Title: The efficacy of dry-off antibiotic treatments in dairy cattle to cure existing intra-mammary infections: A protocol for a systematic review and meta-analysis.

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Author contributions:
CM will serve as review leader responsible for coordinating the review and performing primary and secondary screening, data extraction and risk of bias assessment. CM is responsible for preparing all drafts of the protocol and final manuscript, and content expertise will be provided by DFK when needed. Researchers from the University of York, UK developed the search strategy. JMS, CBW, and AMO will be consulted for methodological expertise and will aid in the statistical analyses. Research assistants (further referred to as RAs in this document) will act as secondary reviewers and participate in primary and secondary screening, data extraction and risk of bias assessment. All authors will review and approve the final version of the review report.

Registration: This protocol is archived in the University of Guelph's institutional repository (The Atrium) available at: https://atrium.lib.uoguelph.ca/xmlui/handle/10214/10046, and registered online with Systematic Reviews for Animals and Food (SYREAF) available at: http://www.syreaf.org. This protocol was developed using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses - Protocols (PRISMA-P) guidelines (Moher et al., 2015), and the PRISMA for Network Meta-Analyses (PRISMA-NMA: http://www.prisma-statement.org/Extensions/NetworkMetaAnalysis.aspx) will be used to report the subsequent systematic review and meta-analysis.

Amendments: Any amendments made to this protocol following its registration will be documented in the final systematic review as protocol deviations.

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INTRODUCTION.

Rationale:

Dry-cow therapy is used to both cure existing intra-mammary infections (IMI) at drying off and to prevent new IMI from occurring during the dry period (Halasa et al., 2009). The early and late dry period represents the time at which dairy cows are most at-risk for IMI (Halasa et al., 2009). IMI also increases the risk of clinical mastitis in the subsequent lactation, most commonly during the first 30 to 60 days in milk (Pantoja et al., 2009). The use of antibiotic dry-cow therapy, environmental pathogen load, and a cow's immune system contribute to risk of developing a new IMI during the dry period (Green et al., 2007). Therefore, dry-off antibiotic use is common during this period to decrease the effect of IMI or clinical mastitis on milk quality and quantity. In the Netherlands, approximately 61% of dairy cattle that were dried off received antibiotic dry-cow therapy in 2013 (Santman-Berends et al., 2016), which can be provided as selective dry-cow treatment (SDCT – treatment is based on presence of infection at the quarter- or cow-level) or blanket dry-cow treatment (BDCT – all quarters are treated regardless of IMI status) (Halasa et al., 2009). A survey of dairy farmers from Germany concluded approximately 79% of farms performed blanket dry-cow treatment, and only 31% of farms performed bacteriologic examination of milk at dry-off (Bertulat et al., 2015). The National Dairy Study (2015) in Canada found similar results in which 84% of herds used BDCT and 11% used SDCT (Kelton, 2015 *[unpublished]*). Recently selective dry-cow therapy has gained popularity for its role in proper antimicrobial stewardship and as a means to reduce antibiotic use in the dairy sector (Lam et al., 2017).

Because the prudent use of antibiotics is an imperative in agriculture, it is important to examine the relative efficacies of all antibiotics used to cure existing IMI at dry-off. A systematic review of randomized controlled trials generates the highest level of evidence for the efficacy of a treatment under field conditions (Sargeant and O'Connor, 2014). The addition of a network meta-analysis creates the opportunity to assess antibiotic products used beyond a pair-wise analysis by using a network meta-analysis approach to estimate the relative efficacy of multiple antibiotic options. This review and network meta-analysis will add to existing knowledge by providing up-to-date comparisons of currently licensed antibiotics used for dry-cow therapy. Conducting a systematic review and meta-analysis in this area of research ensures producers and veterinarians are able to make decisions on the basis of efficacy, and if products are equivalent in efficacy, they can use other factors such as level of importance for human health to improve the effective use of antibiotics.

Objectives: The objective of this protocol is to define the methods for a systematic review and network meta-analysis to assess the relative efficacy of antibiotic dry-cow therapy to cure existing intra-mammary infections. The specific review question to be addressed in this protocol, and the following systematic review, is as follows:

At the individual cow level, what is the relative efficacy of dry-cow antibiotic therapy to cure existing intra-mammary infections?

i. Population: Dairy cows after their first or subsequent lactation with an existing IMI at cessation of milking.
ii. Intervention: Antibiotic dry-cow treatment.
iii. Comparator: Different antibiotic treatment, placebo, a non-antibiotic treatment method, or no treatment.

iv. Outcomes:
   a. Cure risk of IMI from dry-off to calving.
   b. Incidence of clinical mastitis (CM) over the first 30 days in milk (DIM).
   c. Antibiotic use over the first 30 DIM.

METHODS.

Eligibility Criteria: In addition to the PICO elements defined above, other eligibility criteria that will be included are as follows:

Report Characteristics: The study must be published in English and the publication can be both published and non-published if the article reports on primary data.

Study Designs: Only controlled trials with a natural disease exposure are eligible for inclusion.

Information Sources: This review will include a range of biologically relevant databases and other information sources able to identify published and unpublished literature. Table 1 represents the databases/information sources to be searched.

Table 1. Databases and information sources to be searched.

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

In addition, one reviewer will hand-search the table of contents of the following relevant conferences from 1997 to 2019, provided the proceedings are ≥500 words:

- Proceedings of the American Association of Bovine Practitioners;
- World Association for Buiatrics;
- National Mastitis Council Proceedings;
- IDF Mastitis Conference Proceedings

The FDA website containing the Freedom of Information New Animal Drug Approvals (NADA) summaries also will be searched.

Search strategy: The search strategy that will be used for this review was previously developed by researchers at the University of York and was used by researchers at the University of Guelph to gather relevant sources pertaining to dry-cow antibiotic treatment and mastitis on June 28, 2018. In addition to the citations gathered from this existing search strategy, researchers at the University of York will perform an updated search, using the same strategy, to also capture all

relevant articles published after June 28, 2018. This search strategy is presented in Table 2. The conceptual structure of the search is as follows:

- Dairy cows AND dry off AND antibiotics;

OR

- Dry cow AND antibiotics;

OR

- ((Dairy cows AND dry off) OR Dry cow) AND treatment AND intra-mammary infections/mastitis

The current search strategy will be used to update a previous search and will be limited by date to any articles published after June 28, 2018. The search strategy will not be limited by language, or publication type.

Table 2. Search strategy to identify studies of antibiotic treatments during the dry-off period in dairy cattle in Science Citation Index (Web of Science).

#14	#13 OR #12	893
#13	TS=(("dry cow" OR "dry cows") NEAR/3 (therap* OR manag* OR intervention* OR treat* OR strateg*))	411
#12	#11 AND #7	712
#11	#10 OR #9 OR #8	593,401
#10	TS=("albamycin" OR "amoxicillin" OR "amoxycillin" OR "ampicillin" OR "benzathine" OR "cathomycin" OR "cefalexin" OR "cefapirin" OR "cefalonium" OR "cefquinome" OR "ceftiofur" OR "cephalexin" OR "cephapirin" OR "cephalonium" OR "cephapirin" OR "chlortetracycline" OR "cloxacillin" OR "CTC" OR "danofloxacin" OR "dicloxacillin" OR "dihydrostreptomycin" OR "enrofloxacin" OR "erythromycin" OR "florfenicol" OR "framycetin" OR "gamithromycin" OR "gentamicin" OR "gentamycin" OR "lincomycin" OR lincosamide* OR "neomycin" OR "novobiocin" OR "oxytetracycline" OR "penethamate" OR "penicillin" OR "pirlimycin" OR "piroline" OR "spectinomycin" OR "sulfadimethoxine" OR "sulfafurazole" OR "sulfamethoxazole" OR "sulfisoxazole" OR "sulphadimethoxine" OR "tetracycline" OR "tildipirosin" OR "tilmicosin" OR "trimethoprim" OR "tulathromycin" OR "tylosin")	147,813
#9	TS=(antimicrobial* OR "anti-microbial*" OR antibiotic* OR "anti-biotic*" OR antibacterial* OR "anti-bacterial*" OR antiinfect* OR anti-infect* OR bacteriocid* OR bactericid* OR microbicid* OR "anti-mycobacteri*" OR antimycobacteri*)	507,630
#8	TS=("SDCT" OR "BDCT")	140
#7	#6 OR #5	9,647
#6	TS=("dry cow" OR "dry cows")	1,186
#5	#4 AND #3	8,965
#4	TS=("drying off" OR "dry off" OR "dried off" OR "dry up" OR "drying up" OR "dried up" OR "drying period*" OR "dry period*" OR "dry udder*" OR "dry teat*" OR "pre-partum" OR "prepartum" OR (("end" OR finish* OR stop* OR ceas*) NEAR/3 lactat*) OR nonlactat* OR "nonlactat*" OR postlactat* OR "post-lactat*" OR postmilk* OR "post-milk*" OR "involution" OR "steady state")	236,415

#3	#2 OR #1	486,431
#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*")	53,889
#1	TS=("cow" OR "cows" OR "cattle" OR heifer* OR "dairy" OR "milking" OR "bovine" OR "bovinae" OR buiatric*)	460,464

STUDY RECORDS.

Data management: The results from the searches will be downloaded into a bibliographic software (EndNote X7, Clarivate Analytics, Philadelphia) and de-duplicated using several algorithms. Any references that are not compatible for downloading into EndNote will be noted and saved in a Word or Excel file as appropriate. The de-duplicated references from the existing search, conducted on June 28, 2018, and the de-duplicated references from the updated search will be loaded into an online reference management software (DistillerSR®, Ottawa, ON, Canada). Each reviewer involved in the screening and data extraction process will be trained on epidemiologic principles and systematic review methods. Prior to both stages of screening, a pilot test will be conducted to ensure reviewer compatibility and consistent data collection using the forms created in DistillerSR®.

Selection process: This review will entail two stages of screening. First, the titles and abstracts of each article identified by the search strategy will be assessed for eligibility. The pilot test for title and abstract screening will involve all reviewers screening 100 articles for eligibility, followed by a consensus meeting. Any disagreements will be thoroughly discussed to ensure all reviewers are consistent for the remaining articles. The remaining titles and abstracts will be independently assessed for relevance using the following primary screening questions by two independent reviewers:

1) “Is the title and/or abstract available in English?”
2) “Does the title and/or abstract describe a primary research study?”
3) “Does the title and/or abstract examine dry-off antibiotic treatments in dairy cattle with an existing IMI?”

All questions will include a response for YES, NO, and UNCLEAR. A reference will only be excluded if both reviewers give a final decision to exclude (i.e. both reviewers agree the response to any of the above questions is NO). Any conflicts will be resolved by consensus, and if agreement cannot be reached, a third member of the review team will be consulted.

The second stage of screening will involve assessing the full-text of each article for eligibility by two independent reviewers. The full-text articles of the citations deemed eligible by the first stage of screening will be retrieved and uploaded into DistillerSR® with the naming convention:

refID_title. If a full-text article cannot be retrieved online using available University of Guelph resources, articles will be requested via a University of Guelph librarian. All reviewers will conduct the pilot test involving 10 full-text articles to ensure reviewer consistency in applying questions. The following secondary screening questions will be used to assess the full-text of each article by two reviewers working independently:

1) "Is the study available in English?"
2) "Is this a primary research study?"
3) "Does this article examine dry-off antibiotic treatments in dairy cattle with an existing IMI?"
4) "Does this article report using an appropriate comparison group?"
5) "Does the article examine one of the following outcomes: cure risk of IMI from dry-off to calving, clinical mastitis risk in the first 30 DIM, or antibiotic use in the first 30 DIM?"
6) "Is the study a controlled trial with natural disease exposure?"

Answers to Questions 1 - 5 will be YES and NO. Answers to Question 6 will be YES and NO, but an answer of NO will generate a list of possible study designs in order to identify all types, but only controlled trials with natural disease exposure will be included for data extraction. A reference will only be excluded if both reviewers answer NO to any screening question. Any conflicts will be resolved by consensus. If consensus cannot be reached, a third person on the review team will be consulted. We will report on reasons for study exclusion using a flow diagram as outlined by PRISMA (http://www.prisma-statement.org/documents/PRISMA%202009%20flow%20diagram.pdf).

Data extraction process: Two reviewers will extract data from eligible studies independently using a form created in DistillerSR®. This form will be pilot tested using four references by all reviewers in order to ensure consistency in data extraction. Authors will not be contacted to request missing data or to clarify published results.

Data items:

The following information will be extracted:

A) Study information: year of publication, year study was conducted, country, month of data collection, number of farms enrolled in the study
B) Population information: breed of cattle, lactation number (mean by treatment group), definition of IMI at dry-off (level of determination (quarter or cow), method used (culture, SCC, other (specify), number of samples, time samples were taken relative to dry-off)
C) Intervention and comparator information: level of the intervention (quarter or by cow), number of study units enrolled, antibiotic(s) used, route of administration, frequency of administration, dose, any concurrent treatments

Data will be extracted for all controlled trials with natural disease exposure that are identified as relevant at full-text screening.

OUTCOMES AND PRIORITIZATION.

Outcomes:

- IMI cure risk from dry-off to calving
- Incidence of clinical mastitis by 30 DIM
- Antibiotic use over the first 30 DIM

Outcome data to be extracted:

1) Cure risk of dry-off antibiotics for existing IMI
    a. Level at which the outcome was assessed (quarter or cow)
    b. Case definition of IMI (no pathogens isolated at follow-up sample, a different pathogen isolated at follow-up not present at dry-off, or other definition)
        i. Number of milk samples taken and timing of sampling for cultures
        ii. Laboratory methods used and procedures followed (e.g. NMC methods)
    c. Grouping of initial pathogens: data will be extracted for all IMIs at dry-off (all-case cure risk), and if available, by gram-positive or gram-negative bacteria separately as well. If the study reports further subdivision of initial infection, this will be recorded but data will not be extracted for these subgroups.
2) Incidence of clinical mastitis
    a. Case definition of mastitis, if standardized scoring was used
    b. Person assessing clinical mastitis (farmer, researcher, etc.)
    c. Frequency of assessment
    d. Level at which outcome data were measured (quarter or cow)
3) Antibiotic use over the first 30 DIM
    a. Definition, including metrics used
    b. Time period of the measurement of antibiotic use

For each of the outcomes, we will extract the possible metrics in the following order:

- 1st priority: Adjusted summary effect size (adjusted risk ratio or adjusted odds ratio, mean differences for continuous outcomes), variables included in adjustment, and corresponding precision estimate.
- 2nd priority: Unadjusted summary effect size, with corresponding precision estimate.
- 3rd priority: Arm level risk of the outcome, or arm level mean of the outcomes with measure of precision (continuous outcomes).
- For studies where there is non-independence between observations, we will report the approach to the analysis of the non-independent observations (i.e. not reported, 'multilevel model', a 'variance components analysis' or may use 'generalized estimating equations (GEEs)', among other techniques).

If variance estimates are not reported, but the authors provide the data necessary to calculate them using standard formulas, we will calculate these data. If results are provided only in graphical form, we will estimate the numerical results using WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/), if the full text is in a suitable format for using this resource.

Risk of bias in individual studies: Risk of bias assessment will be performed at the outcome level for each outcome using the Cochrane risk of bias 2.0 instrument (Higgins et al., 2016), with the signaling questions modified as necessary for the specific review question. The RoB-2.0 for individual RCTs will be used for all study designs. These tools are available at https://sites.google.com/site/riskofbiastool/welcome/rob-2-0-tool.

DATA SYNTHESIS.

Network meta-analysis: Network meta-analysis will be conducted for each outcome. For IMI cure risk, separate analyses will be done for all-cure risk, gram-positive cure, and gram-negative cure. Network meta-analysis will use the approach described by NICE Decision Support Unit technical document (Dias et al., 2014; O'Connor et al., 2013, O'Connor et al., 2016). The approach to reporting will use the PRISMA-NMA document.

Geometry of the network: The approach described by Salanti et al. (2008) will be used to assess the geometry of the network of evidence. The structure of the network will be assessed visually, to examine if there are pair-wise comparisons which dominate the network, and if there are intervention comparisons that are not linked to a larger network.

Assessment of inconsistency: Consistency between direct and indirect evidence for all pair-wise comparisons will be done using the method by Dias et al. (2010). Mean and standard deviation of log-odds ratio of intervention effects will be calculated using direct, indirect, and combined evidence. Estimates from the indirect and direct models will be compared, along with standard deviations, rather than relying on *P*-values.

Confidence in cumulative evidence: The risk of bias in the overall network of evidence will be assessed using an online platform, the Confidence in Network Meta-Analysis (CINeMA) (CINeMA [Software], University of Bern, 2017), which uses a frequentist approach through the 'metafor' package to determine the basis for the contribution matrix (Viechtbauer, 2010). We will use CINeMA to assess the overall network for within-study bias, indirectness, imprecision, and heterogeneity. Within-study bias will be conducted as described above. Indirectness will be considered based on how closely the study populations resemble the target population for the intervention. Imprecision and heterogeneity will be assessed using 0.8 as a clinically important odds ratio for determining a clinically beneficial (<0.80), neutral (0.8-1.25), or clinically harmful (<1.25) effect.

DISCUSSION.

This systematic review and network meta-analysis will provide a synthesis of current evidence regarding the efficacy of antibiotic-containing dry cow treatments at the individual level for curing IMIs present at dry-off, risk of CM during the first 30 DIM, and total antibiotic use in the first 30 DIM. The results of this review and meta-analysis will aid veterinarians and dairy producers to make evidence-informed decisions regarding the use of antibiotic-containing dry cow treatments. Additional research can be targeted at any gaps in knowledge that will be identifiable by conducting this review related to the efficacy of these products.

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