPP, VPN et une réduction de l'utilisation des antimicrobiens de 86, 86, 58, 96 et 75%, respectivement. Pour les vaches pluripares, le dernier test de CCS pré-tarissement avec un seuil de 200,000 cellules/mL a permis d'obtenir des Se, Sp, VPP, VPN et une réduction de l'utilisation des antimicrobiens de 75, 84, 47, 95 et 77%, respectivement. Cependant, la combinaison de l'utilisation des données de CCS et de la culture de lait à la ferme pour les vaches positives au CCS permet d'améliorer la précision du test diagnostic en termes de spécificité et de valeurs prédictives et le pourcentage de réduction d'antimicrobiens, par rapport à l'utilisation des données de CCS seules.

Pour les troupeaux ayant un CCS < 250,000 cellules/mL, grâce à une bonne identification des IIM au moment du tarissement et par conséquent à l'application d'un traitement approprié, le TS par quartier permettrait de conserver les mêmes niveaux de santé du pis et de production et de

qualité du lait lors de la lactation suivante, en comparaison avec le TU. Cependant, les quartiers qui ne reçoivent pas d'antimicrobiens doivent recevoir du scellant interne à trayon, pour prévenir les NIIM durant la période de tarissement. Les résidus de scellant peuvent être observables dans les premiers jets de lait jusqu'à 12 jours après vêlage avec une moyenne de 4 jours. Ces premiers jets contenant les résidus de scellant doivent être éliminés avant d'envoyer le lait dans le réservoir.

En conclusion, les producteurs laitiers pourraient utiliser les cultures bactériologiques sur Petrifilm® dans leur ferme, pour la prise de décisions de TS par quartier. Toutefois, cette méthode peut présenter des désavantages, pour certains producteurs, comme une demande en personnel qualifié et un besoin de gestion élevé à la ferme. Ils pourraient également appliquer le TS au niveau vache basé sur le dernier CCS seul. Le dernier CCS avec un seuil de 100,000 cellules/mL pour les vaches de première parité et de 200,000 cellules/mL pour les vaches plus âgées représenterait une stratégie de diagnostic optimale du point de vue de la précision. Cependant, le dernier CCS avec un seuil de 200,000 cellules/mL pour toutes les vaches représenterait une stratégie de diagnostic optimale du point de vue de la simplicité et de la réduction d'utilisation d'antimicrobiens, avec une perte de précision relativement très faible. Enfin, le test de tous les quartiers d'une vache identifiée comme infectée à l'aide des données de CCS avec les Petrifilm® pourrait être utilisé pour réduire davantage l'utilisation d'antimicrobiens au moment du tarissement, en appliquant une sélection au niveau quartier. Par exemple, chez les vaches pluripares, en utilisant le dernier test de CCS pré-tarissement avec un seuil de 200,000 cellules/mL, l'ajout de culture de lait sur Petrifilm® a augmenté la réduction de 77 à 90%. Soulignons que les producteurs devraient considérer l'utilisation du scellant interne à trayon dans leur protocole de TS, pour les quartiers qui ne reçoivent pas d'antimicrobiens au tarissement.

Un producteur peut décider d'utiliser les données de CCS ou les cultures bactériologiques de lait sur Petrifilm® seuls ou en les combinant, dépendamment de ses objectifs et motivations en termes de contrôle et prévention des agents de la mammite, d'utilisation judicieuse d'antimicrobiens, de bénéfice économique, de disponibilité de main d'œuvre qualifiée, etc.

Références bibliographiques

Addis, M. F., Tedde, V., Puggioni, G. M. G., Pisanu, S., Casula, A., Locatelli, C., Rota, N., Bronzo, V., Moroni, P. & Uzzau, S. (2016). Evaluation of milk cathelicidin for detection of bovine mastitis. J. Dairy Sci., 99, 8250-8258.

Afifi, M., Kabera, F., Stryhn, H., Roy, J. P., Heider, L. C., Godden, S., Montelpare, W., Sanchez, J. & Dufour, S. (2018). Antimicrobial-based dry cow therapy approaches for cure and prevention of intramammary infections: a protocol for a systematic review and meta-analysis. *Anim. Health Res. Rev.,* 19, 74-78.

Aghamohammadi, M., Haine, D., Kelton, D. F., Barkema, H. W., Hogeveen, H., Keefe, G. P. & Dufour, S. (2018). Herd-Level Mastitis-Associated Costs on Canadian Dairy Farms. Front. Vet. Sci., 5.

Arruda, A. G., Godden, S., Rapnicki, P., Gorden, P., Timms, L., Aly, S. S., Lehenbauer, T. W. & Champagne, J. (2013). Randomized noninferiority clinical trial evaluating 3 commercial dry cow mastitis preparations: I. Quarter-level outcomes. J. Dairy Sci., 96, 4419-4435.

Barkema, H. W., Deluyker, H. A., Schukken, Y. H. & Lam, T. J. (1999). Quarter-milk somatic cell count at calving and at the first six milkings after calving. *Prev Vet Med,* 38, 1-9.

Barkema, H. W., Schukken, Y. H., Lam, T. J., Beiboer, M. L., Wilmink, H., Benedictus, G. & Brand, A. (1998). Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts. *J Dairy Sci,* 81, 411-9.

Bates, A. J., Chambers, G. & Laven, R. A. (2016). Comparison of cephalonium alone and in combination with an internal teat sealant for dry cow therapy in seasonally calving dairy cows. *N Z Vet J,* 64, 95-100.

Bauman, C. A., Barkema, H. W., Dubuc, J., Keefe, G. P. & Kelton, D. F. (2018). Canadian National Dairy Study: Herd-level milk quality. *J Dairy Sci,* 101, 2679-2691.

Berry, E. A. & Hillerton, J. E. (2002a). The effect of an intramammary Teat Seal on new intramammary infections. J. Dairy Sci., 85, 2512-2520.

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

Berry, E. A. & Hillerton, J. E. (2007). Effect of an intramammary teat seal and dry cow antibiotic in relation to dry period length on postpartum mastitis.[Erratum appears in J Dairy Sci. 2007 Aug;90(8):4004]. J. Dairy Sci., 90, 760-5.

Berry, E. A., Johnston, W. T. & Hillerton, J. E. (2003). Prophylactic effects of two selective dry cow strategies accounting for interdependence of quarter. J. Dairy Sci., 86, 3912-3919.

Bhutto, A. L., Murray, R. D. & Woldehiwet, Z. (2011). The effect of dry cow therapy and internal teat-sealant on intra-mammary infections during subsequent lactation. *Research in Veterinary Science,* 90, 316-320.

Bhutto, A. L., Murray, R. D. & Woldehiwet, Z. (2012). California mastitis test scores as indicators of subclinical intra-mammary infections at the end of lactation in dairy cows. *Res. Vet. Sci.,* 92, 13-17.

Bradley, A. (2002). Bovine mastitis: an evolving disease. *Vet. J.,* 164, 116-28.

Bradley, A. J., Barkema, H., Biggs, A., Green, M. & Lam, T. 2012. Chapter 5. Control of Mastitis and Enhancement of Milk Quality (Chapter five). *Dairy herd health.* Wallingford, Oxfordshire, UK: CABI.

Bradley, A. J., Breen, J. E., Payne, B., Williams, P. & Green, M. J. (2010). The use of a cephalonium containing dry cow therapy and an internal teat sealant, both alone and in combination. J. Dairy Sci., 93, 1566-1577.

Bradley, A. J. & Green, M. J. (2000). A study of the incidence and significance of intramammary enterobacterial infections acquired during the dry period. J. Dairy Sci., 83, 1957-1965.

Bradley, A. J. & Green, M. J. (2004). The importance of the nonlactating period in the epidemiology of intramammary infection and strategies for prevention. *Vet. Clin. North Am. Food Anim. Pract.,* 20, 547-68.

Brignardello-Petersen, R., Bonner, A., Alexander, P. E., Siemieniuk, R. A., Furukawa, T. A., Rochwerg, B., Hazlewood, G. S., Alhazzani, W., Mustafa, R. A., Murad, M. H., Puhan, M. A., Schünemann, H. J. & Guyatt, G. H. (2018). Advances in the GRADE approach to rate the certainty in estimates from a network meta-analysis. *J Clin Epidemiol,* 93, 36-44.

Brooks, S. P. & Gelman, A. (1998). General Methods for Monitoring Convergence of Iterative Simulations. *J. Comput. Graph. Stat.,* 7, 434-455.

Browning, J. W., Mein, G. A., Barton, M., Nicholls, T. J. & Brightling, P. (1990). Effects of antibiotic therapy at drying off on mastitis in the dry period and early lactation. *Aust. Vet. J.,* 67**,** 440-2.

Browning, J. W., Mein, G. A., Brightling, P., Nicholls, T. J. & Barton, M. (1994). Strategies of mastitis control: Dry cow therapy and culling. *Aust. Vet. J.,* 71**,** 179-181.

Caldwell, D. M., Ades, A. E. & Higgins, J. P. T. (2005). Simultaneous comparison of multiple treatments: combining direct and indirect evidence. *BMJ-BRIT MED J.,* 331**,** 897-900.

Call, D. R., Davis, M. A. & Sawant, A. A. (2008). Antimicrobial resistance in beef and dairy cattle production. *Anim. Health Res. Rev.,* 9**,** 159-67.

Cameron, M., Barkema, H. W., De Buck, J., De Vliegher, S., Chaffer, M., Lewis, J. & Keefe, G. P. (2017). Identification of bovine-associated coagulase-negative staphylococci by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a direct transfer protocol. *J Dairy Sci,* 100**,** 2137-2147.

Cameron, M., Keefe, G. P., Roy, J. P., Dohoo, I. R., Macdonald, K. A. & Mckenna, S. L. (2013). Evaluation of a 3M Petrifilm on-farm culture system for the detection of intramammary infection at the end of lactation. *Prev Vet Med,* 111**,** 1-9.

Cameron, M., Keefe, G. P., Roy, J. P., Stryhn, H., Dohoo, I. R. & Mckenna, S. L. (2015). Evaluation of selective dry cow treatment following on-farm culture: Milk yield and somatic cell count in the subsequent lactation. J. Dairy Sci*.,* 98**,** 2427-36.

Cameron, M., Mckenna, S. L., Macdonald, K. A., Dohoo, I. R., Roy, J. P. & Keefe, G. P. (2014). Evaluation of selective dry cow treatment following on-farm culture: Risk of postcalving intramammary infection and clinical mastitis in the subsequent lactation. J. Dairy Sci*.,* 97**,** 270-284.

Cameron, M., Perry, J., Middleton, J. R., Chaffer, M., Lewis, J. & Keefe, G. P. (2018). Short communication: Evaluation of MALDI-TOF mass spectrometry and a custom reference spectra expanded database for the identification of bovine-associated coagulase-negative staphylococci. *J Dairy Sci,* 101**,** 590-595.

Cbmqrn. 2010. *Administration technique of intramammary treatment in dairy cows* [Online]. Available: http://www.reseaumammite.org/tactic/wp-content/uploads/2020/02/FR-Fiche-Infusion-intramammaire.pdf [Accessed November 01, 2021].

Cook, N. B., Pionek, D. A. & Sharp, P. (2005). An assessment of the benefits of Orbeseal (R) when used in combination with dry cow antibiotic therapy in three commercial dairy herds. Bov. pract., 39**,** 83-94.

Dendukuri, N. & Joseph, L. (2001). Bayesian approaches to modeling the conditional dependence between multiple diagnostic tests. *Biometrics,* 57**,** 158-67.

Di Girolamo, N., Giuffrida, M. A., Winter, A. L. & Meursinge Reynders, R. (2017). In veterinary trials reporting and communication regarding randomisation procedures is suboptimal. *Vet Rec,* 181**,** 195.

Dias, S., Ades, A. E., Welton, N. J., Jansen, J. P. & Sutton, A. J. (2018a). Generalised Linear Models. *In Network Meta-Analysis for Decision Making (eds S. Dias, A.E. Ades, N.J. Welton, J.P. Jansen and A.J. Sutton). https://doi.org/10.1002/9781118951651.ch4.*

Dias, S., Ades, A. E., Welton, N. J., Jansen, J. P. & Sutton, A. J. (2018b). Meta-Regression for Relative Treatment Effects. *In Network Meta-Analysis for Decision Making (eds S. Dias, A.E. Ades, N.J. Welton, J.P. Jansen and A.J. Sutton). https://doi.org/10.1002/9781118951651.ch8.*

Dingwell, R. T., Kelton, D. F. & Leslie, K. E. (2003). Management of the dry cow in control of peripartum disease and mastitis. *Vet. Clin. North Am. Food Anim. Pract.,* 19**,** 235-65.

Dingwell, R. T., Leslie, K. E., Schukken, Y. H., Sargeant, J. M., Timms, L. L., Duffield, T. E., Keefe, G. P., Kelton, D. F., Lissemore, K. D. & Conklin, J. (2004). Association of cow and quarter-level factors at drying-off with new intramammary infections during the dry period. Prev. Vet. Med., 63**,** 75-89.

Dohoo, I., Stryhn, H. & Sanchez, J. (2007). Evaluation of underlying risk as a source of heterogeneity in meta-analyses: a simulation study of Bayesian and frequentist implementations of three models. *Prev Vet Med,* 81**,** 38-55.

Dohoo, I. R. & Leslie, K. E. (1991). Evaluation of changes in somatic cell counts as indicators of new intramammary infections. Prev. Vet. Med., 10**,** 225-237.

Dohoo, I. R., Martin, S. W. & Stryhn, H. (2009). Screening and diagnostic tests. *Veterinary Epidemiologic Research.* 2nd ed. Charlottetown, P.E.I., Canada: VER Inc., p. 91-133.

Dohoo, I. R., Smith, J., Andersen, S., Kelton, D. F. & Godden, S. (2011). Diagnosing intramammary infections: Evaluation of definitions based on a single milk sample. J. Dairy Sci*.,* 94**,** 250-261.

Dufour, S. & Dohoo, I. R. (2013). Short communication: Relationship between herd intramammary infection incidence and elimination rate during the dry period. J. Dairy Sci*.,* 96**,** 1672-1676.

Dufour, S., Dohoo, I. R., Barkema, H. W., Descôteaux, L., Devries, T. J., Reyher, K. K., Roy, J. P. & Scholl, D. T. (2012). Manageable risk factors associated with the lactational incidence, elimination, and prevalence of Staphylococcus aureus intramammary infections in dairy cows. *J Dairy Sci,* 95**,** 1283-300.

Dufour, S., Fréchette, A., Barkema, H. W., Mussell, A. & Scholl, D. T. (2011). Effect of udder health management practices on herd somatic cell count. J. Dairy Sci*.,* 94**,** 563-579.

Dufour, S., Wellemans, V., Roy, J. P., Lacasse, P., Ordonez-Iturriaga, A. & Francoz, D. (2019). Non-antimicrobial approaches at drying-off for treating and preventing intramammary infections in dairy cows. Part 1. Meta-analyses of efficacy of using an internal teat sealant without a concomitant antimicrobial treatment. *Anim. Health Res. Rev.,* 20**,** 86-97.

Francoz, D., Bergeron, L., Nadeau, M. & Beauchamp, G. (2012). Prevalence of contagious mastitis pathogens in bulk tank milk in Quebec. *Can Vet J,* 53**,** 1071-8.

Francoz, D., Wellemans, V., Dupré, J. P., Karreman, H. J., Roy, J. P., Labelle, F., Lacasse, P. & Dufour, S. (2017). Invited review: A systematic review and qualitative analysis of treatments other than conventional antimicrobials for clinical mastitis in dairy cows. J. Dairy Sci.

Francoz, D., Wellemans, V., Roy, J. P., Lacasse, P., Ordonez-Iturriaga, A., Labelle, F. & Dufour, S. (2016). Non-antibiotic approaches at drying-off for treating and preventing intramammary infections: a protocol for a systematic review and meta-analysis. *Anim. Health Res. Rev.,* 17**,** 169-175.

Godden, S., Rapnicki, P., Stewart, S., Fetrow, J., Johnson, A., Bey, R. & Farnsworth, R. (2003). Effectiveness of an internal teat seal in the prevention of new intramammary infections

during the dry and early-lactation periods in dairy cows when used with a dry cow intramammary antibiotic. J. Dairy Sci., 86, 3899-3911.

Golder, H. M., Hodge, A. & Lean, I. J. (2016). Effects of antibiotic dry-cow therapy and internal teat sealant on milk somatic cell counts and clinical and subclinical mastitis in early lactation. J. Dairy Sci., 99, 7370-7380.

Gradepro, G. D. T. (2015). : GRADEpro Guideline Development Tool [Software]. McMaster University. (developed by Evidence Prime, Inc.). Available from https://gradepro.org/.

Green, M. J., Bradley, A. J., Medley, G. F. & Browne, W. J. (2008). Cow, farm, and herd management factors in the dry period associated with raised somatic cell counts in early lactation. *J Dairy Sci,* 91, 1403-15.

Green, M. J., Green, L. E., Medley, G. F., Schukken, Y. H. & Bradley, A. J. (2002). Influence of dry period bacterial intramammary infection on clinical mastitis in dairy cows. J. Dairy Sci., 85, 2589-2599.

Grindlay, D. J., Brennan, M. L. & Dean, R. S. (2012). Searching the veterinary literature: a comparison of the coverage of veterinary journals by nine bibliographic databases. *J Vet Med Educ,* 39, 404-12.

Guyatt, G., Oxman, A. D., Akl, E. A., Kunz, R., Vist, G., Brozek, J., Norris, S., Falck-Ytter, Y., Glasziou, P., Debeer, H., Jaeschke, R., Rind, D., Meerpohl, J., Dahm, P. & Schunemann, H. J. (2011). GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol,* 64, 383-94.

Haine, D., Dohoo, I., Scholl, D. & Dufour, S. (2018). Diagnosing intramammary infection: Controlling misclassification bias in longitudinal udder health studies. Prev. Vet. Med., 150, 162-167.

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

Halasa, T., Nielen, M., Whist, A. C. & Osteras, O. (2009a). Meta-analysis of dry cow management for dairy cattle. Part 2. Cure of existing intramammary infections. J. Dairy Sci., 92, 3150-3157.

Halasa, T., Osteras, O., Hogeveen, H., Van Werven, T. & Nielen, M. (2009b). Meta-analysis of dry cow management for dairy cattle. Part 1. Protection against new intramammary infections. J. Dairy Sci., 92, 3134-3149.

Hanson, T., Johnson, W. O. & Gardner, I. A. (2003). Hierarchical models for estimating herd prevalence and test accuracy in the absence of a gold standard. *J. Agric. Biol. Environ. Stat.,* 8, 223.

Hassan, Z., Daniel, R. C. W., O'boyle, D. & Frost, A. J. (1999). Effects of dry cow intramammary therapy on quarter infections in the dry period. *Vet. Rec.,* 145, 635-639.

Higgins, J. P. T., Sterne, J. a. C., Savović, J., Hróbjartsson, A., Boutron, I., Reeves, B. & Eldridge, S. (2016). A revised tool for assessing risk of bias in randomized trials. *In:* CHANDLER J, MCKENZIE J & WELCH V (eds.) *Cochrane Methods, Cochrane Database of Systematic Reviews.* Oxford: John Wiley & Sons, Ltd., pp. 29–31.

Hui, S. L. & Walter, S. D. (1980). Estimating the error rates of diagnostic tests. *Biometrics,* 36, 167-71.

Hutton, B., Salanti, G., Caldwell, D. M., Chaimani, A., Schmid, C. H., Cameron, C., Ioannidis, J. P., Straus, S., Thorlund, K., Jansen, J. P., Mulrow, C., Catala-Lopez, F., Gotzsche, P. C., Dickersin, K., Boutron, I., Altman, D. G. & Moher, D. (2015). The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations. *Ann Intern Med,* 162, 777-84.

Huxley, J. N., Green, M. J., Green, L. E. & Bradley, A. J. (2002). Evaluation of the efficacy of an internal teat sealer during the dry period. J. Dairy Sci., 85, 551-61.

Jaeger, S., Virchow, F., Torgerson, P. R., Bischoff, M., Biner, B., Hartnack, S. & Rüegg, S. R. (2017). Test characteristics of milk amyloid A ELISA, somatic cell count, and bacteriological culture for detection of intramammary pathogens that cause subclinical mastitis. J. Dairy Sci., 100, 7419-7426.

Jansen, J. P., Crawford, B., Bergman, G. & Stam, W. (2008). Bayesian Meta-Analysis of Multiple Treatment Comparisons: An Introduction to Mixed Treatment Comparisons. *Value in Health,* 11, 956-964.

Joe, H. (2008). Accuracy of Laplace approximation for discrete response mixed models. *Comput Stat Data Anal.,* 52**,** 5066-5074.

Johnson, W. O., Jones, G. & Gardner, I. A. (2019). Gold standards are out and Bayes is in: Implementing the cure for imperfect reference tests in diagnostic accuracy studies. *Prev Vet Med,* 167**,** 113-127.

Kabera, F., Dufour, S., Keefe, G., Cameron, M. & Roy, J. P. (2020). Evaluation of quarter-based selective dry cow therapy using Petrifilm on-farm milk culture: A randomized controlled trial. *J Dairy Sci,* 103**,** 7276-7287.

Kabera, F., Roy, J.-P., Afifi, M., Godden, S., Stryhn, H., Sanchez, J. & Dufour, S. (2021a). Comparing Blanket vs. Selective Dry Cow Treatment Approaches for Elimination and Prevention of Intramammary Infections During the Dry Period: A Systematic Review and Meta-Analysis. Front. Vet. Sci*.,* 8.

Kabera, F., Roy, J.-P., Keefe, G. & Dufour, S. (2021b). *Bayesian estimation of diagnostic accuracy of somatic cell counts history and on-farm milk culture using Petrifilm® to identify quarters or cows that should be treated with antimicrobials in selective treatment protocols at dry off*. Data file, Scholars Portal Dataverse, V1 ed.: https://doi.org/10.5683/SP3/VYY5TC.

Knapp, G. & Hartung, J. (2003). Improved tests for a random effects meta-regression with a single covariate. *Stat Med,* 22**,** 2693-710.

Kostoulas, P., Nielsen, S. S., Branscum, A. J., Johnson, W. O., Dendukuri, N., Dhand, N. K., Toft, N. & Gardner, I. A. (2017). STARD-BLCM: Standards for the Reporting of Diagnostic accuracy studies that use Bayesian Latent Class Models. *Prev Vet Med,* 138**,** 37-47.

Krattley-Roodenburg, B., Huybens, L. J., Nielen, M. & Van Werven, T. (2021). Dry period management and new high somatic cell count during the dry period in Dutch dairy herds under selective dry cow therapy. J. Dairy Sci*.,* 104**,** 6975-6984.

Landis, J. R. & Koch, G. G. (1977). The measurement of observer agreement for categorical data. *Biometrics,* 33**,** 159-174.

Langan, D., Higgins, J. P. T., Jackson, D., Bowden, J., Veroniki, A. A., Kontopantelis, E., Viechtbauer, W. & Simmonds, M. (2019). A comparison of heterogeneity variance estimators in simulated random-effects meta-analyses. *Res Synth Methods,* 10**,** 83-98.

Lardé, H., Dufour, S., Archambault, M., Léger, D., Loest, D., Roy, J. P. & Francoz, D. (2020). Assignment of Canadian Defined Daily Doses and Canadian Defined Course Doses for Quantification of Antimicrobial Usage in Cattle. *Front Vet Sci,* 7**,** 10.

Lardé, H., Francoz, D., Roy, J. P., Massé, J., Archambault, M., Paradis, M. & Dufour, S. (2021). Comparison of Quantification Methods to Estimate Farm-Level Usage of Antimicrobials Other than in Medicated Feed in Dairy Farms from Québec, Canada. *Microorganisms,* 9.

Laven, R. A., Balcomb, C. C., Tulley, W. T. & Lawrence, K. E. (2014). Effect of dry period length on the effect of an intramammary teat sealant on the risk of mastitis in cattle treated with antibiotics at drying off. *N Z Vet J,* 62**,** 214-20.

Laven, R. A. & Lawrence, K. E. (2008). Efficacy of blanket treatment of cows and heifers with an internal teat sealant in reducing the risk of mastitis in dairy cattle calving on pasture. *N Z Vet J,* 56**,** 171-5.

Lay, A. M., Kolpin, K. M., Sommer, D. A. & Rankin, S. A. (2007). Hot topic: Black spot defect in Cheddar cheese linked to intramammary teat sealant. *J Dairy Sci,* 90**,** 4938-41.

Lindstrom, U. B., Kenttamies, H., Arstila, J. & Tuovila, R. (1981). USEFULNESS OF CELL COUNTS IN PREDICTING BOVINE MASTITIS. *Acta agric. Scand.,* 31**,** 199-203.

Lu, G. & Ades, A. E. (2004). Combination of direct and indirect evidence in mixed treatment comparisons. *Stat Med,* 23**,** 3105-24.

Mahmmod, Y. S., Nonnemann, B., Svennesen, L., Pedersen, K. & Klaas, I. C. (2018). Typeability of MALDI-TOF assay for identification of non-aureus staphylococci associated with bovine intramammary infections and teat apex colonization. J. Dairy Sci*.,* 101**,** 9430-9438.

Mastitis Network. 2020. *Protocole recommandé pour l'administration d'un scellant interne à trayons chez les bovins laitiers* [Online]. Available: http://www.reseaumammite.org/tactic/wp-content/uploads/2020/10/Protocole-recommande%CC%81-pour-ladministration-dun-scellant-interne-a%CC%80-trayons-chez-les-bovins-laitiers_2020.pdf [Accessed November 17, 2021].

Mccarron, J. L., Keefe, G. P., Mckenna, S. L., Dohoo, I. R. & Poole, D. E. (2009). Laboratory evaluation of 3M Petrifilms and University of Minnesota Bi-plates as potential on-farm tests for clinical mastitis. *J Dairy Sci,* 92**,** 2297-305.

Mcdermott, M. P., Erb, H. N. & Natzke, R. P. (1982). Predictability by Somatic Cell Counts Related to Prevalence of Intrammary Infection Within Herds. J. Dairy Sci., 65, 1535-1539.

Mcdougall, S. (2010). A randomised, non-inferiority trial of a new cephalonium dry-cow therapy. *N Z Vet J,* 58, 45-58.

Meaney, W. J. (1977). Effect of a dry period teat seal on bovine udder infection. *Ir. J. Agric. Res.,* 16, 293-299.

Mickey, R. M. & Greenland, S. (1989). The impact of confounder selection criteria on effect estimation. *Am J Epidemiol,* 129, 125-37.

Moher, D., Liberati, A., Tetzlaff, J. & Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Bmj,* 339, b2535.

Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P. & Stewart, L. A. (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst. Rev., 4, 1.

Mütze, K., Wolter, W., Failing, K., Kloppert, B., Bernhardt, H. & Zschoeck, M. (2012). The effect of dry cow antibiotic with and without an internal teat sealant on udder health during the first 100 d of lactation: a field study with matched pairs. *J. Dairy Res.,* 79, 477-484.

National Mastitis Council. 2006. *Recommended mastitis control plan* [Online]. Available: http://www.nmconline.org/wp-content/uploads/2016/08/RECOMMENDED-MASTITIS-CONTROL-PROGRAM-International.pdf [Accessed August 29, 2017].

National Mastitis Council 2016. *Current concepts of bovine mastitis,* 605 Columbus Avenue South, New Prague, Minesota 56071, National Mastistis Council, Inc.

National Mastitis Council (2017a). Chapter one: Sample Collection and Handling. *Laboratory handbook on bovine mastitis.* Third ed. 605 Columbus Avenue South, New Prague, Minesota 56071: National Mastitis Council, Inc.

National Mastitis Council 2017b. *Laboratory handbook on bovine mastitis,* 605 Columbus Avenue South, New Prague, Minesota 56071, National Mastitis Council, Inc.

Neave, F. K., Dodd, F. H., Kingwill, R. G. & Westgarth, D. R. (1969). Control of Mastitis in the Dairy Herd by Hygiene and Management. J. Dairy Sci., 52, 696-707.

Newton, H. T., Green, M. J., Benchaoui, H., Cracknell, V., Rowan, T. & Bradley, A. J. (2008). Comparison of the efficacy of cloxacillin alone and cloxacillin combined with an internal teat sealant for dry-cow therapy. *The Vet. Rec.,* 162**,** 678-84.

O'connor, A. M. & Sargeant, J. M. (2014). Meta-analyses including data from observational studies. *Prev Vet Med,* 113**,** 313-22.

O'connor, A. M., Sargeant, J. M., Gardner, I. A., Dickson, J. S., Torrence, M. E., Dewey, C. E., Dohoo, I. R., Evans, R. B., Gray, J. T., Greiner, M., Keefe, G., Lefebvre, S. L., Morley, P. S., Ramirez, A., Sischo, W., Smith, D. R., Snedeker, K., Sofos, J., Ward, M. P. & Wills, R. (2010). The REFLECT statement: methods and processes of creating reporting guidelines for randomized controlled trials for livestock and food safety. *J Vet Intern Med,* 24**,** 57-64.

Olde Riekerink, R. G., Barkema, H. W., Kelton, D. F. & Scholl, D. T. (2008). Incidence rate of clinical mastitis on Canadian dairy farms. *J Dairy Sci,* 91**,** 1366-77.

Olde Riekerink, R. G., Barkema, H. W., Veenstra, S., Poole, D. E., Dingwell, R. T. & Keefe, G. P. (2006). Prevalence of contagious mastitis pathogens in bulk tank milk in Prince Edward Island. *Can Vet J,* 47**,** 567-72.

Oliver, S. P., Murinda, S. E. & Jayarao, B. M. (2011). Impact of antibiotic use in adult dairy cows on antimicrobial resistance of veterinary and human pathogens: a comprehensive review. *Foodborne Pathog Dis,* 8**,** 337-55.

Pantoja, J. C., Hulland, C. & Ruegg, P. L. (2009). Dynamics of somatic cell counts and intramammary infections across the dry period. *Prev Vet Med,* 90**,** 43-54.

Patel, K., Godden, S. M., Royster, E. E., Timmerman, J. A., Crooker, B. A. & Mcdonald, N. (2017). Pilot study: impact of using a culture-guided selective dry cow therapy program targeting quarter-level treatment on udder health and antibiotic use. Bov. pract.*,* 51**,** 48-57.

Peters, J. L., Sutton, A. J., Jones, D. R., Abrams, K. R. & Rushton, L. (2008). Contour-enhanced meta-analysis funnel plots help distinguish publication bias from other causes of asymmetry. *J Clin Epidemiol,* 61**,** 991-6.

Piessens, V., De Vliegher, S., Verbist, B., Braem, G., Van Nuffel, A., De Vuyst, L., Heyndrickx, M. & Van Coillie, E. (2012). Intra-species diversity and epidemiology varies among coagulase-

negative Staphylococcus species causing bovine intramammary infections. *Vet Microbiol,* 155**,** 62-71.

Poutrel, B. & Rainard, P. (1981). California Mastitis test guide of selective dry cow therapy. J. Dairy Sci*.,* 64**,** 241-8.

Rabiee, A. R. & Lean, I. J. (2013). The effect of internal teat sealant products (Teatseal and Orbeseal) on intramammary infection, clinical mastitis, and somatic cell counts in lactating dairy cows: A meta-analysis. J. Dairy Sci*.,* 96**,** 6915-6931.

Rajala-Schultz, P. J., Torres, A. H. & Degraves, F. J. (2011). Milk yield and somatic cell count during the following lactation after selective treatment of cows at dry-off. *J. Dairy Res.,* 78**,** 489-499.

Reyher, K. K., Dufour, S., Barkema, H. W., Des Coteaux, L., Devries, T. J., Dohoo, I. R., Keefe, G. P., Roy, J. P. & Scholl, D. T. (2011). The National Cohort of Dairy Farms-A data collection platform for mastitis research in Canada. J. Dairy Sci*.,* 94**,** 1616-1626.

Rindsig, R. B., Rodewald, R. G., Smith, A. R. & Spahr, S. L. (1978). Complete versus selective dry cow therapy for mastitis control. J. Dairy Sci*.,* 61**,** 1483-97.

Rindsig, R. B., Rodewald, R. G., Smith, A. R., Thomsen, N. K. & Spahr, S. L. (1979). Mastitis history, California Mastitis Test, and somatic cell counts for identifying cows for treatment in a selective dry cow therapy program. J. Dairy Sci*.,* 62**,** 1335-1339.

Robert, A., Bareille, N., Roussel, P., Poutrel, B., Heuchel, V. & Seegers, H. (2006a). Interdependence of udder quarters for new intramammary infection during the dry period in cows submitted to selective antibiotic therapy. *J. Dairy Res.,* 73**,** 345-352.

Robert, A., Roussel, P., Bareille, N., Ribaud, D., Serieys, F., Heuchel, V. & Seegers, H. (2008). Risk factors for new intramammary infections during the dry period in untreated dairy cows from herds using selective dry cow therapy. *Animal : an international journal of animal bioscience,* 2**,** 247-54.

Robert, A., Seegers, H. & Bareille, N. (2006b). Incidence of intramammary infections during the dry period without or with antibiotic treatment in dairy cows--a quantitative analysis of published data. *Vet. Res.,* 37**,** 25-48.

Robinson, T. C., Jackson, E. R. & Marr, A. (1983). Within herd comparison of teat dipping and dry cow therapy with only selective dry cow therapy in six herds. *Vet. Rec.,* 112**,** 315-319.

Robinson, T. C., Jackson, E. R. & Marr, A. (1988). Mastitis incidence in quarters with different infections status at drying off and calving in two treatment groups. *Br Vet J,* 144**,** 166-73.

Roguinsky, M. & Serieys, F. (1977). Treatment of mastitis in dry cows: treatment of all cows vs treatment of infected cows only (author's transl). *Ann. Rech. Vet.,* 8**,** 327-331.

Rowe, S. M., Godden, S. M., Nydam, D. V., Gorden, P. J., Lago, A., Vasquez, A. K., Royster, E., Timmerman, J. & Thomas, M. J. (2020a). Randomized controlled non-inferiority trial investigating the effect of 2 selective dry-cow therapy protocols on antibiotic use at dry-off and dry period intramammary infection dynamics. *J Dairy Sci,* 103**,** 6473-6492.

Rowe, S. M., Godden, S. M., Nydam, D. V., Gorden, P. J., Lago, A., Vasquez, A. K., Royster, E., Timmerman, J. & Thomas, M. J. (2020b). Randomized controlled trial investigating the effect of 2 selective dry-cow therapy protocols on udder health and performance in the subsequent lactation. *J Dairy Sci,* 103**,** 6493-6503.

Runciman, D. J., Malmo, J. & Deighton, M. (2010). The use of an internal teat sealant in combination with cloxacillin dry cow therapy for the prevention of clinical and subclinical mastitis in seasonal calving dairy cows. J. Dairy Sci.*,* 93**,** 4582-4591.

Saini, V., Mcclure, J. T., Leger, D., Dufour, S., Sheldon, A. G., Scholl, D. T. & Barkema, H. W. (2012). Antimicrobial use on Canadian dairy farms. *J Dairy Sci,* 95**,** 1209-21.

Sanford, C. J., Keefe, G. P., Dohoo, I. R., Leslie, K. E., Dingwell, R. T., Descôteaux, L. & Barkema, H. W. (2006a). Efficacy of using an internal teat sealer to prevent new intramammary infections in nonlactating dairy cattle. *J Am Vet Med Assoc,* 228**,** 1565-73.

Sanford, C. J., Keefe, G. P., Sanchez, J., Dingwell, R. T., Barkema, H. W., Leslie, K. E. & Dohoo, I. R. (2006b). Test characteristics from latent-class models of the California Mastitis Test. *Prev Vet Med,* 77**,** 96-108.

Santman-Berends, I. M. G. A., Swinkels, J. M., Lam, T. J. G. M., Keurentjes, J. & Van Schaik, G. (2016). Evaluation of udder health parameters and risk factors for clinical mastitis in Dutch dairy herds in the context of a restricted antimicrobial usage policy. J. Dairy Sci.*,* 99**,** 2930-2939.

Sargeant, J. M., O'connor, A. M., Dohoo, I. R., Erb, H. N., Cevallos, M., Egger, M., Ersbøll, A. K., Martin, S. W., Nielsen, L. R., Pearl, D. L., Pfeiffer, D. U., Sanchez, J., Torrence, M. E., Vigre, H., Waldner, C. & Ward, M. P. (2016). Methods and processes of developing the strengthening the reporting of observational studies in epidemiology - veterinary (STROBE-Vet) statement. *Prev Vet Med,* 134**,** 188-196.

Sargeant, J. M., O'connor, A. M., Gardner, I. A., Dickson, J. S., Torrence, M. E., Dohoo, I. R., Lefebvre, S. L., Morley, P. S., Ramirez, A. & Snedeker, K. (2010). The REFLECT statement: reporting guidelines for randomized controlled trials in livestock and food safety: explanation and elaboration. *J Food Prot,* 73**,** 579-603.

Schabauer, A., Pinior, B., Gruber, C. M., Firth, C. L., Käsbohrer, A., Wagner, M., Rychli, K. & Obritzhauser, W. (2018). The relationship between clinical signs and microbiological species, spa type, and antimicrobial resistance in bovine mastitis cases in Austria. *Vet Microbiol,* 227**,** 52-60.

Schepers, A. J., Lam, T. J. G. M., Schukken, Y. H., Wilmink, J. B. M. & Hanekamp, W. J. A. (1997). Estimation of Variance Components for Somatic Cell Counts to Determine Thresholds for Uninfected Quarters. J. Dairy Sci*.,* 80**,** 1833-1840.

Scherpenzeel, C. G. M., Den Uijl, I. E. M., Van Schaik, G., Olde Riekerink, R. G. M., Keurentjes, J. M. & Lam, T. J. G. M. (2014). Evaluation of the use of dry cow antibiotics in low somatic cell count cows. J. Dairy Sci*.,* 97**,** 3606-14.

Scherpenzeel, C. G. M., Den Uijl, I. E. M., Van Schaik, G., Riekerink, R. G. M. O., Hogeveen, H. & Lam, T. J. G. M. (2016a). Effect of different scenarios for selective dry-cow therapy on udder health, antimicrobial usage, and economics. J. Dairy Sci*.,* 99**,** 3753-3764.

Scherpenzeel, C. G. M., Tijs, S. H. W., Den Uijl, I. E. M., Santman-Berends, I. M. G. A., Velthuis, A. G. J. & Lam, T. J. G. M. (2016b). Farmers' attitude toward the introduction of selective dry cow therapy. J. Dairy Sci*.,* 99**,** 8259-8266.

Schukken, Y. H., Smit, J. a. H., Grommers, F. J., Vandegeer, D. & Brand, A. (1989). Effect of Freezing on Bacteriologic Culturing of Mastitis Milk Samples. J. Dairy Sci*.,* 72**,** 1900-1906.

Schukken, Y. H., Wilson, D. J., Welcome, F., Garrison-Tikofsky, L. & Gonzalez, R. N. (2003). Monitoring udder health and milk quality using somatic cell counts. *Vet Res,* 34**,** 579-96.

Schünemann, H. J., Brożek, J., Guyatt, G. & Oxman, A., (Eds) (2013). GRADE Handbook for Grading Quality of Evidence and Strength of Recommendations. Available from www.guidelinedevelopment.org/handbook (accessed January 2017).

Schunemann, H. J., Oxman, A. D., Vist, G. E. & Higgins, J. P. T. 2017. Chapter 12: interpreting results and drawing conclusions. *In:* HIGGINS J.P.T, CHANDLER J & CUMPSTON M.S (eds.) *Cochrane Handbook for Systematic Reviews of Interventions Version 5.2.0. pp. 12:1–12:26. Available from www.training.cochrane.org/handbook (accessed September 2017).*

Seeth, M. T., Wente, N., Paduch, J. H., Klocke, D., Vries, E. M. D., Hoedemaker, M. & Kromker, V. (2017). Different selective dry cow therapy concepts compared to blanket antibiotic dry cow treatment. *Tierarztl Prax Ausg G Grosstiere Nutztiere,* 45**,** 343-349.

Sergeant, E. S. G. (2018). Epitools Epidemiological Calculators. Ausvet. Available at: http://epitools.ausvet.com.au.

Serieys, F. & Roguinsky, M. (1975). Comparative tests with dry cow therapy for some or all cows in a herd. *Annual Bulletin, International Dairy Federation***,** 349-351.

Shamseer, L., Moher, D., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P. & Stewart, L. A. (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *Bmj,* 350**,** g7647.

Shook, G. E. (1993). Genetic improvement of mastitis through selection on somatic cell count. *Vet Clin North Am Food Anim Pract,* 9**,** 563-81.

Smith, K. L., Todhunter, D. A. & Schoenberger, P. S. (1985a). Environmental mastitis: cause, prevalence, prevention. J. Dairy Sci*.,* 68**,** 1531-53.

Smith, K. L., Todhunter, D. A. & Schoenberger, P. S. (1985b). Environmental pathogens and intramammary infection during the dry period. J. Dairy Sci*.,* 68**,** 402-17.

Spiegelhalter, D. J., Best, N. G., Carlin, B. P. & Van Der Linde, A. (2002). Bayesian measures of model complexity and fit. *J R Stat Soc Series B Stat Methodol,* 64**,** 583-639.

Sterne, J. A., Hernán, M. A., Reeves, B. C., Savović, J., Berkman, N. D., Viswanathan, M., Henry, D., Altman, D. G., Ansari, M. T., Boutron, I., Carpenter, J. R., Chan, A. W., Churchill, R., Deeks, J. J., Hróbjartsson, A., Kirkham, J., Jüni, P., Loke, Y. K., Pigott, T. D., Ramsay, C. R., Regidor, D., Rothstein, H. R., Sandhu, L., Santaguida, P. L., Schünemann, H. J., Shea, B., Shrier, I.,

Tugwell, P., Turner, L., Valentine, J. C., Waddington, H., Waters, E., Wells, G. A., Whiting, P. F. & Higgins, J. P. (2016). ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *Bmj,* 355**,** i4919.

Swinkels, J. M., Leach, K. A., Breen, J. E., Payne, B., White, V., Green, M. J. & Bradley, A. J. (2021). Randomized controlled field trial comparing quarter and cow level selective dry cow treatment using the California Mastitis Test. *J Dairy Sci,* 104**,** 9063-9081.

Thomson, K., Rantala, M., Hautala, M., Pyorala, S. & Kaartinen, L. (2008). Cross-sectional prospective survey to study indication-based usage of antimicrobials in animals: results of use in cattle. *BMC Vet Res,* 4**,** 15.

Toft, N., Akerstedt, J., Tharaldsen, J. & Hopp, P. (2007a). Evaluation of three serological tests for diagnosis of Maedi-Visna virus infection using latent class analysis. *Vet Microbiol,* 120**,** 77-86.

Toft, N., Innocent, G. T., Gettinby, G. & Reid, S. W. (2007b). Assessing the convergence of Markov Chain Monte Carlo methods: an example from evaluation of diagnostic tests in absence of a gold standard. *Prev Vet Med,* 79**,** 244-56.

Toft, N., Jørgensen, E. & Højsgaard, S. (2005). Diagnosing diagnostic tests: evaluating the assumptions underlying the estimation of sensitivity and specificity in the absence of a gold standard. *Prev Vet Med,* 68**,** 19-33.

Torres, A. H., Rajala-Schultz, P. J., Degraves, F. J. & Hoblet, K. H. (2008). Using dairy herd improvement records and clinical mastitis history to identify subclinical mastitis infections at dry-off. *J. Dairy Res.,* 75**,** 240-247.

Vanhoudt, A., Van Hees-Huijps, K., Van Knegsel, A. T. M., Sampimon, O. C., Vernooij, J. C. M., Nielen, M. & Van Werven, T. (2018). Effects of reduced intramammary antimicrobial use during the dry period on udder health in Dutch dairy herds. J. Dairy Sci*.,* 101**,** 3248-3260.

Vasquez, A. K., Nydam, D. V., Foditsch, C., Wieland, M., Lynch, R., Eicker, S. & Virkler, P. D. (2018). Use of a culture-independent on-farm algorithm to guide the use of selective dry-cow antibiotic therapy. J. Dairy Sci*.,* 101**,** 5345-5361.

Vissio, C., Dieser, S. A., Agnelli, H. L., Odierno, L. M. & Larriestra, A. J. (2014). Accuracy of the composite somatic cell count to detect intra-mammary infection in dairy cows using latent class analysis. Prev. Vet. Med., 113, 547-555.

Vuong, Q. H. (1989). Likelihood Ratio Tests for Model Selection and Non-Nested Hypotheses. *Econometrica,* 57, 307-333.

Ward, G. E. & Schultz, L. H. (1974). Incidence and control of mastitis during the dry period. J. Dairy Sci., 57, 1341-9.

White, I. R., Barrett, J. K., Jackson, D. & Higgins, J. P. (2012). Consistency and inconsistency in network meta-analysis: model estimation using multivariate meta-regression. *Res Synth Methods,* 3, 111-25.

Williamson, J. H. 2001. Strategies for protecting the teat at dry-off. *The National Mastitis Council 40th Annual Meeting, February 11–14, 2001 2001 Reno, Nevada.* National Mastitis Council, Verona, WI., 88 - 94.

Williamson, J. H., Woolford, M. W. & Day, A. M. (1995). The prophylactic effect of a dry-cow antibiotic against Streptococcus uberis. *N Z Vet J,* 43, 228-34.

Winder, C. B., Sargeant, J. M., Kelton, D. F., Leblanc, S. J., Duffield, T. F., Glanville, J., Wood, H., Churchill, K. J., Dunn, J., Bergevin, M. D., Dawkins, K., Meadows, S. & O'connor, A. M. (2019). Comparative efficacy of blanket versus selective dry-cow therapy: a systematic review and pairwise meta-analysis. *Anim. Health Res. Rev.,* 20, 217-228.

Woolford, M. W., Williamson, J. H., Day, A. M. & Copeman, P. J. (1998). The prophylactic effect of a teat sealer on bovine mastitis during the dry period and the following lactation. *N Z Vet J,* 46, 12-9.

Zadoks, R., Gillespie, B., Barkema, H., Sampimon, O., Oliver, S. & Schukken, Y. (2003). Clinical, epidemiological and molecular characteristics of Streptococcus uberis infections in dairy herds. *Epidemiol Infect,* 130, 335-349.

Annexes

Annexe 1:

Boolean search algorithm for identifying literature of antimicrobial-based dry cow therapy approaches for cure and prevention of intramammary infections

The search strategy has been developed after an iterative process that involved test searches of different search terms and combinations of terms. Starting with general free-text words describing each component of our PICO questions, refinements have been made to get search index terms that fit each bibliographic database such as MESH terms for MEDLINE and thesaurus terms CAB Abstracts.

General keys words:

Population

Cow* OR Cattle OR Bovine* OR Bovidae

Interventions

Antibiotic* OR antibacterial* OR anti-bacterial* OR anti-microbial* OR antimicrobial* OR anti-infective* OR antiinfective* OR therap* OR treatment* OR seal*

Outcomes

Intramammary infection* OR Intra mammary infection* OR Intra-mammary infection* OR Mastitis OR udder health OR IMI

Period

Dry-off OR Dry off OR Drying-off OR Drying off OR Dry period* OR Drying period*

Medline (https://www.ncbi.nlm.nih.gov/pubmed) Mesh terms

Population

"Cattle"[Mesh]

Interventions

"Anti-Infective Agents"[Mesh]

Outcomes

"Mastitis, Bovine"[Mesh]

CAB Abstracts (http://ovidsp.ovid.com/autologin.cgi)
Thesaurus terms
Population

exp dairy cows/ OR exp dairy cattle/

Interventions

exp antiinfective agents/ or exp antibiotics/

Outcomes

exp mastitis/

Period

exp dry period/

Web of Science (https://login.webofknowledge.com)
(Cow* OR Cattle OR Bovine* OR Bovidae) AND (Antibiotic* OR antibacterial* OR anti-bacterial* OR anti-microbial* OR antimicrobial* OR anti-infective* OR antiinfective* OR therap* OR treatment* OR seal*) AND (Intramammary infection* OR Intra mammary infection* OR Intra-mammary infection* OR Mastitis OR udder health OR IMI) AND (Dry-off OR Dry off OR Drying-off OR Drying off OR Dry period* OR Drying period*)

Annexe 2:

Screening tool for eligibility of articles to be included in reviews on antimicrobial-based dry cow therapy approaches for cure and prevention of intramammary infections.

Six-question checklist with three different options: *Yes* (Y); *Unsure* (US); or *No* (N). Answering *Yes* or *Unsure* for all the questions will pass the citation to the next review stage and the article will be procured. Consequently, by selection of *No* for at least one question the article will be excluded.

Questions	Response (Y/N/US)
Q1. Does the title/abstract describe original research (as opposed to a review)?	
Q2. Does the title/abstract describe a field-based study?	
Q3. Does the title/abstract describe dairy cows at drying-off?	
Q4. Does the title/abstract evaluate at least one antibiotic?	
Q5. Does the title/abstract evaluate at least one of the dry cow therapy approaches (SDCT, BDCT)?	
Q6. Does the title/abstract report at least one outcome related to preventing or curing IMI?	

Annexe 3: Example of the OpenBUGS code where cow-level SCC data are compared with quarter-level milk culture on Petrifilm and quarter-level milk culture using standard bacteriology in laboratory.

```
# Model used to estimate sensitivity and specificity of the last DHI test SCC at a threshold of 200,000 cells/mL, Petrifilm® on-farm milk culture and standard bacteriological culture
# And disease prevalence assuming conditional dependence between Petrifilm® on-farm milk culture and standard bacteriological culture
# Nine populations
#============================================================#
model{
for (i in 1:9){
t12_z0[i,1:8] ~ dmulti(p12_z0[i, 1:8], n_z0[i])
n_z0[i] <- sum(t12_z0[i, 1:8])
p12_z0[i,1] <- pi[i]*(Se_petri*Se_lab+covp)*Se_scc_z0 + (1-pi[i])*((1-Sp_petri)*(1-Sp_lab)+covn)*(1-Sp_scc_z0)
p12_z0[i,2] <- pi[i]*(Se_petri*(1-Se_lab)-covp)*Se_scc_z0 + (1-pi[i])*((1-Sp_petri)*Sp_lab-covn)*(1-Sp_scc_z0)
p12_z0[i,3] <- pi[i]*((1-Se_petri)*Se_lab-covp)*Se_scc_z0 + (1-pi[i])*(Sp_petri*(1-Sp_lab)-covn)*(1-Sp_scc_z0)
p12_z0[i,4] <- pi[i]*((1-Se_petri)*(1-Se_lab)+covp)*Se_scc_z0 + (1-pi[i])*(Sp_petri*Sp_lab+covn)*(1-Sp_scc_z0)
p12_z0[i,5] <- pi[i]*(Se_petri*Se_lab+covp)*(1-Se_scc_z0) + (1-pi[i])*((1-Sp_petri)*(1-Sp_lab)+covn)*Sp_scc_z0
p12_z0[i,6] <- pi[i]*(Se_petri*(1-Se_lab)-covp)*(1-Se_scc_z0) + (1-pi[i])*((1-Sp_petri)*Sp_lab-covn)*Sp_scc_z0
p12_z0[i,7] <- pi[i]*((1-Se_petri)*Se_lab-covp)*(1-Se_scc_z0) + (1-pi[i])*(Sp_petri*(1-Sp_lab)-covn)*Sp_scc_z0
```

```
p12_z0[i,8] <- pi[i]*((1-Se_petri)*(1-Se_lab)+covp)*(1-Se_scc_z0) + (1-pi[i])*(Sp_petri*Sp_lab+covn)*Sp_scc_z0


t12_z1[i,1:8] ~ dmulti(p12_z1[i, 1:8], n_z1[i])

n_z1[i] <- sum(t12_z1[i, 1:8])

p12_z1[i,1] <- pi[i]*(Se_petri*Se_lab+covp)*Se_scc_z1 + (1-pi[i])*((1-Sp_petri)*(1-Sp_lab)+covn)*(1-Sp_scc_z1)

p12_z1[i,2] <- pi[i]*(Se_petri*(1-Se_lab)-covp)*Se_scc_z1 + (1-pi[i])*((1-Sp_petri)*Sp_lab-covn)*(1-Sp_scc_z1)

p12_z1[i,3] <- pi[i]*((1-Se_petri)*Se_lab-covp)*Se_scc_z1 + (1-pi[i])*(Sp_petri*(1-Sp_lab)-covn)*(1-Sp_scc_z1)

p12_z1[i,4] <- pi[i]*((1-Se_petri)*(1-Se_lab)+covp)*Se_scc_z1 + (1-pi[i])*(Sp_petri*Sp_lab+covn)*(1-Sp_scc_z1)

p12_z1[i,5] <- pi[i]*(Se_petri*Se_lab+covp)*(1-Se_scc_z1) + (1-pi[i])*((1-Sp_petri)*(1-Sp_lab)+covn)*Sp_scc_z1

p12_z1[i,6] <- pi[i]*(Se_petri*(1-Se_lab)-covp)*(1-Se_scc_z1) + (1-pi[i])*((1-Sp_petri)*Sp_lab-covn)*Sp_scc_z1

p12_z1[i,7] <- pi[i]*((1-Se_petri)*Se_lab-covp)*(1-Se_scc_z1) + (1-pi[i])*(Sp_petri*(1-Sp_lab)-covn)*Sp_scc_z1

p12_z1[i,8] <- pi[i]*((1-Se_petri)*(1-Se_lab)+covp)*(1-Se_scc_z1) + (1-pi[i])*(Sp_petri*Sp_lab+covn)*Sp_scc_z1


# PRIOR FOR PREVALENCE

pi[i] ~ dbeta(alpha, beta)

}

#=== PRIORS ===#

#HYPERPRIORS
```

```
alpha <- mu*psi                  ## a parameter for the hierarchical beta distribution

beta <- psi*(1-mu)               ## a parameter for the hierarchical beta distribution

mu ~ dbeta(20.3, 88.8)        # median (of the mean) prevalence is 0.18 and the 95% quantile (of the mean) is 0.25

                               ## Mode at 0.18 (mean of Cameron and Rowe) and 95th percentile at 0.25 (most extreme upper 95%CI)

psi ~ dgamma(3.2, 0.31)    # 50th percentile for the 90th herd prev percentile = 0.50 (approximation assuming that contemporary herds have lower
                             herd prev than in 2007-2008)

                           # 95th percentile for the 90th herd prev percentile = 0.83 (based on the 53 CBMRN herds with SCC LT 250,000)

                           # Using mode=0.18 and 95th percentile=0.50 we obtain beta(2.3, 7.0). Mode of Psi is 2.3+7.0=9.3

                           # Using mode=0.18 and 95th percentile=0.83 we obtain beta(1.2, 1.8). 5th percentile of Psi is 1.2+1.8=3.0

                           # A gamma distribution with mode=9.3 and 5th percentile=3.0 would be dgamma()


#PRIORS FOR SENSITIVITY AND SPECIFICITY

#Petrifilm (petri)

#Se_petri ~ dbeta(1, 1) ## Vague

#Sp_petri ~ dbeta(1, 1) ## Non-informative

Se_petri ~ dbeta(91.1229, 16.6551) ## mode=0.852, 5/95 percentile=0.785 [Se=85.2% (78.5–90.5)]

Sp_petri ~ dbeta(98.5337, 36.7091) ## mode=0.732, 5/95 percentile=0.664 [Sp=73.2% (66.4–79.3)]
```

#Lab bacteriology (lab)

#Se_lab ~ dbeta(1, 1) ## Non-informative

#Sp_lab ~ dbeta(1, 1) ## Non-informative

Se_lab ~ dbeta(46.4238, 5.8238) ## mode=0.904, 5/95 percentile=0.81 [Mean Se=90.4% (81.0–100)]

Sp_lab ~ dbeta(37.0772, 14.6501) ## mode=0.7255, 5/95 percentile=0.61 [Mean Sp=72.55% (61–89)]

#SCC_z0 (first parity cows)

#Se_scc_z0 ~ dbeta(1, 1) ## Non-informative

#Sp_scc_z0 ~ dbeta(1, 1) ## Non-informative

Se_scc_z0 ~ dbeta(16.5203, 3.5266) ## mode=0.86, 5/95 percentile=0.94 [Mean Se=86.0% at cow level and 94% at quarter level]

Sp_scc_z0 ~ dbeta(280.0703, 419.6055) ## mode=0.40, 5/95 percentile=0.37 [Mean Sp=40.0% at cow level and 37% at quarter level]

#SCC_z1 (older cows; parity > 1)

#Se_scc_z1 ~ dbeta(1, 1) ## Non-informative

#Sp_scc_z1 ~ dbeta(1, 1) ## Non-informative

Se_scc_z1 ~ dbeta(16.5203, 3.5266) ## mode=0.86, 5/95 percentile=0.94 [Mean Se=86.0% at cow level and 94% at quarter level]

Sp_scc_z1 ~ dbeta(280.0703, 419.6055) ## mode=0.40, 5/95 percentile=0.37 [Mean Sp=40.0% at cow level and 37% at quarter level]

```
#=== CONDITIONAL DEPENDENCE STRUCTURE ===#

  covp ~ dunif(minp,maxp)

  covn ~ dunif(minn,maxn)

  minp <- (1-Se_lab)*(Se_petri-1)

  minn <- (Sp_lab-1)*(1-Sp_petri)

  maxp <- min(Se_lab,Se_petri) - Se_lab*Se_petri

  maxn <- min(Sp_lab,Sp_petri) - Sp_lab*Sp_petri


#===OTHER EPIDEMIOLOGICAL VALUES===#

Petri_VPP <-(mu*Se_petri)/(mu*Se_petri+(1-mu)*(1-Sp_petri))

Petri_VPN <-((1-mu)*Sp_petri)/((1-mu)*Sp_petri+mu*(1-Se_petri))

Lab_VPP <-(mu*Se_lab)/(mu*Se_lab+(1-mu)*(1-Sp_lab))

Lab_VPN <-((1-mu)*Sp_lab)/((1-mu)*Sp_lab+mu*(1-Se_lab))

scc_z0_VPP <-(mu*Se_scc_z0)/(mu*Se_scc_z0+(1-mu)*(1-Sp_scc_z0))

scc_z0_VPN <-((1-mu)*Sp_scc_z0)/((1-mu)*Sp_scc_z0+mu*(1-Se_scc_z0))

scc_z1_VPP <-(mu*Se_scc_z1)/(mu*Se_scc_z1+(1-mu)*(1-Sp_scc_z1))

scc_z1_VPN <-((1-mu)*Sp_scc_z1)/((1-mu)*Sp_scc_z1+mu*(1-Se_scc_z1))

PetriSCCz0_Se <-(Se_scc_z0*Se_petri)
```

```
PetriSCCz0_Sp <-(Sp_scc_z0+Sp_petri)-(Sp_scc_z0*Sp_petri)

PetriSCCz1_Se <-(Se_scc_z1*Se_petri)

PetriSCCz1_Sp <-(Sp_scc_z1+Sp_petri)-(Sp_scc_z1*Sp_petri)

PetriSCCz0_VPP <-(mu*PetriSCCz0_Se)/(mu*PetriSCCz0_Se+(1-mu)*(1-PetriSCCz0_Sp))

PetriSCCz0_VPN <-((1-mu)*PetriSCCz0_Sp)/((1-mu)*PetriSCCz0_Sp+mu*(1-PetriSCCz0_Se))

PetriSCCz1_VPP <-(mu*PetriSCCz1_Se)/(mu*PetriSCCz1_Se+(1-mu)*(1-PetriSCCz1_Sp))

PetriSCCz1_VPN <-((1-mu)*PetriSCCz1_Sp)/((1-mu)*PetriSCCz1_Sp+mu*(1-PetriSCCz1_Se))

}
```