Title: Evaluating the efficacy of internal teat sealants at dry-off for the prevention of new intra-mammary infections during the dry-period or clinical mastitis during early lactation in dairy cows: A protocol for a systematic review and cumulative meta-analysis.

Authors: Sydney D. Pearce[1], Jan M. Sargeant[1,2], Charlotte B. Winder[1], Jane Parmley[1], Dave F. Kelton[1].

[1]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, N1G 2W1.
[2]Centre for Public Health and Zoonoses, University of Guelph, Guelph, ON, Canada, N1G 2W1.

The current protocol acts to update and expand upon a systematic review conducted by Winder et al. (2019) by increasing the eligible time range and incorporating alternative analysis methods. It adopts various aspects of the methodology outlined in the original study's protocol (Sargeant et al., 2018).

Author contributions:

SDP wrote the protocol and will be responsible for final manuscript writing. Current protocol and future manuscript review and revision is conducted by all authors. Content expertise will be provided by DFK, JMS, and CBW. Researchers from the University of York, UK developed the original search strategy which was modified by SDP for this protocol. JMS and CBW will provide methodological/statistical expertise. JMS and CBW will provide data from the original review for inclusion in the data synthesis phase. SDP will be responsible for project coordination, primary and secondary screening, data extraction and the risk of bias assessment for new records (citations and articles published after the search date of the previous review), as well as data synthesis. Secondary reviews for new records in the primary and secondary screening, data extraction, and risk of bias assessment stages will be conducted by research assistants.

Registration:

This protocol will be archived in the University of Guelph's institutional repository (https://atrium.lib.uoguelph.ca/xmlui/handle/10214/10046) and published online with Systematic Reviews for Animals and Food (SYREAF) available at: http://www.syreaf.org/. The systematic review will use the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2015) for reporting guidelines.

Introduction.

Rationale: With growing scrutiny of antibiotic usage broadly among health and agricultural sectors, the dairy industry experiences increasing pressure to implement novel, evidence-based antibiotic use practices as they develop. Antibiotic administration for the prevention or treatment of intramammary infections (IMI) and mastitis is a large component of antibiotic use in the dairy industry (Lam et al., 2012). Adoption of antibiotic alternatives can contribute to reduced dairy industry antibiotic use and more effective antimicrobial stewardship. Identifying the efficacy of teat sealants as a non-antibiotic

treatment alternative for the prevention of IMI and mastitis was the rationale for the original protocol (Sargeant et al., 2018) for the systematic review and network meta-analysis conducted by Winder et al. (2019).

The current review protocol describes an update to the search strategy to identify relevant articles published after the original search, modified eligibility, and adds a temporal component to the existing knowledge via cumulative meta-analysis. Cumulative meta-analysis, also known as sequential meta-analysis, analyses summary effects of exposures of interest sequentially, starting with the earliest dated publication and re-analysing at the addition of each study in ascending order (Spence et al., 2016). This analysis method will provide additional support for dairy antibiotic stewardship in two ways. First, it will determine the point in time that the available literature provided sufficient evidence of teat sealant use reducing IMI and/or clinical mastitis incidence for recommendation to producers and veterinarians for inclusion in their treatment plans, provided that point has already been reached. Secondly, understanding the changing magnitude of impact that studies can have on this recommendation over time may enable research funding groups focused on antibiotic alternatives to redirect resources into other methods in an informed manner. Should the efficacy literature on teat sealants be sufficient, this allows the continued evaluation of other alternative options that provide the most value to current knowledge for more efficient antimicrobial stewardship research. The second component is key for ensuring research goals are aligned with the current, up-to-date needs of the dairy industry and that research bodies continue to move innovation forward to support it.

The original review used network meta-analysis to evaluate the relative efficacy of teat sealants for the prevention of IMI and clinical mastitis, using individual and combination teat sealant therapies to build the network establishing its efficacy (Winder et al., 2019). The current protocol outlines a cumulative pair-wise meta-analysis evaluating the efficacy of internal teat sealant treatment on its own in comparison to no treatment in preventing two of the same critical outcomes as the original review; incidence of IMI in the pre-calving to at calving period and incidence of clinical mastitis in the first 30 days of subsequent lactation.

The original review showed that internal teat sealants at dry-off were protective against incidence of IMI at calving compared to groups receiving no treatment (RR=0.36, 95% CI 0.25-0.72), (Winder et al., 2019). Teat sealants were numerically protective against incidence of mastitis during the first 30 days of lactation, but the difference was not statistically significant and limited eligible studies were found for analysis (Winder et al., 2019).

The review outlined in this protocol can attain additional value from the body of literature as 2 years will have passed from the time of the literature search in the original study to the current review's search. Internal teat sealant technology has also been off patent since December 2017 which allowed the development of new teat sealant products that may have been targeted for research during the two-year timespan. This new product variability may impact the evidence for cumulative efficacy and changes in recommendations over time should be assessed and documented if they occur. If changes in recommendations correspond with the timing of new products coming to market, whether it contributes to greater or lesser efficacy, additional research may be required to determine factors in why this occurred. Additionally, the added body of literature may provide sufficient data for narrowing the confidence intervals of outcomes previously assessed to establish a more accurate estimate of their efficacy.

Objectives: The current review objective is to sequentially assess literature evaluating the efficacy of internal teat sealant treatment in comparison to no treatment at dry-off in dairy cows, or prepartum in heifers, for their ability to prevent new IMI from intervention to calving and clinical mastitis in early lactation. The temporal feature – if stopping points are reached due to either intervention futility or significant effect on the outcome (Spence et al., 2016) – will be determined to inform future research goals.

Methods

Eligibility criteria:

The PICOS elements defining eligibility for this review are similar to the original protocol (Sargeant et al., 2018), with the modifications highlighted below to limit to a single comparator and study design. One outcome of interest (incidence of IMI in the first 30 days of lactation) was removed due to insufficient eligible studies measuring this outcome in the original review.

i.   Population: Pre-partum heifers and dairy cows after their first (or greater) lactation without existing IMI.
ii.   Intervention: Internal teat sealant with no additional therapies applied to dairy cows at the time of dry off or pre-partum in heifers.
iii.   Comparator: No treatment or placebo control group
iv.   Outcomes: Critical outcomes will include i) incidence of IMI during the pre-calving period immediately following the intervention, ii) incidence of clinical mastitis during the first 30 days of the subsequent lactation.
v.   Study Design: Controlled trials with natural disease exposure

Eligibility criteria and information sources will follow the original protocol (Sargeant et al., 2018) with modifications to limit to a single study design, intervention (teat sealants alone without antibiotics), and no treatment or placebo comparators as outlined by the PICOS elements above. Additionally, database and relevant conference proceeding searches will be limited to 2018-2020 to reduce overlap with the original review's search date on 26-06-2018. The search will not be limited by publication type or language and will be conducted in the multiple databases listed in the original protocol (Sargeant et al., 2018) and shown below.

Table 1: Databases and information sources to be searched (Sargeant et al., 2018)

Database / Information Source	Interface / URL
MEDLINE, MEDLINE In-Process and MEDLINE Daily Epub Ahead of Print	Ovid SP
CAB Abstracts	CAB Interface
Science Citation Index	Web of Science
Conference Proceedings Citation Index – Science	Web of Science
Agricola	ProQuest

A hand-search of the table of contents of the following relevant conferences from 2018 to 2020:

• Proceedings of the American Association of Bovine Practitioners;

- World Association for Buiatrics;
- National Mastitis Council Proceedings

Search strategy:

The search string and structure from the original protocol (Sargeant et al., 2018) was used with minimal additions that are highlighted in Table 1. All additions are relevant only to the current review's search date range of 2018-2020 as they include names of new products not available before the original search in 2018. Terms specifically associated with external teat sealants were removed due to lack of relevant, eligible literature identified in the previous review, as well as the differences in application methods (Huvepharma, n.d.; University of Delaware, 2019) and potential differences in efficacy between the two types. The conceptual structure is as follows:

- **Dairy cattle & IMI/Mastitis;**

AND

- **Internal teat sealants.**

Table 2: Search strategy to identify studies using teat sealants during the dry-off period in dairy cattle between 2018-2020: Tested in Web of Science as of May 2020

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10 #9 AND #4 **63**

9 #8 OR #7 OR #6 OR #5 7,972

8 TS=(bismuth* OR Teatseal* OR "Teat-seal*" OR Orbeseal* OR "Orbe-seal*" OR LockOut* OR "Lock Out*" OR Boviseal* OR "Bovi-seal*" OR Cepralock* OR "Cepra-lock*" OR Noroseal* OR "Noro-seal*" UdderLife* OR "Udder-Life*" OR OptiShield* OR "Opti-Shield*" OR "U-Seal*" OR USeal* OR DrySeal* OR "Dry-Seal*" OR BoviBlock* OR "Bovi-Block*" OR "Masti-Shield*" OR MastiShield* OR Dryzen* OR "Dry-Zen*" OR Vetoseal* OR "Veto-Seal*" OR "Shut-Out*" OR ShutOut*) 7,734

7 TS=(("teat" OR "teats" OR intramammar* OR "intra-mammar*") NEAR/5 barrier*) 2

6 TS=((internal* OR persistent*) NEAR/5 ("seal" OR "seals" OR sealant* OR "sealed" OR "sealing" OR sealer* OR plug*)) 151

5 TS=(("teat" OR "teats" OR intramammar* OR "intra-mammar*" OR "barrier") NEAR/5 ("seal" OR "seals" OR sealant* OR "sealed" OR "sealing" OR sealer* OR plug*)) 115

4 #3 OR #2 OR #1 54,753

3 TS=(mastiti* OR ((intramammar* OR "intra-mammar*") NEAR/3 (infect* OR inflamm*))) 2,352

2 TS=(ayrshire* OR "brown swiss*" OR "busa" OR "busas" OR canadienne* OR dexter* OR "dutch belted*" OR "estonian red*" OR fleckvieh* OR friesian* OR girolando* OR guernsey* OR holstein* OR illawarra* OR "irish moiled*" OR jersey* OR "meuse rhine issel*" OR montbeliarde* OR normande* OR "norwegian red*" OR "red poll" OR "red polls" OR shorthorn* OR "short horn*") 8,446

1 TS=("cow" OR "cows" OR "cattle" OR heifer* OR "dairy" OR "milking" OR "bovine" OR "bovinae" OR buiatric*) 49,809

Study records:

Data management:

Records from the previous review conducted by Winder et al. (2019) involving the relevant pairwise comparison of interest will be identified and combined with the dataset extracted from new reviews for inclusion in the data analysis phase. The screening and collection phases will not be conducted for this group of original records as they were eligible and had their data extracted in the previous review. Removal of irrelevant data items collected due to modified eligibility criteria and de-duplication with new records will be made in the combined dataset.

Records identified from the 2018-2020 search will be captured as follows. All records will be uploaded into bibliographic software (EndNote, Clarivate Analytics, Philadelphia, USA) and de-duplicated. After de-duplication, records will be uploaded into DistillerSR® systematic review software (Evidence Partners Inc., Ottawa, Canada).

Selection process:

The selection process for all new records identified in the 2018-2020 search will follow the original protocol (Sargeant et al., 2018) in DistillerSR® with moderate changes. Changes include question 1, where use of teat sealants alone has been specified, and question 6, where the option of comparators including antimicrobials will be removed. This was done to identify pair-wise analysis as opposed to the broader comparative efficacy network analysis of interventions used in the previous project. Questions 2, 3, and 5 were added to accommodate the modified eligibility criteria and outcomes of the current review.

The following questionnaire will be used for title and abstract screening to select studies for inclusion in full-text screening. This process will be conducted in duplicate by two reviewers from the current research team. Studies will be excluded if they receive a NO from both reviewers on any question, disagreements will be resolved via consensus and a third reviewer will be enlisted if one cannot be reached.

> 1) Does the study evaluate the use of internal teat sealants, <mark>alone and not in combination therapy</mark>, in pre-partum dairy heifers or at dry-off in dairy cows following the first or greater lactation?
>
> > *YES (neutral response), NO (EXCLUDE), UNCLEAR (neutral response)*
>
> 2) Is the study a controlled trial with natural disease exposure?
>
> > *YES (neutral response), NO (EXCLUDE), UNCLEAR (neutral response)*
>
> 3) Was it published or available online in January 2018 or later?
>
> > *YES (neutral response), NO (EXCLUDE), UNCLEAR (neutral response)*
>
> 4) Is the full-text available in English?
>
> > *YES (neutral response), NO (EXCLUDE), UNCLEAR (neutral response)*

Records passing title and abstract screening will be included for full-text screening using the following questionnaire. This process will be conducted in duplicate by two reviewers. Studies will be excluded if they receive a NO from both reviewers on any question, disagreements will be resolved via consensus and a third reviewer will be enlisted if one cannot be reached.

1) Does the study evaluate the use of internal teat sealants, ==alone and not in combination therapy==, in pre-partum dairy heifers or at dry-off in dairy cows following the first or greater lactation?

YES (neutral response), NO (EXCLUDE)

2) Is the study a controlled trial with natural disease exposure?

YES (neutral response), NO (EXCLUDE)

3) Was it published or available online in January 2018 or later?

YES (neutral response), NO (EXCLUDE)

4) Is the full-text available in English?

YES (neutral response), NO (EXCLUDE)

5) Does it examine one of the outcomes of interest (incidence of IMI during the pre-calving period immediately following the intervention, incidence of clinical mastitis during the first 30 days of the subsequent lactation)?

YES (neutral response), NO (EXCLUDE)

6) Is there a ==concurrent no treatment or placebo== comparison group?

YES (neutral response), NO (EXCLUDE)

New records passing both levels of screening will be admitted to the data collection process.

Data collection process:
Data collection will follow the original protocol (Sargeant et al., 2018) with minimal changes that are outlined in this section.

Records identified in the current screening will undergo data extraction conducted in duplicate by two reviewers. DistillerSR® forms will be used for extraction, disagreement between the reviewers will be resolved by consensus or by a third reviewer if one cannot be reached.

Changes to the study level data previously outlined in the original protocol (Sargeant et al., 2018) to account for the modified eligibility criteria are highlighted.

- Country
- Commercial versus research trial
- Number of herds enrolled
- Year the study data was collected
- Months of data collection

- Breed of cattle
- Whether the study population is comprised of pre-partum heifers, first lactation or greater dairy cows, or both
- Description of the <mark>individual teat sealant treatment not included in a combination therapy</mark> (brand used)
  - Additionally, for pre-partum heifers, the time pre-partum when the intervention was applied
- Description of comparison group <mark>(no treatment or placebo control)</mark>

Arm level data to be collected are outlined in the original protocol (Sargeant et al., 2018) with minimal changes. Changes include **the removal of arm level data on additional concurrent treatments given to intervention groups.**

Data to be collected on outcomes relevant to this review and their prioritization are outlined in the original protocol (Sargeant et al., 2018).

Risk of bias in individual studies: Risk of bias (RoB) will follow the same methods as the original protocol (Sargeant et al., 2018). Studies included in the original study will not be reassessed: their original RoB information will be used. The RoB of new studies not included in the original review will be assessed by two reviewers working independently. The Cochrane risk of bias instrument, ROB2.0 for RCTs (Higgins et al., 2016), will be used for this process with modifications to signaling questions to address the topic area as per the original study (Winder et al., 2019).

Data synthesis

Irrelevant records and data items from the original review's dataset will be removed by applying the modified eligibility criteria from the current review. Remaining records will be combined with the dataset obtained from the current data collection process and manually de-duplicated. The combined dataset of new and relevant original records will undergo cumulative meta-analysis.

Cumulative meta-analysis: Cumulative meta-analysis (meta-analysis that builds its estimate by adding one study at a time, sequentially) will be conducted for both of the outcomes of interest. The cumulative meta-analysis will use the approach and R code described by Spence et al. (2016). A Bayesian method was chosen for its ability to use posterior distributions of previous interim analyses as well-informed prior distributions for the following analysis' effect size and heterogeneity parameters. Diffuse priors will be used for the first interim analysis on the earliest-dated study without previous posterior effect size and heterogeneity values to inform its priors. A Bayesian approach to cumulative meta-analysis was also chosen as it is typically subject to fewer type II errors and similar type I errors to other methods (frequentist and semi-Bayesian) in interventions that have non-zero true effects (Spence et al., 2016). This is beneficial for the current review as non-zero effects for at least one outcome are suspected based on the previous network meta-analysis results (Winder et al., 2019). Stopping recommendations, for futility or effect of the exposure, are calculated using posterior effect size and heterogeneity, with threshold values of $\varepsilon_{effect} = 0.002$ and $\varepsilon_{futility} = 0.01$. The conservative thresholds were informed by simulations conducted by Spence et al. (2016) and account for the heterogeneity expected due to substantial heterogeneity seen in the original review (Winder et al., 2019).

A priori sub-group analysis will be conducted for heifers vs. single or multiparous cows and for randomized vs. non-randomized trials.

Conclusion

This review will provide the most current evidence for teat sealant use as a non-antibiotic treatment option for preventing IMI during the dry period and clinical mastitis in early lactation by synthesizing current efficacy literature. If significant evidence for teat sealant efficacy is found, this may help decision-makers (e.g., producers, veterinarians, researchers) employ evidence-based decisions for inclusion of teat sealants in their dry cow treatment plans and reduce the burden of dairy industry antibiotic use. Additionally, changes in teat sealant use recommendations over time will be assessed, which will identify at what point the literature established an effective stopping point in teat sealant research or if more evidence is still needed to ensure cumulative significance of its efficacy to prevent new IMI and/or mastitis. If a stopping point has been reached, this work can help inform future research areas. Funding sources have limited resources to distribute and must make informed decisions regarding where to allocate them. The addition of cumulative meta-analysis can provide these groups, as well as non-antibiotic alternative research labs, with evidence tailored to this decision-making.

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