Protocol for a systematic review and network meta-analysis: Relative efficacy of antibiotics for the treatment of mastitis in dairy cattle

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INTRODUCTION

Rationale

Mastitis is one of the most costly diseases of dairy cattle. It is painful, can result in premature culling, reduced milk production, and decreased fecundity at the cow level, and is often accompanied by herd-level consequences such as poor milk quality and increased risk of antibiotic residues in marketed milk. Approximately one in four dairy cows suffer from clinical mastitis each lactation. While the bacterial etiology varies, a significant proportion of these cases benefit from prompt administration of an effective antibiotic, with or without other therapy. Canadian dairy farmers and veterinarians have a considerable number of antibiotic treatments available, including some products of greater importance to human medicine. Veterinarians need information about relative efficacy among products in order to help facilitate their choices and, where possible, select efficacious products with the lowest human medical importance.

For many clinical mastitis treatments, comparative efficacy estimates are available for only one or two antibiotic products. Ideally, producers and veterinarians would have comparative efficacy of all possible treatment options, in order to include relative efficacy with other treatment decision parameters such as cost, convenience, and importance to human medicine. Normally, information about efficacy would be obtained from randomized controlled trials; however, with so many treatment options for clinical mastitis, a trial that concurrently included all possible treatment options is not feasible. A robust alternative is to conduct a network meta-analysis that combines all of the information from multiple trials and enables accurate and valid comparisons to be made for all available treatments. The statistical methods for this approach are well established and have been used extensively in human health (Bero et al., 1998) and bovine respiratory disease (O'Connor et al., 2013).

Objectives

The objective of this review is to evaluate the comparative efficacy of antibiotics or antibiotic treatment regimes for the treatment of clinical mastitis in milking dairy cows, using bacteriological and clinical cures as the primary outcomes. This aim will be met through two stages: 1.) Conduct a systematic review and meta-analyses to identify and summarize the results of all clinical trials conducted to evaluate the efficacy of antibiotics for the treatment of clinical mastitis in dairy cattle, and 2.) Conduct a network meta-analysis to evaluate the comparative efficacy of different antibiotic treatments or regimes for both bacteriologic and clinical cure.

MATERIALS AND METHODS

Study registration

This protocol is archived in the University of Guelph online repository and published online with Systematic Reviews for Animals and Food (**SYREAF**) and is prepared in accordance to Preferred Reporting Items for Systematic review and Meta-Analysis Protocols (**PRISMA-P**) 2015 Checklist (Shamseer et al., 2015).

Eligibility criteria

Studies will be eligible if they are clinical trials of naturally occurring clinical mastitis in lactating dairy-breed cattle that evaluate the efficacy of one or more antibiotic treatments compared to another antibiotic, dose of antibiotic, or to a placebo or non-treated control group. Studies evaluating alternative treatments (e.g., non-antibiotics) as the treatment group or as the comparison group and studies evaluating dry cow antibiotic treatments or treatments intended to prevent the incidence of mastitis will be excluded.

Information sources & search strategy

Search terms are listed in Table 1, with the controlled vocabulary option used where available. Electronic searches will be completed using CAB Direct (via CABI), Medline (via Ovid), and ProQuest (via ProQuest Central) databases. Data from conference proceedings will be located by searching the National Mastitis Council (**NMC**) proceedings library and the American Association of Bovine Practitioners (**AABP**) conference proceedings and Bovine Practitioner journal. The literature search will be conducted in January 2018, and limited to English language publications. No restriction on publication date will be placed aside from that of the database (CAB Direct, 1973; Medline, 1950; ProQuest, 1997; NMC, 1961; AABP, 1997). Ten relevant studies will be pre-selected by DFK to determine if they were located in the initial search. If these are not all included, the search strategy will be modified and reported as a protocol deviation.

Study records

Data management. Studies will be exported from EndNoteX7 (Clarivate Analytics; Philadelphia, PA, United States) into a commercial systematic review management program (DistillerSR, Evidence Partners Inc.; Ottawa, ON, Canada) and de-duplicated.

Selection process. Two reviewers (CBW and one other reviewer) working independently will screen publications for eligibility. Initially, the title and abstract will be screened for relevance using the following questions, with 'yes', 'no', or 'unclear' as all possible answers.

1.) Does the title and/or abstract describe a controlled trial?
2.) Does the title and/or abstract describe a study of naturally occurring clinical mastitis in lactating dairy cows?
3.) Does the title and/or abstract describe one or more intervention groups of an antimicrobial therapy, as compared to either another antimicrobial treatment, a placebo treatment, or no treatment?
4.) Are at least one of the mastitis treatments described above NOT a long-acting, single treatment therapy designed to be given to cows at dry-off?

Studies will be excluded if both reviewers agree the study does not meet one or more of these descriptions by answering 'no' to any of the above questions. Full text screening will then be conducted on all studies passing the primary round, using the initial three questions as well as the following questions:

5.) Is the full text available in English?
6.) Are at least one of the mastitis treatments described above given after the diagnosis of clinical mastitis (i.e. not a preventative product)?
7.) Does the study describe an outcome of either bacteriologic or clinical cure?

At full text screening, answers must be 'yes' or 'no' to all questions. Studies will be excluded if both reviewers agree the study does not meet the inclusion criteria by answering 'no' to any of the above questions. Conflicts on inclusion/exclusion at all stages will be resolved by consensus and mediated by another author if a decision cannot be reached.

Data collection process and data items. Full text copies of eligible publications will be acquired and relevant data will be extracted using a structured form created in DistillerSR. This will include study level data on the population (location, year, animal characteristics), inclusion/exclusion criteria, who determined the diagnosis of clinical mastitis (researcher/owner/veterinarian/laboratory/other), how severity was scored (Wenz et al., 2001), who collected the sample (researcher/owner/veterinarian/other), how the bacterial diagnosis was determined (culture (specify media used (e.g. 5 % sheep blood/1 % esculin/MacConkey/other, NMC guidelines Y/N/not reported), if MALDI-TOF mass spectrometry was used Y/N, if PCR was used Y/N), who determined the bacterial diagnosis (researcher/owner/veterinarian/laboratory/other), what the bacterial diagnosis was (most detailed level available; species > species group > gram stain), who was responsible for treatment (researcher/owner/veterinarian/other), when treatment was

given relative to day of diagnosis, what treatment was given (antibiotic, dose, route, frequency), what outcomes were measured (bacteriologic or clinical cure), level outcomes were measured on (quarter/cow), and when outcomes were measured relative to the day of treatment (specify day after treatment for re-sampling). Details of the intervention and comparator group(s) (antibiotic, route, dose, frequency), and results for each of the outcomes of interest (binary for both potential outcomes; bacteriologic species if diagnosed) will be extracted (sample sizes including losses to follow up, raw data or relative measure (RR, OR), relevant measure of variation for each effect or effect size, other variables controlled in the analysis such as days in milk, lactation number, or other (specify)).

Combining bacteriologic diagnostic information. While examining the effect of treatment on clinical mastitis caused by a unique bacterial species would be ideal, we anticipate grouping similar species that would likely have a similar response to treatment. Misclassification of *Enterococcus* species as *Streptococcus* prior to use of MALDI-TOF mass spectrometry is a concern, and data will not be combined for these species groups between studies incorporating MALDI-TOF and those that do not. *Staphylococcus aureus* will be considered separate from coagulase-negative *Staph* species, the latter of which will be grouped. *Streptococcus aglactiae, uberis,* and *dysgalactiae* will be considered unique diagnoses, with remaining species considered environmental *Strep* species and grouped, while *Enterococcus* species will be considered a unique group. *Escherichia coli* and *Klebsiella* species will be considered as separate diagnoses.

Risk of bias in individual studies

Risk of bias will be performed at the outcome level using the validated Cochrane risk of bias instrument for evaluating randomized controlled trials (Higgins et al., 2011), modified by also including an assessment of reporting of randomization (in addition to random sequence generation), and reported alongside the data synthesis.

Data synthesis

Pairwise meta-analysis. A standard meta-analysis will be conducted for each antibiotic-placebo combination and for each outcome (bacteriological cure for each species group, clinical cure) where multiple pairwise comparisons exist. Random effects meta-analysis with inverse variance weighting will be used to create a forest plot. Heterogeneity will be assessed using the Q-test and I-squared statistic (Higgins et al., 2003). A summary of findings table will be produced to put the results in the context of the strength of evidence available from the synthesized literature

Network meta-analysis. Network meta-analysis (aka mixed treatment comparison meta-analysis) will use the approach described by NICE Decision Support Unit technical document (Dias et al., 2014). The approach uses a generalized linear modeling framework with a logit link for the binary outcomes (clinical cure and bacteriological cure). The outcome is the log of the odds ratio. The model has a mean (u) which is assumed to be normally disturbed, and Î1⁄4i are the trial-specific baseline effects in a trial i (treated as nuisance parameters). The model also has an additive trial-specific treatment

effect of the treatment in arm k relative to the control treatment in arm b (b=1) in that trial. The study effects will be treated as unrelated nuisance parameters with starting priors: Î1⁄4jb~N(0,10000) . The treatment effects of B, C, and D etc. relative to treatment A (placebo) are the basic parameters with starting priors: dAB, dAC, dAD ~ N(0,10000). We will obtain the remaining contrasts of interest for the comparisons (referred to as functional parameters) using the basic parameters such as dBC = dAC-dAB; dBD = dAD-dAB; dCD = dAD-dAC. The assumption is that the variance of the treatment effects is consistent across treatments. To estimate the posterior distribution of the treatment effects, we will use a Bayesian approach to estimation in JAGS for R using Markov chain Monte Carlo (MCMC) simulation. The GLM framework enables us to assess model fit using Deviance Information Criterion (DIC) and goodness of fit tests based on residual deviance. The geometry of the network of evidence will be assessed using the approach described by Salanti et al. (2008). The consistent assumption will be tested using the back calculation method (Dias et al., 2010). The results will be reported in a manner consistent with current guidelines for reporting network meta-analysis. Outputs of these analyses will include a figure showing the number of network treatment arms and comparisons, a table showing the relative efficacy of all possible comparisons, and a ranked list of antibiotics by efficacy for each critical outcome showing mean rank and 95 % credibility interval.

DISCUSSION

We anticipate the results from this network meta- analysis approach to evaluating the comparative efficacy of antibiotic treatments for clinical mastitis to be beneficial to the dairy industry. This work will provide science-based input on the relative efficacy of different antibiotic treatment options to aid producers in making prudent antibiotic use decisions, which is based on both efficacy and importance to human medicine, an increasingly important societal demand, while supporting continued prompt treatment of clinical mastitis, which is known to be a painful condition.

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244 Table 1. Initial search strategy conducted in CABI (via CAB Direct) on 12/09/17.

#	**Search terms**	**Results**
1	dairy OR cow OR cattle OR bovine OR holstein OR friesian OR jersey OR "brown swiss" OR ayrshire OR guernsey OR "milking shorthorn" OR "norwegian red"	920,047
2	mastitis OR mastitic	38,812
3	**1** AND **2**	34,340
4	antibiotic OR antimicrobial OR "lactating therapy" OR treatment OR intramammary OR dicloxacillin OR cloxacillin OR cephalexin OR ceftiofur OR cephapirin OR pirlimycin OR penicillin OR novobiocin OR amoxicillin OR ampicillin OR penethamate OR sulfisoxazole OR trimethoprim OR sulfa* OR dihydrostreptomycin OR erythromycin OR florfenicol OR oxytetracycline OR tetracycline OR tylosin OR lincosamide OR linco*	2,035,401
5	bacter* OR culture OR cure OR resolution OR resolve	2,064,990
6	**4** AND **5**	572,304
7	**3** AND **6**	**10,178**

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