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Selective treatment of nonsevere clinical mastitis does not adversely affect cure, somatic cell count, milk yield, recurrence, or culling: A systematic review and meta-analysis

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ABSTRACT

Treatment of clinical mastitis (CM) contributes to antimicrobial use on dairy farms. Selective treatment of CM based on bacterial diagnosis can reduce antimicrobial use, as not all cases of CM will benefit from antimicrobial treatment, e.g., mild and moderate gram-negative infections. However, impacts of selective CM treatment on udder health and culling are not fully understood. A systematic search identified 13 studies that compared selective versus blanket CM treatment protocols. Reported outcomes were synthesized with random-effects models and presented as risk ratios or mean differences. Selective CM treatment protocol was not inferior to blanket CM treatment protocol for the outcome bacteriological cure. Noninferiority margins could not be established for the outcomes clinical cure, new intramammary infection, somatic cell count, milk yield, recurrence, or culling. However, no differences were detected between selective and blanket CM treatment protocols using traditional analyses, apart from a not clinically relevant increase in interval from treatment to clinical cure (0.4 d) in the selective group and higher proportion of clinical cure at 14 d in the selective group. The latter occurred in studies co-administering nonsteroidal anti-inflammatories only in the selective group. Bias could not be ruled out in most studies due to suboptimal randomization, although this would likely only affect subjective outcomes such as clinical cure. Hence, findings were supported by a high or moderate certainty of evidence for all outcome measures except clinical cure. In conclusion, this review supported the assertion that a selective CM treatment protocol can be adopted without adversely influencing bacteriological and clinical cure, somatic cell count, milk yield, and incidence of recurrence or culling.

Key words: antimicrobial stewardship, clinical mastitis, rapid diagnostic test, bacteriological cure, noninferiority

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Table 1. Overview of selected on-farm rapid diagnostic tests

Item	System	Detection of
CHROMagar (CHROMagar)	Bi culture plate	*Streptococcus agalactiae*, *Streptococcus uberis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Proteus*, *Pseudomonas*, *Candida albicans*
mastDecide (Quidee GmbH)	Tubes with reactants	Gram-positives, gram-negatives
Accumast (FERA Animal Health LCC)	Tri culture plate	*Staph. aureus*, staphylococci, streptococci, enterococci, *E. coli*, *Pseudomonas* spp., other gram-negatives
Minnesota Easy Culture System (University of Minnesota Udder Health Laboratory)	Bi culture plate	*Staph. aureus*, gram-positives, gram-negatives
	Tri culture plate	*Staph. aureus*, streptococci or *Streptococcus*-like species, *Staphylococcus* spp., or other non-*Streptococcus* gram-positives, gram-negatives
Hardy Diagnostics Mastitis plates (Hardy Diagnostics)	Bi culture plate	Gram-positives, gram-negatives
	Tri culture plate	Gram-positives, streptococci, gram-negatives
VétoRapid (Vetoquinol)	Tri culture plate	Staphylococci, streptococci, gram-negatives
BACT Gram +/− test (FluimediX ApS)	Paper with reactants	Gram-positives, gram-negatives
Mastatest (Mastaplex Ltd.)	Wells with reactants	*Strep. uberis*, *Staph. aureus*, CNS, gram-negatives
Petrifilm Coliform and Aerobic Count Plates (3M)	Count plates	Gram-positives, gram-negatives
Check-Up (Farm Medix)	Quad plate	*Staph. aureus*, NAS, staphylococci/streptococci mix, *Strep. uberis*, *Streptococcus dysgalactiae*, *Strep. agalactiae*, *Trueperella* spp., enterococci, streptococci-like organisms, *Bacillus*, *Corynebacterium* spp., micrococci, *E. coli*, *Klebsiella* spp., *Enterobacteriaceae*, *Serratia* spp., *Proteus* spp., *Pasteurella* spp., *Pseudomonas* spp.

INTRODUCTION

Clinical mastitis (**CM**) is a major contributor to overall farm antimicrobial use (**AMU**) (Kuipers et al., 2016; Firth et al., 2017; Lardé et al., 2021). Many available CM treatment products contain antimicrobial agents listed by the World Health Organization as highly important (e.g., cloxacillin, cephapirin, pirlimycin, and procaine benzylpenicillin) or critically important (e.g., ceftiofur) for human health (WHO, 2018). Although critically important antimicrobials are often not superior to other antimicrobial treatment of nonsevere CM (Nobrega et al., 2020), their continued use along with highly important antimicrobials contributes to social pressure for judicious AMU in livestock (WHO, 2015; OIE, 2016; FAO, 2021).

Decreasing mastitis-related AMU is possible because antimicrobial treatment is not required for all mild or moderate CM cases. High rates of spontaneous clinical cure occur with gram-negative CM (Wilson et al., 1999; Leininger et al., 2003; Schmenger and Krömker, 2020), culture-negative CM, as well as CM cases arising from nonbacterial causes (Ruegg, 2018). Accordingly, treatment of CM is ideally guided by the pathogen identified. Although such an approach has long been implemented in certain regions such as the Nordic countries (Rajala-Schultz et al., 2021), blanket treatment of CM still remains the norm in many other countries with large dairy industries (e.g., United States and Brazil; USDA, 2016; Tomazi and dos Santos, 2020). In the past decade, many rapid diagnostic tests (Table 1) have become available that can be used by veterinarians and producers to identify bacteria in CM milk samples and provide information regarding pathogen groupings (i.e., gram-positive vs. gram-negative). Therefore, selective antimicrobial treatment protocols for nonsevere cases of CM can be effectively guided by rapid diagnostic testing of milk samples from all CM cases. Additional information such as CM history and SCC records can also be included, although clear thresholds are lacking. Regardless, selective CM treatment protocols need to minimize risks of withholding antimicrobial therapy from cases that may benefit and demonstrate a similar performance in terms of udder health compared with blanket CM treatments.

Among commonly used study designs to measure associations, randomized clinical trials (**RCT**) provide the highest grade of evidence if done well. Since 2011 (Lago et al., 2011a,b), several positive-controlled RCT have compared selective versus blanket treatment of CM, comparing udder health outcomes such as bacteriological cure, clinical cure, SCC, and recurrence. However, all of these studies used a superiority approach, i.e., they were intended to demonstrate that 1 treatment was better than the other. Failing to demonstrate superiority does not mean that 1 treatment is no worse in terms of udder health outcomes. Accordingly, a noninferiority approach is more appropriate (Schumi and Wittes, 2011). Consequently, we conducted a series of meta-analyses using a noninferiority approach to

examine if selective treatment of CM on dairy farms resulted in similar health outcomes (i.e., that it would be no worse than blanket CM treatment). Formulated using the PICO statement, our focused research question was: For dairy cows with nonsevere CM (Population), do selective CM treatment protocols (Intervention) perform worse than blanket CM treatment protocols (Comparator) in terms of udder health parameters: clinical cure, bacteriological cure, SCC, milk yield, recurrence, and culling (Outcomes)?

MATERIALS AND METHODS

Systematic Search and Data Extraction

We searched CAB Abstracts (EBSCOhost; https://search.ebscohost.com/), MEDLINE (Ovid; https://ovidsp.ovid.com/ovidweb.cgi), Web of Science (https://webofknowledge.com/WOS), and Scopus (Elsevier; https://www.scopus.com/) from January 1, 2010, to December 1, 2021, with the following strategy: Mastitis AND (treatment* OR therap*) AND (culture* OR rapid test* OR targeted OR selective) AND (cattle OR cow*) AND NOT (dry cow* or dry period). A full overview of search terms is presented in Supplemental Tables S1 to S4 (https://data.mendeley.com/datasets/hd7gh8nc46; de Jong, 2022). Nine key articles were used to validate the search terms used. No language restrictions were used. Studies explicitly addressing dry cow therapy were excluded. Conference proceedings from the National Mastitis Council and American Dairy Science Association were hand searched for relevant studies from 2010 through January 19, 2022. An additional citation check was performed on July 7, 2022, to capture any additional articles. This review was not registered beforehand. As a literature review, this research did not require approval of the Research Ethics Board or Animal Care Committee.

Two reviewers (MB and EdJ) screened each record and resolved any disagreements. Articles were included if they described original research, used dairy cow or udder as the unit of interest, mentioned CM, described a field-based study, and did a comparison with 1 or more groups predetermined as a control or baseline. Full-text review was done by EdJ, during which articles were retained only if they compared a selective to a blanket CM treatment protocol (regardless of administration route) and reported effects for at least 1 of the following udder health outcomes: bacteriological cure, clinical cure, new IMI, SCC, milk yield, recurrence, or culling. If a title, abstract, or full text was non-English, Google Translate (Google) was used to determine its relevance. Relevant data were extracted from each article by EdJ, including location, study design, number of farms, treatment protocols, and pathogen distribution. Data for all reported outcome measures were extracted, including short-term outcomes such as time to bacteriological cure, clinical cure, and SCC, and long-term outcomes such as milk yield, recurrence risk, and culling rate. When necessary information was not reported, authors were contacted via email.

Risk of bias was assessed by EdJ and LC using the Cochrane Collaboration's Risk-of-Bias tool for randomized trials (RoB2; Higgins et al., 2021a). Each manuscript was scored on the following 5 domains: (1) bias arising from the randomization process, (2) bias due to deviations from intended interventions, (3) bias due to missing outcome data, (4) bias in outcome measurement, and (5) bias in selection of reported results. Changes were made in the judgment of domains 1 and 5. When the sequence allocation was not concealed but baseline characteristics suggested no imbalances (Questions 1.2 and 1.3), studies were categorized as "some concerns" instead of "high risk." Similarly, when no information was available regarding existence of a prespecified analysis plan that was finalized before unblinding of participants (Question 5.1), studies were categorized as "low risk" instead of "some concerns." Total bias risk was determined using the algorithm specified by Higgins et al. (2021a), where the total bias risk reflected the most severe level of bias indicated for any of the 5 domains.

Data Synthesis and Meta-Analyses

Noninferiority Margins. Instead of demonstrating a difference (i.e., superiority), the aim of this study was to demonstrate noninferiority: selective CM treatment protocols did not perform worse than the positive control (blanket CM treatment protocols) in terms of clinical cure, bacteriological cure, SCC, milk yield, recurrence, or culling. Therefore, the null and alternative hypotheses were as follows (FDA, 2016):

- Ho: $B - S \geq M$ [selective (S) is inferior to blanket (B) by M or more],
- Ha: $B - S < M$ [selective (S) is inferior to blanket (B) by less than M].

M was the noninferiority margin, which was specified for each reported outcome measure in advance of the analysis and was calculated as follows (Tsui et al., 2019; Trone et al., 2020):

$$M = \left(\frac{1}{RR_{BvsNA}}\right)^{1-\alpha}.$$

M is based on the estimated risk ratio (**RR**) of the positive control (blanket treatment protocol) with the negative control (i.e., no antimicrobial treatment for any CM cases, NA) (RR_{BvsNA}). Where possible, the RR_{BvsNA} was determined for each outcome variable by evaluating historical accounts. Authors relied heavily on articles identified in the review by Francoz et al. (2017). Relevant studies after 2015 were identified using Google Scholar (Google). Results were pooled with random-effects models to estimate RR_{BvsNA} as suggested by Kaul and Diamond (2006). For outcome measures where RR_{BvsNA} was similar for blanket and no antimicrobial treatment groups, or RR_{BvsNA} was worse for the blanket treatment group, noninferiority margins could not be calculated because it was no longer important to maintain the treatment effects of the blanket protocol.

In addition, M also included a prespecified percentage of effect retention (α) based on clinical significance. Alpha was set at 50%, representing the percentage of the blanket treatment protocol's effect that needed to be retained when applying a selective treatment protocol. This is common in studies evaluating noninferiority of CM treatments (Schukken et al., 2013; Vasquez et al., 2016; Tomazi et al., 2018).

Pooled Effect Estimates. Risk ratios or mean differences (**MD**) were obtained for each outcome variable via a series of meta-analyses using the software package Meta (Schwarzer, 2007; Balduzzi et al., 2019) in RStudio version 1.2.5033 (R Core Team 2019, R Foundation for Statistical Computing). Available data were pooled using a random-effects model (DerSimonian and Laird, 1986) and synthesized as RR (events) or MD (continuous measures).

The I^2 statistic was used to quantify total variability due to between-study differences (Higgins et al., 2021b). Tau^2 was also used to assess heterogeneity, as a measure of between-study variances (Higgins et al., 2021b). Results were displayed in forest plots and sorted according to their observation interval, or year published. Where possible, stratified analyses were performed according to differences in follow-up duration as a potential source of heterogeneity.

For each study, it was recognized that baseline characteristics in selective and blanket groups needed to be similar for valid comparisons, especially with respect to factors that could influence treatment outcomes. Therefore, sensitivity analyses were performed, when possible, to assess effects of an uneven pathogen distribution and use of nonsteroidal antiinflammatory drugs (**NSAID**) on the pooled effect measure by removing those studies and comparing the pooled effect measure. Similarly, variations between studies in selective treatment protocols could also influence effects reported. Therefore, sensitivity analyses were also used to investigate the influence of varying protocols on the pooled effect measure. The influence of rapid on-farm testing system used was not assessed, as not enough studies used the same testing approach to properly assess its effect on the pooled effect measures.

Certainty of Evidence. The GRADE tool (Grading of Recommendations, Assessment, Development and Evaluations; Schünemann and Santesso, 2010) was used by EdJ and LC to evaluate certainty in the body of evidence for each outcome measure in the meta-analysis. Confidence in the reported results were judged using the following 5 domains: (1) risk of bias, (2) inconsistencies across studies, (3) use of indirect evidence, (4) possible imprecisions, and (5) presence of publication bias. More specifically, publication bias was assessed by producing 3 types of funnel plots: standard funnel plots to assess symmetry in the reported results, contour plots to identify if asymmetry was due to publication bias (Peters et al., 2008), and trimmed and filled plots for sensitivity analyses (Duval and Tweedie, 2000).

RESULTS

The systematic search yielded 1,444 citations with 679 duplicates (Figure 1). Titles and abstracts of 765 references were screened for eligibility, of which 132 were retrieved for full-text review. One additional study was identified via reference screening. Thirteen studies, of which 1 was published in 2 papers and 2 were described in an additional third paper (total n = 15), encompassing 97 dairy herds, met all eligibility criteria for evaluating associations between a selective versus blanket CM treatment protocol and udder health (Table 2).

Description of the Included Studies

All studies included used a split-herd design, where CM cases were allocated to either a blanket or a selective treatment group. An on-farm rapid diagnostic test was used on all cases allocated to the selective treatment group to identify the causal pathogen, with level of detail varying across studies [ranging from cell wall level (i.e., gram-positive or negative) to speciation]. Seven of the 13 studies applied a selective CM treatment protocol where antimicrobial treatment was withheld for all gram-negative or culture-negative cases. Modified protocols were used by the remaining studies (Table 3): either moderate mastitis cases caused by gram-negative cases (Bazzanella et al., 2020) or all gram-negative CM cases also received antimicrobial treatment (Griffioen

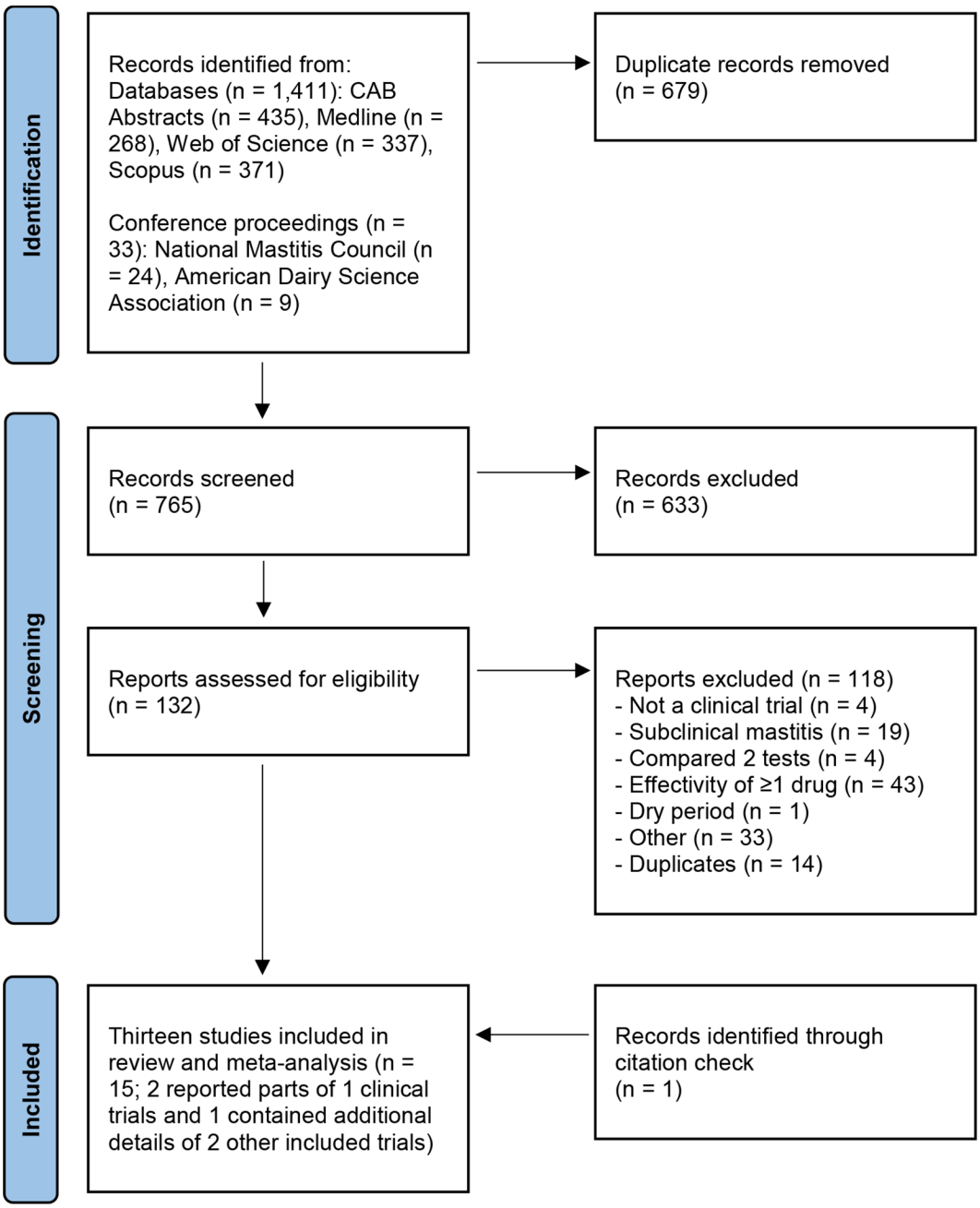

Figure 1. Flow diagram of the systematic search and selection of abstracts and manuscripts.

et al., 2021), and those with CM caused by anything other than streptococci were excluded from antimicrobial treatment (Lago et al., 2016b). Three studies excluded cows deemed to be unworthy of treatment with gram-positive rapid test results from antimicrobials (Mansion-de Vries et al., 2016; Kock et al., 2018; Schmenger et al., 2020), where unworthy of treatment was defined as those cows with >2 CM cases in quarter in current lactation or >700,000 cells/mL milk at all 3 previous DHIs recordings.

Severe CM cases were included in 3 studies, where immediate systemic antimicrobial treatment was provided irrespective of pathogen and culture results were awaited before CM cases were allocated to the selective treatment group (Mansion-de Vries et al., 2016; Kock et al., 2018; Schmenger et al., 2020). One study allowed severe cases to enroll (Bazzanella et al., 2020), but those cases were evaluated separately and thus not included in this review. Five studies provided an NSAID to mild and moderate cases in the selective treatment

Table 2. Study characteristics of studies evaluating the impacts of selective clinical mastitis (CM) treatment protocols

Study	Demographics	Diagnostic procedure in selective treatment group	Exclusion
Lago et al. (2011a,b)	8 herds (2 in Minnesota, 5 in Wisconsin, 1 in Ontario, Canada)	On-farm culture with Minnesota Easy Culture System Bi-plate (University of Minnesota Udder Health Laboratory). Results in 18 to 24 h; if culture-negative, re-read after an additional 18 to 24 h	Severe cases and cows with <3 functional quarters
MacDonald (2011)	48 herds in Canada	On-farm culture with Petrifilm AerobicCount and ColiformCount (3M). Results in 22 to 24 h	Severe cases
Mansion-de Vries et al. (2016)	1 herd in Germany	On-farm culture with Petrifilm AerobicCount and ColiformCount	Cows suffering from another disease, or with teat injury
Lago et al. (2016a, 2019)	1 herd in California	Culture in laboratory, 24 h	Severe cases
Lago et al. (2016b, 2019)	1 herd in California	Culture in laboratory, 24 h	Severe cases
Vasquez et al. (2017)	1 herd in New York State	Culture in laboratory, 18 to 24 h	Severe cases and those caused by *Prototheca* spp., *Mycoplasma* spp., *Staphylococcus aureus*, or *Streptococcus agalactiae*
Kock et al. (2018)	1 herd in Germany	On-farm culture with Petrifilm and MastDecide (Quidee GmbH), 12 h	
McDougall et al. (2018)	7 herds in New Zealand	On-farm culture with Check-up (Farm Medix), 24 to 48 h	Severe cases and cases diagnosed solely via SCC reports or via elevated rapid mastitis test scores (subclinical cases)
Bates et al. (2020)	7 herds in New Zealand	On-farm culture with Mastatest (Mastaplex Ltd.), 24 h	Severe cases and cases diagnosed solely via SCC reports or via elevated rapid mastitis test scores (subclinical cases)
Schmenger et al. (2020)	5 herds in Germany	On-farm culture with MastDecide, 12 h	
Bazzanella et al. (2020)	1 herd in Slovakia	On-farm culture with VetoRapid (Vetoquinol). Results in 8 to 24 h; reconfirmation after 48 h	Severe cases and those caused by *Staph. aureus*
Griffioen et al. (2021)	15 herds in the Netherlands	On-farm culture in 2 groups: • CHROMagar (CHROMagar), 18 to 24 h • Minnesota Easy Culture System II Tri-plate (University of Minnesota Udder Health Laboratory), 18 to 24 h	Severe cases, CM in >1 quarters, cows with previous antimicrobial treatment during the last 30 d, or cows close to dry off (21 d)
Borchardt and Heuwieser (2022)	1 herd in Germany	On-farm culture with Accumast (FERA Animal Health LCC), 24 h	Severe cases, cows with previous antimicrobial or anti-inflammatory treatment during the last 15 d, cows on cull list

group (either to a large portion or all cases) and to all severe cases included (Mansion-de Vries et al., 2016; Kock et al., 2018; Bazzanella et al., 2020; Schmenger et al., 2020; Borchardt and Heuwieser, 2022).

Various antimicrobial treatments were used across studies, which is expected considering the availability of products in countries where the studies were conducted. Almost all studies used antimicrobials that included activity against gram-negative bacteria (Table 3) except for McDougall et al. (2018), who used penicillin for blanket treated cases and penicillin or cloxacillin for selectively treated cases, and Bates et al. (2020), where penicillin was given to blanket treated cases. Four studies used the same antimicrobial treatment for their selective and blanket treatment groups: cephapirin (Lago et al., 2011a; MacDonald, 2011), cephalexin and kanamycin combination (Borchardt and Heuwieser, 2022) or ceftiofur (Lago et al., 2016a,b).

There was a large variety in dosage and treatment duration, although most treatments provided were according to label instructions. Mansion-de Vries et al. (2016) described a longer treatment duration (5 vs. 1.5 d) for cows in their first or second lactation with mild or moderate CM that had tested gram-positive and were deemed worthy of therapy. Vasquez et al. (2017) provided a longer treatment duration for cases in the blanket group (5 d of intramammary ceftiofur vs. 1 d of intramammary cephapirin). Bates et al. (2020) provided longer treatments for cows with >1 quarter affected. Two studies used existing on-farm protocols and only provided information regarding administration route and general class of compounds used (Schmenger et al., 2020; Griffioen et al., 2021).

Three studies reported significant differences in pathogen distributions between selective and blanket treatment groups (Table 4). When comparing both

Table 3. Protocol details and type of antimicrobials used in studies evaluating impacts of selective clinical mastitis (CM) treatment protocols[1]

	Selective group			
Study	No antimicrobial treatment	Antimicrobial treatment	Blanket group	NSAID used
Lago et al. (2011a,b)[2]	Gram-negative and culture-negative	Gram-positive and mixed results: IMM cephapirin 200 mg (1 d, 2×)	IMM cephapirin 200 mg (1 d, 2×)	
MacDonald (2011)[2]	Gram-negative and culture-negative	Gram-positive: IMM cephapirin 200 mg (1 d, 2×)	IMM cephapirin 200 mg (1 d, 2×)	
Mansion-de Vries et al. (2016)[2]	Mild/moderate gram-negative, culture-negative, and therapy unworthy gram-positive	- Gram-positive, mild/moderate, therapy worthy and <3 lactations: IMM cefquinome 75 mg (5 d, 10×) - Gram-positive, mild/moderate, therapy worthy and ≥3 lactations: IMM cefquinome 75 mg (1.5 d, 3×) - Severe: immediate SYS cefquinome 1 mg/kg BW (3 d, 3×) while awaiting culture results	- Mild: IMM ampicillin 75 mg + cloxacillin 200 mg (max 5 d, 10×) - Moderate: [IMM cefquinome 75 mg (1.5 d, 3×)] or [IMM ampicillin 75 mg + cloxacillin 200 mg (max 5 d, 10×)] - Severe: IMM cefquinome 75 mg (1.5 d, 3×) + SYS cefquinome 1 mg/kg BW (3 d, 3×)	- Selective: ketoprofen 3 mg/kg BW (max 3 d, 3×) - Blanket, severe cases only: [meloxicam 0.5 mg/kg BW (1 d, 2×)] OR [ketoprofen 3 mg/kg BW (max 3 d, 3×)]
Lago et al. (2016a, 2019)	Gram-negative and culture-negative	Gram-positive: IMM ceftiofur 125 mg (3 d, 3×)	IMM ceftiofur 125 mg (3 d, 3×)	
Lago et al. (2016b, 2019)	Gram-negative and culture-negative	Gram-positive: IMM ceftiofur 125 mg (3 d, 3×)	IMM ceftiofur 125 mg (3 d, 3×)	
Vasquez et al. (2017)	Culture-negative and bacteria other than CNS, *Streptococcus* group G, *Streptococcus* group C, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Enterococcus* spp.	CNS, *Streptococcus* group G, *Streptococcus* group C, *Strep. dysgalactiae*, *Strep. uberis*, *Enterococcus* spp.: IMM cephapirin 200 mg (1 d, 2×)	IMM ceftiofur 125 mg (5 d, 5×)	
Kock et al. (2018)[3]	Mild and moderate gram-negative, culture-negative, and therapy unworthy gram-positive	- Gram-positive, mild/moderate, therapy worthy: IMM cephapirin 300 mg (2 d, 4×) - Severe cases: immediate SYS cefquinome 29 mg (2 d, 2×) while awaiting culture results	- Mild/moderate: [IMM amoxicillin 200 mg + clavulanic acid 50 mg (2.5 d, 5×)] or [SYS cefquinome 29 mg (2 d, 2×)] - Severe: [SYS marbofloxacin 2 mg/kg of BW (3 d, 3×)] or [SYS marbofloxacin 10 mg/kg BW (1 d, 1×)]	- Selective, all cases: flunixin 33 mg/kg BW (1 d, 1×) - Selective, gram-positive: IMM prednisolone 20 mg (2 d, 4×) - Selective, severe: IMM prednisolone 20 mg (2.5 d, 5×) - Blanket, mild/moderate: IMM prednisolone 10 mg (2.5 d, 5×) + NSAID (not specified)
McDougall et al. (2018)	Gram-negative and culture-negative (re-assess culture at 48 h)	- *Staphylococcus aureus* in 1 quarter: IMM cloxacillin 200 mg (max 2 d, 6×) - *Staph. aureus* in >1 quarter: [IMM cloxacillin 200 mg (max 2 d, 6×)] or [SYS tylosin 10 mg/kg BW (3 d, 3×)] - Other gram-positive in 1 quarter: IMM penicillin 1,000 mg (1.5 d, 3×) - Other gram-positive in >1 quarter: SYS penethamate hydrioxide 5 g (3 d, 3×)	- 1 quarter: IMM penicillin 1,000 mg (1.5 d, 3×) - >1 quarters: SYS penethamate hydrioxide 5 g (3 d, 3×)	

Continued

groups, Kock et al. (2018) and Bazzanella et al. (2020) reported more staphylococci (mainly *Staphylococcus aureus*) in the selective treatment group and more streptococci (mainly *Streptococcus uberis*) in the blanket treatment group. Griffioen et al. (2021) reported more streptococci (mainly *Strep. uberis* and *Streptococ-*

Table 3 (Continued). Protocol details and type of antimicrobials used in studies evaluating impacts of selective clinical mastitis (CM) treatment protocols[1]

	Selective group			
Study	No antimicrobial treatment	Antimicrobial treatment	Blanket group	NSAID used
Bates et al. (2020)[3]	Gram-negative and culture-negative	- Gram-positive, based on antimicrobial sensitivity: [IMM penicillin 1,000 mg (1.5 d, 3×)] or [IMM cloxacillin 200 mg (3 d, 3×)] or [IMM lincomycin 330 mg + neomycin 100 mg (1.5 d, 3×)] - >1 quarter and IMM penicillin indicated, also provide SYS penethamate hydrioxide 5 g (3 d, 3×)	- 1 quarter: IMM penicillin 1,000 mg (1.5 d, 3×) - >1 quarters: SYS penethamate hydrioxide 5 g (3 d, 3×)	
Schmenger et al. (2020)	Gram-negative, culture-negative, and treatment unworthy gram-positive	- Gram-positive, therapy worthy: IMM antimicrobial (not specified) - Severe cases: immediate SYS antimicrobials (not specified) while awaiting culture results	- Mild/moderate: immediate IMM antimicrobials (not specified) - Severe: SYS antimicrobials (not specified)	- Selective, all cases: NSAID (not specified) - Selective, severe: SYS NSAID (not specified) - Blanket, severe cases: SYS NSAID (not specified)
Bazzanella et al. (2020)[3]	Mild gram-negative and culture-negative	- Mild (gram-positive) and moderate (gram-negative and gram-positive): [IMM penicillin procaine 3 g (3 d, 3×)] or [IMM lincomycin 330 mg + neomycin 100 mg (5 d, 10×)[4]] or [IMM kanamycin 100,000 IU + cephalexin 200 mg (2 d, 2×)[4]] - Severe: immediate SYS enrofloxacin 5 mg/kg BW (max 5 d, 5×)[4]	- Mild and moderate: [IMM kanamycin 100,000 IU + cephalexin 200 mg (2 d, 2×)[3]] or [IMM amoxicillin 200 mg + clavulanic acid 50 mg (1.5 d, 3×)[3]] or [IMM cefquinome 75 mg (1.5 d, 3×)[3]] - Severe and repeated CM: SYS enrofloxacin 5 mg/kg BW (max 5 d, 5×)[3]	- Selective, moderate and severe cases: meloxicam 0.5 mg/kg BW (1 d, 1×)[4] - Blanket, severe case and repeated CM: meloxicam 0.5 mg/kg BW (1 d, 1×)[4]
Griffioen et al. (2021)	Culture-negative	- Gram-positive: narrow spectrum IMM antimicrobials (not specified) - Gram-negative: broad spectrum IMM antimicrobials (not specified)	Antimicrobial treatment (not specified)	
Borchardt and Heuwieser (2022)	Culture-negative, gram-negative	- *Strep. uberis* or Streptococci spp., first CM: IMM cephalexin 200 mg + kanamycin 100,000 IU (5 d, 5×) - *Strep. uberis* or *Streptococcus* spp., repeated CM: IMM cefoperazone 100 mg (5 d, 5×) - Other gram-positive, first CM: IMM cephalexin 200 mg + kanamycin 100,000 IU (3 d, 3×) - Other gram-positive, repeated CM: IMM cefoperazone 100 mg (3 d, 3×)	- First CM: IMM cephalexin 200 mg + kanamycin 100,000 IU (5 d, 5×) - Repeated CM: IMM cefoperazone 100 mg (3 d, 3×)	- All cases: meloxicam 0.5 mg/kg BW 20 mg (1 d, 1×) - Selective, gram-negative: additional meloxicam 0.5 mg/kg BW 20 mg (2 d, 2×)

[1]IMM = intramammary administration; SYS = systemic administration; max = maximum; NSAID = nonsteroidal antiinflammatory drug.
[2]Extended treatment allowed.
[3]Extended treatment only allowed for cases in blanket group.
[4]According to label instructions, specific duration was not specified.

cus dysgalactiae) in selective and more *Escherichia coli* in the blanket treatment group. Borchardt and Heuwieser (2022) did not perform bacteriological testing on the blanket treatment group, hence their pathogen distribution is unknown.

Risk-of-Bias Assessment

Outcomes of the risk of bias assessment are presented in Table 5. Although it is recommended to perform a separate assessment per outcome measure, 1 overall

Table 4. Predominant bacteria isolated in studies evaluating impacts of selective clinical mastitis treatment protocols

Study	Predominant bacteria isolated	Remarks
Lago et al. (2011a,b)	• *Escherichia coli* (18%) • CNS (9%) • *Staphylococcus aureus* (7%)	
MacDonald (2011)	• *Staph. aureus* (14%) • *Streptococcus* spp. other than *Streptococcus uberis*, *Strep. agalactiae*, and *Streptococcus dysgalactiae* (9%) • *E. coli* (7%)	
Mansion-de Vries et al. (2016)	• *E. coli* (22%) • *Strep. uberis* (16%)	
Lago et al. (2016a, 2019)	• Streptococci (30%) • CNS (19%) • Coliforms (10%)	
Lago et al. (2016b, 2019)	• Streptococci (26%) • CNS (12%) • Coliforms (7%)	
Vasquez et al. (2017)	• *E. coli* (23%) • *Strep. uberis* (14%) • *Strep. dysgalactiae* (13%)	
Kock et al. (2018)	• *Strep. uberis* (26%) • *Staph. aureus* (9%) • *Prototheca* spp. (9%)	More staphylococci in the selective and more streptococci in the blanket CM treatment group.
McDougall et al. (2018)	• *Strep. uberis* (60%) • *Staph. aureus* (13%) • *E. coli* (8%)	
Bates et al. (2020)	• *Strep. uberis* (50%) • *Streptococcus* spp. other than *Strep. uberis* (35%) • *Staph. aureus* (10%)	
Schmenger et al. (2020)	• *Strep. uberis* (19.4%) • *E. coli* (7.8%) • NAS (7.2%)	
Bazzanella et al. (2020)	• *Strep. uberis* (17.7%) • *E. coli* (15.9%) • *Staph. aureus* (7.5%)	More staphylococci in the selective and more streptococci in the blanket CM treatment group.
Griffioen et al. (2021)	• *E. coli* (20%) • NAS (16%) • *Strep. uberis* (15%)	More streptococci in the selective and more *E. coli* in the blanket CM treatment group.
Borchardt and Heuwieser (2022)	• *Strep. uberis* (40%) • Gram-positive other than *Strep. uberis* (30%) • Gram-negative (13%)	Results from rapid diagnostic test, for selective treatment group only.

assessment per study was done because of similarity in risk across the various outcome measures. If discrepancies existed between 2 or more outcome measures within 1 study, overall risk was based on the worst performing outcome measure.

Eight studies had an elevated risk of selection bias introduced by suboptimal randomization: 4 studies randomized based on even or odd ID numbers (Lago et al., 2016a,b; Mansion-de Vries et al., 2016; Kock et al., 2018), 1 alternated cases between the selective or blanket group (McDougall et al., 2018), and 1 used a randomized list that was not concealed (Bates et al., 2020). Of these 7 studies, only Kock et al. (2018) reported differences in pathogen distribution between the 2 groups. Bates et al. (2020) reported a higher average parity in the selective group. McDougall et al. (2018) provided no information regarding baseline characteristics such as DIM and parity. Two studies did not randomize at all as they evaluated the blanket protocol first and the

Table 5. Risk-of-bias assessment of studies evaluating the effects of selective clinical mastitis (CM) treatment protocols[1]

Study	A	B	C	D	E	Total
Lago et al. (2011a,b)	+	+	+	+	−	−
MacDonald (2011)	+	+	+	+	+	+
Mansion-de Vries et al. (2016)	!	+	+	+	−	−
Lago et al. (2016a, 2019)	!	+	+	+	+	!
Lago et al. (2016b, 2019)	!	+	+	+	+	!
Vasquez et al. (2017)	+	+	−	!	+	−
Kock et al. (2018)	−	+	+	+	+	−
McDougall et al. (2018)	−	+	+	+	+	−
Bates et al. (2020)	−	+	+	+	+	−
Schmenger et al. (2020)	−	+	−	+	+	−
Bazzanella et al. (2020)	−	+	+	+	+	−
Griffioen et al. (2021)	+	+	+	+	+	+
Borchardt and Heuwieser (2022)	+	+	+	+	+	+

[1]Level of bias risk indicated by + (low), ! (some concerns), – (high). A = bias arising from the randomization process; B = bias due to deviations from intended interventions; C = bias due to missing outcome data; D = bias in measurement of the outcome; E = bias in selection of the reported result.

selective treatment protocol second (Bazzanella et al., 2020; Schmenger et al., 2020). Although no differences between the 2 groups in terms of DIM and parity were reported, differences in pathogen distribution existed (both reported more cases with culture-negative results in the selective treatment group).

Bias due to missing outcome data was infrequent. In Vasquez et al. (2017), some data were missing with respect to days to clinical cure, as some cows were not placed in hospital pens and thus not monitored. Schmenger et al. (2020) had a higher proportion of missing data in the blanket CM treatment group for recurrent cases and a higher proportion of missing data in the selective CM treatment group for bacteriological cure, SCC, and incidence of new IMI. For both studies, missing data had the potential of creating recording bias.

Potential bias from selective reporting was uncommon. Lago et al. (2011a) reported the results of Generalized Linear Models for all outcome variables except for risk of primary antimicrobial treatment. Mansion-de Vries et al. (2016) described performing a logistic regression but did not present the results. No biases were identified regarding deviations from intended interventions.

Outcome Meta-Analyses

Outcome measures reported in sufficient detail in at least 2 studies included short-term outcome measures bacteriological cure, clinical cure, and new IMI risk, and long-term outcomes SCC, milk yield, recurrence, and culling rate. A summary of each meta-analysis is presented in Table 6; underlying data are presented in Supplemental Table S5 (https://data.mendeley.com/datasets/hd7gh8nc46; de Jong, 2022). Data supporting the inferiority margin M for each of the outcome measures are presented in Supplemental Table S6 (https://data.mendeley.com/datasets/hd7gh8nc46; de Jong, 2022).

Bacteriological Cure. Ten studies assessed bacteriological cure either at 14 and 21 d or solely at 21 d after CM diagnosis (Figure 2a), with bacteriological cure defined by all studies as absence of original mastitis pathogen in all available samples after CM diagnosis. There was a similar risk for no bacteriological cure in selective versus blanket treatment groups, indicating noninferiority, as the 95% CI spanned R = 1 and the upper bound of the 95% CI was lower than the noninferiority margin M (RR 1.09; 95% CI: 0.87 to 1.36; M = 1.42). A sensitivity analysis was conducted where studies with an uneven pathogen distribution were removed (Kock et al., 2018; Bazzanella et al., 2020; Griffioen et al., 2021), but the conclusion of noninferiority remained (RR 1.11; 95% CI: 0.89 to 1.38). However, after excluding studies that co-administered more NSAID to the selective group (Mansion-de Vries et al., 2016; Kock et al., 2018; Bazzanella et al., 2020; Schmenger et al., 2020), results were inconclusive, such that noninferiority could not be demonstrated due to wide confidence intervals (RR 1.27; 95% CI: 0.96 to 1.66).

New Intramammary Infection Risk. Four studies assessed new IMI risk at 14 d, 21 d, or both 14 and 21 d (Figure 2b), with new IMI defined by all studies as isolation of bacterial species not present in the original (d 0) sample. The comparison of selective versus blanket protocols did not indicate a difference in proportion of cases that developed a new IMI (RR 0.96; 95% CI: 0.81 to 1.14). A noninferiority margin could not be calculated because the only available study did not include gram-positive cases (Supplemental Table S6, https://data.mendeley.com/datasets/hd7gh8nc46; de Jong, 2022).

Clinical Cure. Five studies reported days to clinical cure (Figure 3a), of which none co-administered NSAID to the selective group. Time to clinical cure was approximately a half-day longer in the selective group compared with the blanket group (MD 0.43; 95% CI: 0.19 to 0.69). A noninferiority margin could not be calculated because the only available study did not include gram-positive cases (Supplemental Table S6, https://data.mendeley.com/datasets/hd7gh8nc46; de Jong, 2022).

Five studies assessed proportion of cases that reached clinical cure, with observation intervals ranging from 5 to 21 d after CM diagnosis (Figure 3b). In the blanket treatment groups, the risk of not being cured clinically was lower compared with the selective treatment group (RR 0.75; 95% CI: 0.58 to 0.97). However, after removing studies that co-administered NSAID (Mansion-de Vries et al., 2016; Kock et al., 2018; Bazzanella et al., 2020), this effect was no longer present in the 2 remaining studies (RR 1.03; 95% CI: 0.79 to 1.34). Furthermore, when studies with an uneven pathogen distribution were removed (Kock et al., 2018; Bazzanella et al., 2020; Griffioen et al., 2021), no differences in risk of cure were noticed in the remaining 2 studies (RR 0.91; 95% CI: 0.70 to 1.19). Regardless, a noninferiority margin was not calculated because the supporting literature comparing blanket CM treatment protocols with a nonantimicrobial CM treatment protocol lacked evidence of any benefit of antimicrobial therapy on clinical cure (Supplemental Table S6).

Somatic Cell Count. Nine studies assessed SCS (a log 2 base transformation of SCC) either as a single measure at 21, 43, or 150 d after CM diagnosis, 30 to 35

Table 6. Summary of findings from meta-analyses comparing selective versus blanket clinical mastitis (CM) treatment protocols[1]

Outcome	No. of herds and cows (studies)	Relative effect (95% CI)	Noninferiority margin	Conclusion	Certainty of evidence (GRADE)
Bacteriological cure Proportion not cured within 21 d	88 herds 2,626 cows (10 studies)	RR 1.09 (0.87 to 1.36)	M 1.42	Blanket is not inferior to selective treatment	⊕⊕⊕⊕ (High)
Clinical cure Proportion not cured within 5 to 14 d	66 herds 1,543 cows (5 studies) 63 herds 784 cows (2 studies) 3 herds 767 cows (3 studies)	All studies[2] RR 0.75 (0.58 to 0.97) No NSAID use RR 1.03 (0.79 to 1.34) NSAID use RR 0.66 (0.54 to 0.82)	Not available	No evidence for a difference between selective and blanket	⊕⊖⊖⊖ (Very low)[3]
Days to clinical cure	11 herds 1,477 cows (4 studies)	MD 0.43 (0.19 to 0.69)	Not available	Slight increase (0.4 d) in days to clinical cure in selective group	⊕⊕⊖⊖ (Low)[4]
New IMI Proportion with new IMI within 21 d	15 herds 1,464 cows (4 studies)	RR 0.96 (0.81 to 1.14)	Not available	No evidence for a difference between selective and blanket	⊕⊕⊕⊖ (Moderate)[5]
SCC Proportion high SCC within 14 to 21 d	22 herds 1,001 cows (4 studies)	RR 1.00 (0.94 to 1.06)	Not available	No evidence for a difference between selective and blanket	⊕⊕⊕⊕ (High)
Average SCS after CM within 21 to 305 d	24 herds 2,374 cows (7 studies)	MD 0.004 (−0.10 to 0.11)	Not available	No evidence for a difference between selective and blanket	⊕⊕⊕⊖ (Moderate)[6]
Milk yield Average production within 150 to 305 d	12 herds 1,892 cows (5 studies)	MD 0.34 (−0.67 to 1.35)	Not available	No evidence for a difference between selective and blanket	⊕⊕⊕⊖ (Moderate)[7]
Recurrence Proportion with new CM within 60 to 305 d	79 herds 4,102 cows (9 studies)	RR 0.91 (0.73 to 1.13)	Not available	No evidence for a difference between selective and blanket	⊕⊕⊕⊖ (Moderate)[8]
Culling Proportion culled within 21 to 305 d	14 herds 2,553 cows (7 studies)	RR 0.87 (0.70 to 1.08)	Not available	No evidence for a difference between selective and blanket	⊕⊕⊕⊕ (High)

[1]RR = risk ratio; MD = mean difference; M = noninferiority margin; CM = clinical mastitis; GRADE = Grading of Recommendations, Assessment, Development and Evaluations; Schünemann and Santesso (2010).

[2]Including 3 studies with higher nonsteroidal antiinflammatory drug (NSAID) used in selective group.

[3]Randomization issues (3 out of 5 studies), NSAID used in the selective group (3 out of 5 studies), uneven pathogen distribution (2 out of 5 studies) and differences in time point of measurement, which affects clinical cure and measurement of clinical cure.

[4]Randomization issues (2 out of 4 studies), which affects clinical cure and measurement of clinical cure, 3 studies are from the same research group.

[5]Three studies are from the same research group.

[6]Wide CI with clinical implications.

[7]Three studies from the same research group.

[8]Randomization issues (7 out of 9 studies).

d before drying-off, or as an average over the remainder of the lactation (Figure 4a). There was no evidence of a difference in SCS between selective and blanket treatment groups (MD 0.004; 95% CI: −0.10 to 0.11), regardless of observation period.

Three studies reported proportion of cows with SCC <200,000 cells/mL at 14 and 21 d; 1 study reported proportion of cows with SCC <100,000 cells/mL (Figure 4b). Furthermore, comparison of selective versus blanket CM treatment protocols did not indicate a difference in proportion of cases with high SCC (RR 1.00; 95% CI: 0.94 to 1.06). A noninferiority margin could not be calculated because the supporting literature comparing blanket CM treatment versus no antimicrobial CM treatment protocols reported that antimicrobial therapy did not affect the proportion with high SCC (Supplemental Table S6).

Milk Yield. Five studies assessed milk yield with observation intervals ranging from 43 d to the remainder of lactation (Figure 5a). Milk yield was not different when comparing selective and blanket protocols (MD 0.34; 95% CI: −0.67 to 1.35). A noninferiority margin could not be calculated as the supporting literature that compared blanket CM protocols with a no anti-

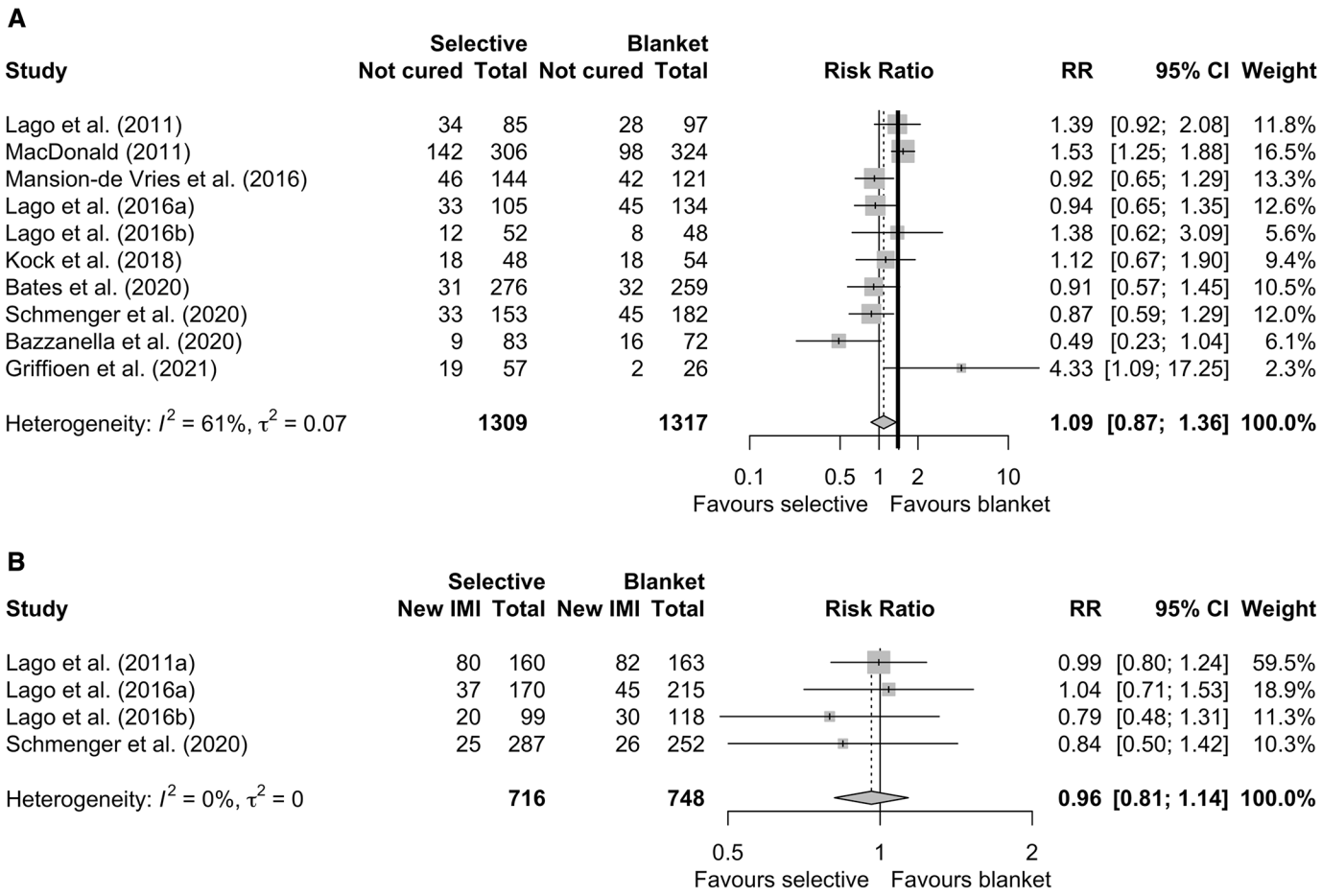

Figure 2. Pooled risk ratio (RR) of proportion of cases not cured bacteriologically (A) and new IMI (B) following treatment of clinical mastitis (CM) according to selective versus blanket treatment protocols. Noninferiority margin is indicated as a solid black line.

microbial treatment demonstrated that antimicrobials did not influence milk yield (Supplemental Table S6). A sensitivity analysis was conducted where the study that only treated streptococci in the selective group was removed, but this did not change the conclusion (MD 0.34; 95% CI: −0.88 to 1.56).

Recurrence. Nine studies assessed risk of CM recurrence with observation periods ranging from 60 to 305 d (Figure 5b). The comparison of selective versus blanket protocols did not indicate a difference in recurrence of CM (RR 0.91; 95% CI: 0.73 to 1.13). A noninferiority margin could not be calculated because the only available study did not include gram-positive cases (Supplemental Table S6). A sensitivity analysis where the study with an uneven pathogen distribution was removed did not lead to a different conclusion (RR 0.89; 95% CI: 0.72 to 1.10).

Culling. Seven studies assessed risk of culling with observation periods ranging from 21 d to the remainder of lactation (Figure 5c). The comparison of selective versus blanket protocols did not indicate a difference in proportion culled (RR 0.87; 95% CI: 0.70 to 1.08). A noninferiority margin could not be calculated, as the supporting literature that compared blanket CM protocols with a no antimicrobial treatment demonstrated that antimicrobials did not influence culling (Supplemental Table S6). A sensitivity analysis was conducted where the study with an uneven distribution of pathogens was removed, but this did not change the conclusion (RR 0.87; 95% CI: 0.69 to 1.09).

Other Variables. Three studies assessed the number of secondary treatments following initial CM treatment decision (Supplemental Table S5); they were higher for cases treated according to a blanket CM treatment protocol (Lago et al., 2011a; MacDonald, 2011; Kock et al., 2018). Four studies assessed days milk was kept out of the bulk tank (Lago et al., 2011a, 2016a,b; Bates et al., 2020), but there was a high level of unexplained heterogeneity present (84%), so a pooled effect measure is not displayed (Supplemental Table S5).

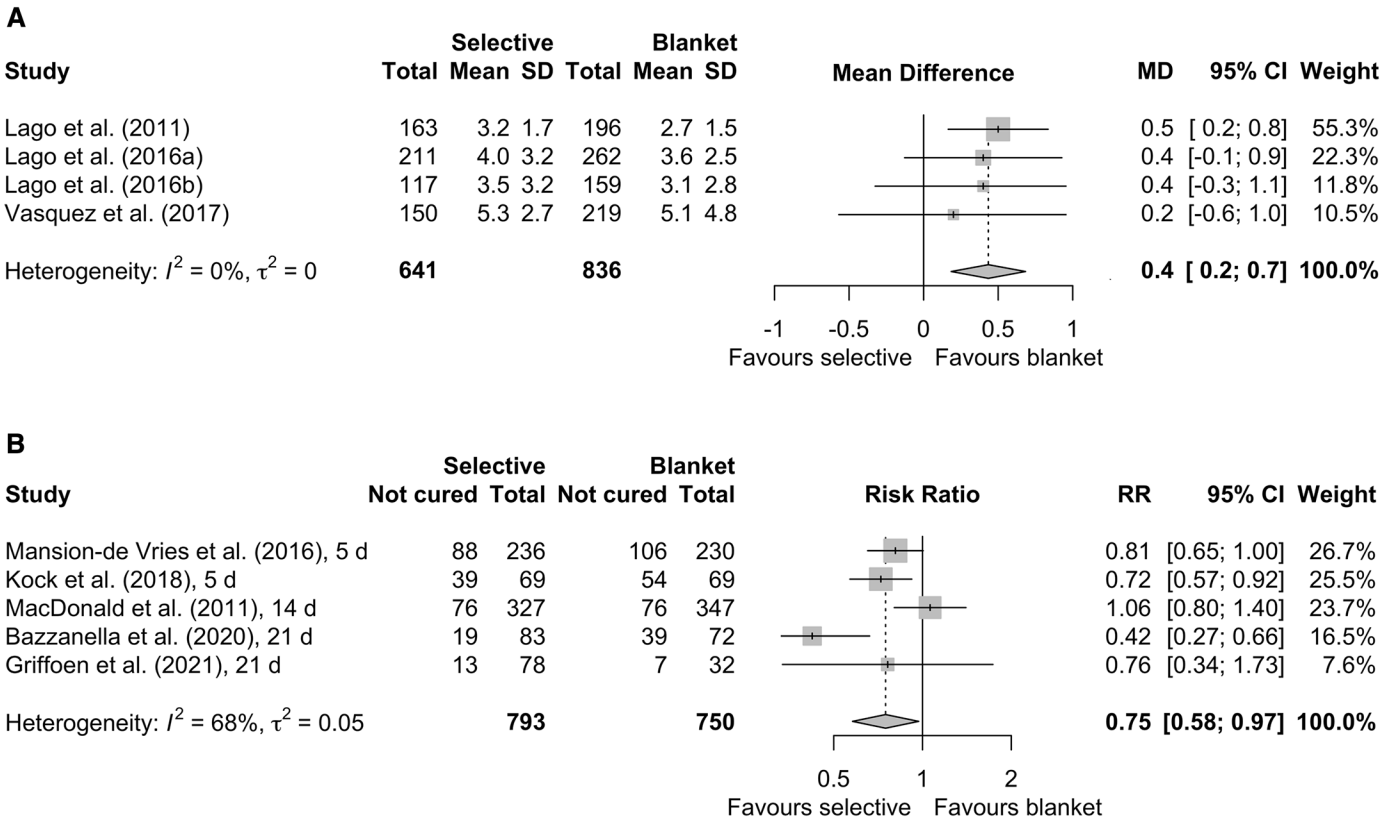

Figure 3. Pooled mean difference (MD) of days to clinical cure (A) and risk ratio (RR) of proportion of cases not cured clinically (B) following treatment of clinical mastitis (CM) according to selective versus blanket treatment protocols.

Publication Bias

Symmetry in the funnel plots was observed for bacteriological cure, clinical cure, proportion high SCC, and milk yield and recurrence (Supplemental Figures S1 to S5, https://data.mendeley.com/datasets/hd7gh8nc46; de Jong, 2022). Asymmetry was observed for days to clinical cure, new IMI, SCS, and culling (Supplemental Figures S6 to S9, https://data.mendeley.com/datasets/hd7gh8nc46; de Jong, 2022). However, missing studies did not influence reported RR and MD to the extent that conclusions presented in this review would be altered (data not shown).

Certainty of Evidence

The GRADE assessment was carried out to evaluate the certainty of reported results from the meta-analysis (Table 6). Authors placed a high level of confidence in reported effects of selective CM treatment on the following dairy cattle health indicators: bacteriological cure, proportion high SCC, or culling. They placed a moderate level of confidence in new IMI, recurrence, SCS, or milk yield; a low level of confidence in days to clinical cure; and a very low level of confidence in proportion of clinical cure.

DISCUSSION

Selective CM treatment was not inferior to blanket treatment for bacteriological cure using a conservative margin and supported by a high certainty of evidence, regardless of variations in study locations that reflected multiple management styles. A longer interval to clinical cure and larger proportion that reached cure at 14 d was observed in the selective group, but these results were due to NSAID use in selectively treated cows, and thus supported by a low certainty of evidence. Regardless, with high and moderate certainties of evidence, all other important outcomes, including new IMI, SCC, milk yield, recurrence, or culling were not different in cows with CM treated selectively than when blanket treatment was used. Our findings indicated that selective use of antimicrobial therapy for CM, as guided by rapid diagnostic tests, can be effective with similar outcomes and without excess risk.

Using noninferiority margins to compare selective CM treatment with blanket CM treatment protocols is

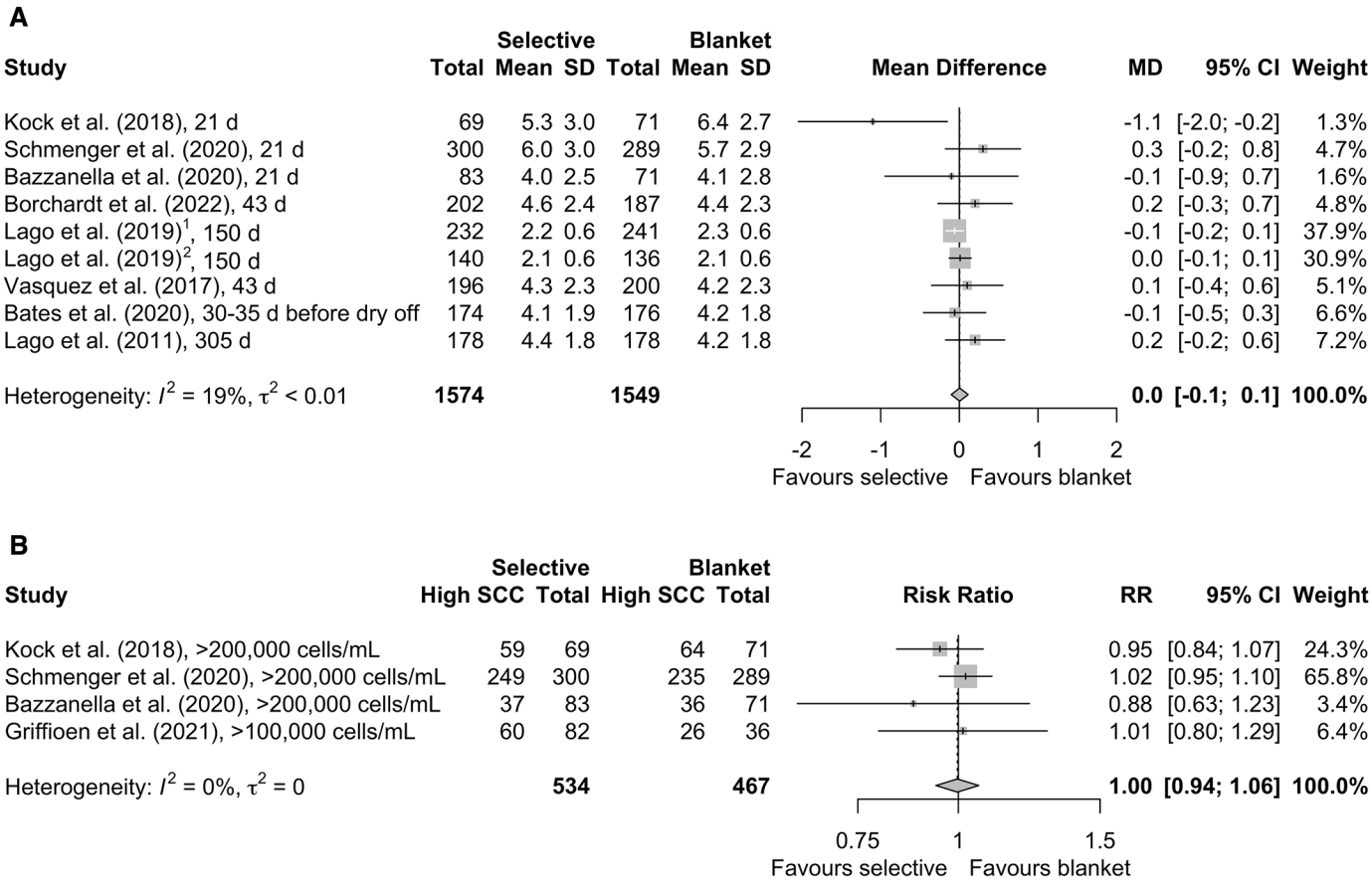

Figure 4. Pooled mean difference (MD) of SCS (A) and risk ratio (RR) or proportion of cases with high SCC within 14 or 21 d (B) following treatment of clinical mastitis (CM) according to selective versus blanket treatment protocols. [1]Corresponding to Lago et al. (2016a); [2]corresponding to Lago et al. (2016b).

an alternative to the traditional superiority approach used in most clinical trials. The noninferiority approach can be used to evaluate treatment efficacies and is particularly suitable for the current study where the relevant question was whether selective treatment of CM would result in less favorable results than when using blanket treatment of CM. Here, historical accounts of nonsevere CM disease course in the negative control group (e.g., CM cases that do not receive antimicrobial treatment) were used to establish a baseline efficacy of blanket CM protocols (positive control) that were used to determine the efficacy of selective treatment protocols. Unfortunately, not enough literature was available to establish margins for outcome days to clinical cure, new IMI, and recurrence. Furthermore, there was limited literature that included both gram-negative and gram-positive cases for SCC, culling, and milk yield. All these outcomes are important considerations in CM treatment protocol evaluations and are frequently used by farmers and veterinarians (Ruegg, 2021).

Most included studies used a per-protocol analysis, where cases were omitted from conclusions when the allocated protocol was not followed. In superiority trials, a focus on intention to treat is recommended as it produces a smaller effect estimate, which can be expected in real-life situations. Only McDougall et al. (2018) followed an intention to treat analysis where possible. In contrast, a per-protocol analysis is justified in a noninferiority trial as an intention to treat approach will limit the chances of determining noninferiority (Scott, 2009). When implementing selective CM treatments on dairy farms, we do not expect the results presented in this study to change, as no differences were detected between selective and blanket CM treatments for the majority of outcome measures. These results will not be affected by a reduction in effect size, a common feature of reviewing intention to treat protocols. In addition, in real-life situations, sample sizes will be smaller, and any identified differences will most likely be due to confounding factors.

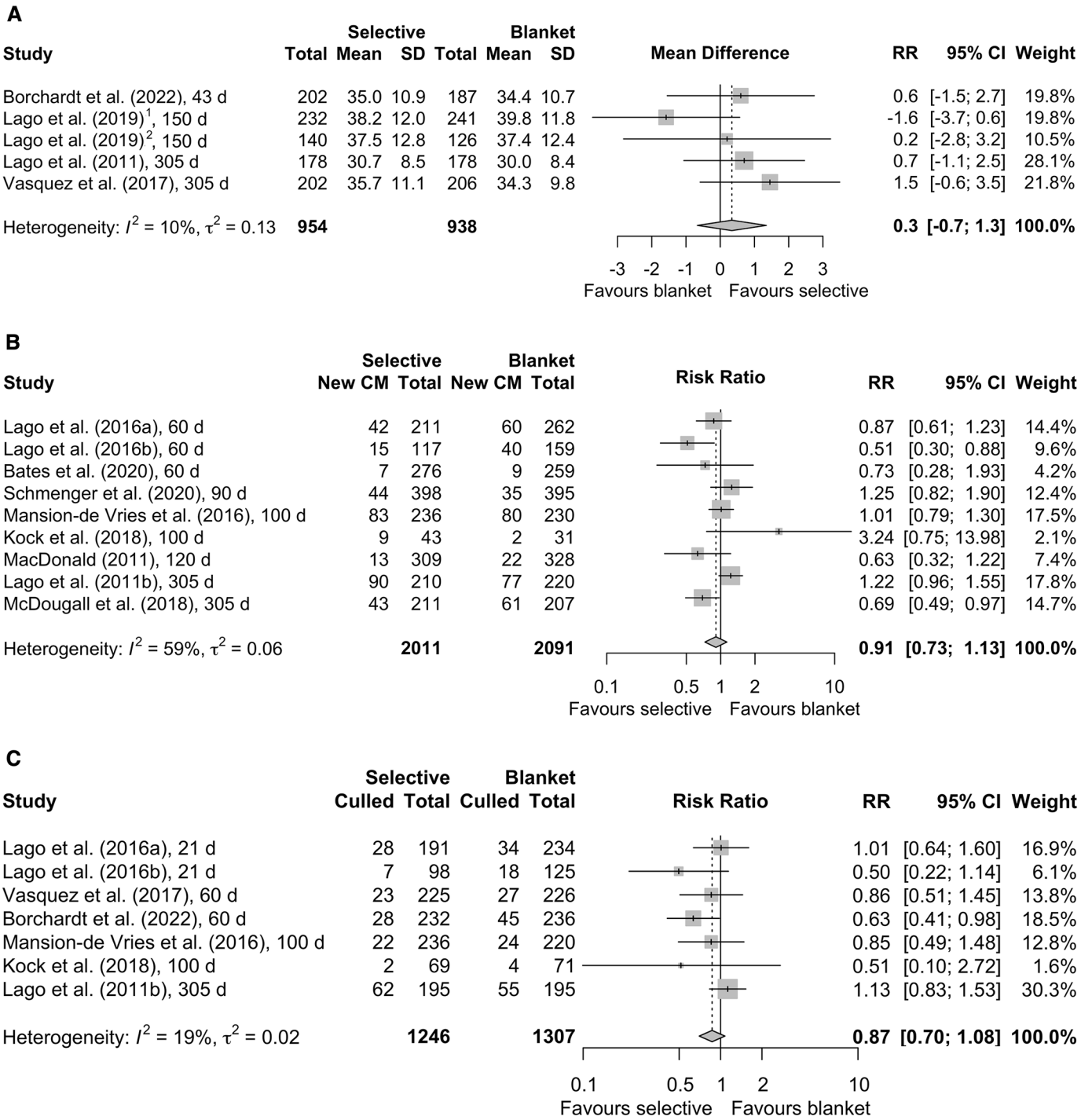

Figure 5. Pooled mean difference (MD) of milk yield (A), risk ratio (RR) of recurrence of clinical mastitis (CM) (B), and RR of culling rate (C) after CM following treatment of CM according to selective versus blanket treatment protocols. [1]Corresponding to Lago et al. (2016a); [2]corresponding to Lago et al. (2016b).

The included studies used a variety of rapid diagnostic tests. The tests used were based on the culture and identification of mastitis-causing pathogens via selective media on plates or in tubes. This variety can potentially impact the results, as the sensitivity of these tests to identify gram-positive bacteria is known to range from 59 to 98%, and specificity ranges from 48 to 97% (Malcata et al., 2020). However, a sensitiv-

ity analysis was not possible, as few studies used the same rapid diagnostic tests. In addition, the included studies used varying selective treatment protocols as a result of variations in specific herd goals (e.g., culling all *Staph. aureus* cases, Vasquez et al., 2017), by including cow-specific factors [e.g., by categorizing cows as worthy or unworthy of treatment, Mansion-de Vries et al. (2016), Kock et al. (2018), Schmenger et al. (2020)] and other factors. Hence, 4 types of selective treatment protocols were included and described. Although the differences in selective CM treatment protocols will influence on-farm prospective AMU reduction, the sensitivity analyses did not indicate an effect on the 9 outcome measures.

Randomization methods differed among studies, with lack of randomization in 2 studies (Bazzanella et al., 2020; Schmenger et al., 2020). Three of the 7 studies with suboptimal randomization reported uneven distributions between groups of either pathogens or average parity, confirming the likelihood that selection bias occurred as a result of predictability in assigning cases to each treatment group. Correct randomization using sealed envelopes minimizes possible confounding and reduces the risk of exaggerating treatment effects. The latter is especially associated with subjective outcomes such as clinical cure. However, it is unlikely to affect objective outcomes such as bacteriological cure, milk yield, or SCC. In each study, bias could also have been introduced during assessment of outcomes as farm workers were aware of which cows belonged to which groups after enrollment. Especially for clinical cure, recurrence, and culling, knowledge of which intervention was received can affect attention to clinical signs in favor of or disadvantageous to selective treatment of CM. We do not believe that this would be the case for bacteriological cure, SCC, and milk yield. Evidence that this might be the case was only reported by Vasquez et al. (2017), who had more missing data in the selective treatment group. Effects of randomization and measurement of the outcome on reported results was taken into account in the GRADE assessment of certainty of evidence (Table 6).

For bacteriological cure, noninferiority was demonstrated between selective and blanket treatment groups, except when studies were excluded that administered NSAID in the selective group (regardless of NSAID use in the blanket treatment group). Exclusion of those studies led to a wider CI, which spanned the noninferiority margin. Although this might be indicative of confounding factors, a similar distribution of severity, DIM, and parity between the 2 treatment groups were reported by Kock et al. (2018) and Schmenger et al. (2020). Only Bazzanella et al. (2020) reported more severe cases in the blanket CM treatment group, but those cases were not included in their reported results. Thus, a hypothesis could not be formed to explain the effect of administrating NSAID on diminishing evidence of noninferiority.

A slight increase in time to clinical cure was observed in selective treatment group (0.4 d). In contrast, there was an absence of evidence for a difference in proportion of cases reaching clinical cure within either 14 or 21 d except for studies that co-administered NSAID to cases in the selective treatment group and not in the blanket treatment group. In those studies, a higher proportion of cases reached clinical cure within the stated observation period. This was, however, in contrast to the majority of studies that did not identify a positive effect of NSAID (ketoprofen or penethamate hydrioxide) on clinical cure in mild gram-negative CM (Latosinski et al., 2020), mild and moderate gram-positive CM (McDougall et al., 2007), or in mild or moderate chronic CM (Krömker et al., 2021). Only Banting et al. (2008) reported less clinical signs in endotoxin-challenged CM treated with ketoprofen compared with no treatment. Without co-administering NSAID, available literature suggested that antimicrobial treatment did not affect clinical cure (Supplemental Table S6), consistent with our findings. Hence, it has been recommended to move away from using clinical cure as an indicator of a successful CM treatment (Ruegg, 2021). Regardless, producers frequently include clinical cure in their assessment to extend CM treatments as they regard it as one of the most tangible measures of therapy success (Swinkels et al., 2015).

For proportion of new IMI, the meta-analysis also provided no evidence to assume a difference between selective and blanket treatment groups. Only 1 study was available that compared blanket to no antimicrobial treatment of naturally occurring *E. coli* CM in which no difference in the proportion of new IMI was indicated (Suojala et al., 2010).

For proportion of cases failing to reach a low SCC threshold, only 2 studies were available that reviewed effects of AMU using the California Mastitis Test (CMT) (Roberson et al., 2004; Persson et al., 2015). The cut-off of CMT <3 was used to indicate the low SCC threshold, although sensitivity of CMT for detecting any mastitis pathogen using a single sample was 50%, with sensitivity declining with increasing CMT score (Middleton et al., 2004). Both studies indicated no difference between those CM cases treated with and without antimicrobials. Additionally, another study detected no effect of microbiological diagnosis or treatment duration on likelihood of reaching a low SCC threshold (Pinzón-Sánchez and Ruegg, 2011). This is in line with the outcome of the meta-analysis, which did not provide evidence for a difference in proportion with

low SCC (i.e., <200,000 cells/mL) between CM cases treated according to a blanket or selective treatment protocol. There was also no evidence of difference in average SCC between the selective and blanket treatment groups for 21 to 305 d after CM. No difference in average milk yield was present between cases in the selective and blanket treatment groups, in line with literature evaluating antimicrobial treatment (Supplemental Table S6, https://data.mendeley.com/datasets/hd7gh8nc46; de Jong, 2022).

This meta-analysis provided no evidence to assume a difference in recurrence of CM between selective and blanket groups. This was in line with Fuenzalida and Ruegg (2019), who reported no differences in recurrence between gram-negative cases treated with and without antimicrobials; data comparing blanket with no antimicrobial treatment of gram-positive cases were not available. A narrative review concluded that more aggressive and extended treatments seemed to be more important determinants of CM recurrence (Jamali et al., 2018).

The outcome of the meta-analysis provided no evidence to assume a difference in culling risk between selective and blanket groups, which was in line with reports that for gram-negative cases that there was no effect of AMU (blanket or selective) on culling (Suojala et al., 2010; Schukken et al., 2011; Persson et al., 2015). No studies were available that considered gram-positive CM cases. It has been reported that length of antimicrobial treatment does not influence risk of culling; only milk yield before CM was identified as an explanatory variable by Pinzón-Sánchez and Ruegg (2011) when evaluating treatment outcomes of mild and moderate CM cases.

All studies except for 2 that were presented at conferences (Lago et al., 2016a,b) acknowledged the source of funding. These funding sources varied from government, nongovernmental organizations, and industry. Industry funding was received by Vasquez et al. (2017), Kock et al. (2018), and Bates et al. (2020). In our opinion, this has not impacted their study design, analysis, or conclusions drawn.

The results of this systematic review and meta-analysis can lead to an increased uptake of selective CM treatment protocols and subsequently reduce mastitis-related AMU. More specifically, this review contributes to an informed discussion surrounding CM treatment protocols for both veterinarians and dairy farmers by highlighting the impact of selective CM treatment protocols compared with blanket CM treatment protocols. However, other factors will influence adaptation of the described protocols, such as the costs of the added labor, availability and accessibility to rapid diagnostic tests (either on-farm or via veterinary laboratory), and the expected reduction in AMU. These factors have not been discussed in the current review but should be carefully considered when developing on-farm treatment protocols.

Drawbacks of the conclusions drawn in this study include the absence of studies assessing the impacts of selective CM treatment protocols in dairy countries outside of North America, Europe, and Australasia, such as Brazil, Pakistan, Iran, and China. However, those countries experience a higher proportion of cases caused by gram-positive bacteria (Hameed et al., 2008; Hashemi et al., 2011; Gao et al., 2017). Hence, the added labor associated with the selective treatment protocol related to taking milk samples and performing the rapid diagnostic test might outweigh the benefit of the reduced AMU. Nonetheless, dairy farms in these countries can benefit from implementing selective treatment protocol as it informs pathogen distribution and spot cases that do not benefit from treatment. However, differences in climate, feed, production systems, and management should be taken into consideration when interpreting the results from this systematic review and meta-analysis.

CONCLUSIONS

Meta-analyses of 13 studies identified through systematic search and selection demonstrated that a selective treatment protocol of CM was noninferior to a blanket treatment protocol in terms of bacteriological cure. Some differences between selective and blanket groups were observed with regards to clinical cure, but these results were confounded by use of NSAID in the selective treatment group only. Furthermore, we did not identify any differences between selective and blanket treated CM cases for the outcome measures clinical cure, proportion new IMI, SCC, milk yield, recurrence, and culling, although noninferiority margins could not be determined. When studies that co-administered NSAID were excluded, conclusions remained similar. Hence, this review supported that selective treatment of nonsevere CM can be adopted without negative udder health consequences.

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