Invited review: Effectiveness of precalving treatment on postcalving udder health in nulliparous dairy heifers: A systematic review and meta-analysis

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ABSTRACT

Clinical mastitis affects 3% of primiparous dairy cattle (heifers) in the first month after calving. Additionally, the prevalence of intramammary infection (IMI) in the months before first calving is high, resulting in a high prevalence of heifers calving with IMI. Precalving therapy is an accepted recommendation for reducing mastitis in multiparous cows, but prophylactic treatment for heifers is uncommon in North America. Objectives of this study were to (1) quantify changes in postcalving udder health in heifers following application of a precalving treatment; (2) compare effectiveness among various types of treatments; and (3) compare effectiveness of various types of treatments against specific pathogens. A systematic review was conducted comparing interventions aimed at improving udder health in heifers. Of 62 included studies, 48 clinical trials were used in a meta-analysis. Data were synthesized using a random effects model for meta-analysis, followed by sub-group analyses comparing treatment types, and specific pathogens with statistical testing using meta-regression. Occurrence of mastitis (defined as elevated somatic cell count, clinical mastitis, and IMI) was reduced in treated heifers compared with untreated controls with a pooled risk ratio of treated to untreated heifers of 0.56 (95% confidence interval: 0.47 to 0.67). Upon stratification by treatment types, teat sealants and combination therapies (vaccines and antimicrobials; antimicrobials and teat sealants; and all 3) were most effective at improving udder health with pooled risk ratios of 0.40 (95% confidence interval: 0.30 to 0.52) and 0.34 (95% confidence interval: 0.25 to 0.45), respectively. Antimicrobials and vaccines also reduced occurrence of IMI and subclinical and clinical mastitis when compared with untreated heifers. Al-

though variation was observed in the pathogen-specific effectiveness of treatments at reducing rates of disease, antimicrobials, teat sealants, and combinations of vaccines or teat sealants with antimicrobials were consistently effective, whereas vaccines were only effective for contagious pathogens. Recommendations for use of antibiotics should consider their relative benefit while also considering potential for increasing antimicrobial resistance.

Key words: heifer, mastitis, prepartum, treatment, meta-analysis

INTRODUCTION

Mastitis is one of the most economically important diseases in the dairy industry (Seegers et al., 2003) as it has implications not only for animal health and welfare, but also for milk quality and production. The occurrence of mastitis is not only high in lactating cows, but also in heifers in the first weeks after calving (De Vlieghe et al., 2012; Naqvi et al., 2018). Heifers with (sub)clinical mastitis, IMI, or both are often not identified because they are not milked, and therefore their teats and milk typically are not examined (De Vlieghe et al., 2012). However, the prevalence of IMI in the months before the first calving is high, resulting in a high prevalence of heifers calving with IMI (De Vlieghe et al., 2012).

Although nonlactating heifers are not included in standard mastitis prevention plans like the 5-point schedule (Neave et al., 1969) or the relatively recent NMC 10-point plan (NMC, 2004), a variety of treatments to prevent or cure IMI in these heifers has been studied. Following a review on heifer mastitis (De Vlieghe et al., 2012), the NMC published a 10-point plan specifically for controlling heifer mastitis. In this new set of recommendations, only 2 were specific to heifers, whereas the others were similar to recommendations in the first 10-point plan. Some commonly used precalving treatments are vaccines, non-antimicrobial teat sealants, short- and long-term antimicrobials, and combinations of these treatments. Many studies have

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been conducted on effects of a single type of treatment in heifers (Edinger et al., 2000; Santos et al., 2004; Roy et al., 2007; Pellegrino et al., 2008). However, very few studies have compared types of therapies, and their results have been inconsistent (McDougall et al., 2009).

Previous studies have demonstrated and quantified a net positive benefit of various precalving treatments (Ataee et al., 2009; Duplessis et al., 2014; Passchyn et al., 2014), as well as characterizing pathogens causing ensuing infections. Systematic reviews have been conducted on the effectiveness of antimicrobial treatments in heifers (Nickerson, 2009), non-antibiotic strategies for prevention and control of mastitis in heifers (McDougall et al., 2009), and vaccines for lactating cows (Middleton et al., 2009), whereas reviews of postpartum mastitis and mastitis control have also described preventive treatments in heifers and lactating cows (Pyörälä, 2008; De Vliegher et al., 2012). However, no review has focused on quantifying treatment effectiveness in heifers, or compared efficacy of precalving treatment types using a meta-analysis to estimate overall effect size. Objectives of this study were therefore to (1) synthesize results from previous studies to determine and compare effectiveness of various types of precalving therapies aimed at improving udder health; and (2) determine and compare effectiveness of various types of precalving therapies against types of pathogens, grouped by etiology.

MATERIALS AND METHODS

This systematic review and meta-analysis was conducted following a pre-specified protocol created using the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols guidelines (Shamseer et al., 2015).

Data Sources and Literature Search

Online databases searched were CAB Abstracts, Web of Science (complete collection), AGRICOLA, MedLine, and SCOPUS, from inception to September 2017. In addition, 2 individuals (SAN and DBN) hand-searched references of existing reviews on the subject. Gray literature consisted of a search through all available conference proceedings in relevant conferences, as well as websites of pharmaceutical companies and the Proceedings of the World Buiatrics Congress (2000–2014) on The International Veterinary Information Service website. In addition, proceedings of the American Dairy Science Association (1957–2017) and National Mastitis Council (NMC; 1989–2017) annual meetings were searched. Experts in the field were also

contacted for information about potential ongoing or unpublished studies, and were identified through the review process or through direct communications at conferences (Mastitis Research Worker's meeting in November 2016, and NMC annual meeting in January 2017). No publication date or language filter was applied to the search, and the search was conducted as recommended (Egger et al., 2008).

The search strategy was split into 3 main components corresponding to the population of interest, intervention type, and study outcome. The population of interest included primigravid to primiparous dairy cattle, with the intervention having to be applied during pregnancy and udder health measured after calving. The interventions of interest were any kind of treatment applied precalving aimed specifically at improving udder health. This was intended to exclude interventions such as feed additives intended to improve overall health and growth, as it did not address the primary question of targeted and individual treatment of heifers. Full details of the search terms used are provided (Appendix).

Study Eligibility and Selection

An initial screen of all titles was performed by 2 individuals (SAN and DBN) to determine if manuscripts were eligible for full text review for inclusion in the systematic review. If the title was not detailed enough to decide about moving forward with full-text screening, the abstract was scrutinized by one of the reviewers (SAN) to determine whether it was eligible. A manuscript needed to meet 2 conditions to be considered for a full text review: (1) The manuscript had to report on primary data; and (2) the manuscript had to report on an intervention aimed at improving udder health (no vaccines for other conditions such as leucosis, or treatment with anthelmintic products), although it may not necessarily be the primary aim of the study (comparing mastitis in 2 regions where a particular product may not be allowed).

This initial screen was designed to be fairly broad to encompass as many potentially relevant manuscripts for full text review. A full text review was then conducted on all of these screened manuscripts to ensure that some aspect of intervention was addressed in the manuscript. The reason for this full text screening step in the systematic review was that the second condition described above could have resulted in manuscripts whose primary goal was not assessing intervention effectiveness, but was included as a covariate in their analysis. As it was not the primary goal of the study, it may not have been reported in the abstract and a full text screening would capture these manuscripts.

Identification of Manuscripts for Eligibility in the Systematic Review and Meta-Analysis

The same 2 individuals (SAN and DBN) reviewed full text manuscripts identified after the 2 screening stages described in the previous section using the following criteria to select manuscripts eligible for inclusion in the systematic review and meta-analysis.

- Population = primigravid/primiparous dairy cattle (if the primary study population was not primiparous dairy cattle, the manuscript must stratify and report on them separately from older animals).
- Intervention = any sort of treatment given after confirmed pregnancy, before calving or on the day of calving that was aimed specifically at improving udder health. Studies that did not assess treatment effectiveness as a primary goal (described in the previous section) were excluded from the systematic review and meta-analysis because they would not be designed around that variable and pooling would not be appropriate.
- Comparison = a treatment must be compared with either another treatment, or no treatment at all.
- Outcome = postcalving prevalence or incidence of (1) clinical mastitis (CM); (2) subclinical mastitis (SCM; elevated SCC); or (3) IMI (presence of bacteria in the milk) reported within 2 mo after first calving.
- Study design = randomized controlled trials or observational cohort studies.

The screening of manuscripts was designed to capture studies comparing precalving treatments in heifers to each other or to untreated heifers in their association with incidence of udder disease postcalving. Only randomized controlled trials were meta-analyzed, whereas results from observational cohort studies were included descriptively in the discussion. Agreement between reviewers (SAN and DBN) regarding study inclusion was quantified using a kappa score and percent agreement, and any disagreements were resolved by the last author (HWB).

Study Quality Assessment and Data Extraction

Study quality and risk of bias was assessed using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool (Hooijmans et al., 2014). The SYRCLE tool is based on the Cochrane Risk of Bias tool (Higgins et al., 2011), with some items

modified or added to fit better with risk of bias in experimental trials in animals. Briefly, the SYRCLE tool consists of 10 items, corresponding to 5 types of bias: selection, performance, detection, attrition, and reporting bias. Risk of selection bias is evaluated through considering allocation sequence generation, subject baseline characteristics, and allocation concealments. Performance bias is evaluated by considering random housing of heifers and blinding when assigning treatments. Detection bias is assessed by evaluating randomization of subjects during outcome assessment and blinding of outcome assessors. Attrition bias is assessed by evaluating the study's reporting on incomplete outcome data. Lastly, reporting bias is assessed by evaluating outcomes on which studies reported, as well as outcomes that were excluded. A study was considered to be at high risk of a given type of bias if it had failed to address over half of the sources of error contributing to that given type; moderate risk of bias if they failed to address at least 1, but not more than half; and low or no risk of bias if they did not have any sources of error from a given type of bias.

Data were extracted using a single electronic form using Microsoft Excel (Microsoft Corp., Redmond, WA). Study characteristics extracted were (1) country of study, (2) year of publication, (3) breed, (4) intervention type(s), and (5) time of intervention application. As management decisions are based on anticipated calving dates, the intended or median of intended application times was recorded in the extraction form (e.g., for 2 to 3 wk precalving, the number taken was 17.5 d). Outcome characteristics recorded, often multiple for a study, were (1) type of outcome defined as cure rate of existing IMI, SCM, IMI (and its definition), and CM; (2) time of outcome measurement or follow-up in the case of CM; and (3) numbers of individuals (animals or quarters depending on the study) and cases per treatment arm of the study. For studies where postcalving samples were collected on multiple days, the earliest sample that was not the day of calving was used for outcome extraction. Numbers in treatment arms were pooled by intervention or control and type of intervention depending on possible stratification in meta-analyses.

Statistical Analyses

All statistical analyses were performed using the "metafor" package (Viechtbauer, 2010) in R version 3.4.2 (R Core Team 2017, R Foundation for Statistical Computing, Vienna, Austria). For all analyses, a P -value < 0.05 was considered significant, and a $0.05 < P$ -value < 0.10 was considered a tendency for significance.

Results from randomized clinical trials were pooled using a random-effects meta-analysis, as no observational studies were included in the systematic review. Randomized clinical trials that did not report numbers of individuals and only ratios were not included in the meta-analysis but were included only descriptively. A mixed effects model was selected given the underlying heterogeneity in intervention and population characteristics, and the fact that studies often had multiple treatment types and outcomes. Effect sizes were pooled as risk ratios (RR) because it is most intuitively interpretable in terms of detecting a benefit/harm. All pooled estimates and stratified meta-analyses were visualized using forest plots, and homogeneity was assessed using the Cochrane Q statistic for significance and I^2 statistic to quantify the amount of variation due to study heterogeneity. The primary question concerning therapy type was statistically tested using meta-regression, and heterogeneity was then explored by stratifying on relevant variables of practical interest: type of outcome and bacterial species of infection. Variation in effectiveness by pathogen type was addressed through a combination of meta-regression and subgroup analyses. Sensitivity and specificity to detect IMI varies considerably among IMI definitions (Andersen et al., 2010; Dohoo et al., 2011a,b), so a meta-regression was conducted to determine whether effect size estimates with rates of IMI differed between studies that described their IMI definition and those that only provided details for culturing. Questions related to effects of study quality or design (abstract versus full text, disease definitions) were also addressed using meta-regression. Publication bias for the primary outcome of interest (mastitis of any form) was assessed using a funnel plot of the RR with the standard error of the RR. Asymmetry was tested using Egger's regression test for small study effects, and visualized with a graph of study variance against the natural log of the RR.

RESULTS

Description of Studies

Search Results. Numbers of studies included at various stages of the review process are shown (Figure 1). Briefly, a total of 974 unique records were identified, with most coming from the database search ($n = 1,245$ before duplicate removal) and a small number being identified through the described gray literature review ($n = 44$ before duplicate removal). After screening abstracts, 82 peer-reviewed manuscripts were identified for a more thorough full-text review, along with 31 records from conference proceedings. A small number of

non-English manuscripts were identified: Portuguese ($n = 1$), German ($n = 3$), Dutch ($n = 1$), Czech ($n = 1$), and Italian ($n = 1$). These manuscripts were reviewed using the pre-specified criteria by colleagues with fluency in the respective languages.

Studies Eligible for Inclusion in the Systematic Review. Forty studies from published manuscripts were included in the systematic review, of which 38 were eligible for inclusion in the meta-analysis. All included peer-reviewed manuscripts reported results from clinical trials; 2 could not be included in the meta-analysis because they did not report numbers of outcomes, rather relative measures such as RR and odds ratios. In addition, 22 studies from conference proceedings or abstracts were included in the systematic review. Ten conference proceedings provided enough detail in their results to be eligible for meta-analysis, whereas others were discussed only descriptively. Records from 48 studies were included in the meta-analysis, although multiple records were included for many studies because they compared multiple treatment types (Table 1). A detailed description of the specific products used in each study is provided (Appendix Table A1).

Risk of Bias in Studies Included. Design characteristics of all full-text manuscripts that are important for risk of bias are described (Table 2). Based on the SYRCLE guidelines, all studies were at high risk for at least one type of bias. The majority of studies were at moderate risk of selection bias ($n = 27$), with 2 being at low/no risk of selection bias. Allocation sequence generation was often systematic rather than completely random, and baseline animal characteristics were described in most studies ($n = 30$). The largest type of bias that this pool of studies was at risk for was detection/performance bias, as most did not describe blinding or randomized animal housing. Most ($n = 31$) studies adequately addressed incomplete data by providing numbers of heifers at each stage of the study, with reasons provided for their removal or exclusion from the trial minimizing risk of attrition bias. No studies were at risk of reporting bias, as they all either provided justification for outcomes measured or they were selected in part based on their reporting of outcomes.

Another aspect of variability among studies and a possible source of bias was how they defined outcomes (Table 3), IMI in particular. Many studies ($n = 16$) only described culturing details for their milk samples, without a strict definition of what was considered an IMI. Risk ratio for IMI did not differ between treated and untreated heifers when comparing the 2 groups of studies ($P = 0.93$). Definition of IMI was not used in any subsequent analyses, or considered for stratification.

Effectiveness of Treatment

Overall Effect Size. The overall RR of udder disease of any sort in treated compared with untreated heifers was 0.60 (95% CI: 0.47 to 0.67), demonstrating a reduction in udder diseases after calving in treated compared with untreated heifers ($P < 0.0001$). Effect size estimates from abstracts/proceedings tended to be slightly lower and had more variance than estimates from full-text manuscripts ($P = 0.08$; Figure 2), but

their inclusion did not have an effect on overall effect size (RR = 0.60 for full-text manuscripts alone; RR = 0.57 for a combined meta-analysis). All further analyses were conducted on results from both full-text manuscripts and abstracts/conference proceedings without stratification.

Effect Size by Outcome Type. The majority of records ($n = 34$) used IMI as the outcome, whereas 15 records assessed CM and 2 assessed SCM. Effect size estimates for CM tended to be lower than those for IMI

Figure 1. Flow diagram of the manuscript selection process. ADSA = American Dairy Science Association; NMC = National Mastitis Council.

Table 1. Study characteristics of the 62 included studies¹

Reference	Country	Heifer breed	Intervention type					Time of intervention application (d before calving)	Outcome type				Outcome measurement time (DIM)			Pathogens studied ²		
			AM	TSE	TD	Other	VAC	COM	CM	IMI	SCM	CR				Cont.	CNS	Env.
Acuña and Chertcoff (2003) ^{3,4}	Argentina	Holando Argentino	✓					45	✓				3					
Acuña et al. (2015) ³	Argentina	Holando Argentino						60	✓				30					
Acuña Pasqualina et al. (2006) ³	Argentina	Argentino Holstein		✓				12		✓			3–6		✓			✓
Alaniz et al. (2013) ^{3,4}	US	Mixed Holstein	✓				✓	75	✓				21					✓
Ataee et al. (2009)	Iran	Mixed	✓					10–14				✓	3		✓			
Barto and Bush (1978)	US	Mixed	✓					10–14		✓			3					
Bassel et al. (2003) ^{3,4}	Canada	Mixed			✓					✓			1					✓
Bastan et al. (2010)	Turkey	Holstein	✓					45		✓			10		✓			✓
Borm et al. (2006)	US/Canada	—	✓					10–21		✓			4		✓			✓
Bowers et al. (2006)	US	Mixed			✓			Starting at 21		✓			Up to 60					
Bray et al. (1989) ³	US	—	✓					21		✓		✓	1					
Bryan and Friton (2005)	New Zealand	—	✓					0	✓				4					
Bryan and Taylor (2009)	New Zealand	—	✓					0	✓				4					
Bush et al. (1977)	US	Mixed			✓			14		✓			4					
Compton and McDougall (2008) ³	New Zealand	—				✓		0	✓				4–21					
Compton et al. (2014)	New Zealand	Mixed	✓					21	✓	✓			3 for IMI; 30 for CM					
Contreras et al. (2013)	US	—	✓					12–18		✓			4		✓			✓
Edinger et al. (2000)	Germany	Holstein			✓			Starting at 15	✓	✓			4		✓			✓
Galphin and Patrick (1996) ³	US	Holstein				✓		300	✓				0					
Gentilini et al. (2012)	Brazil	Mixed					✓	60	✓	✓			7 for IMI; 100 for CM		✓			✓
Gilbert (2014) ³	Wales	—		✓				60	✓	✓			30					
Girardo et al. (1997)	Argentina	Holstein					✓	56	✓	✓	✓		15		✓			
Han and Park (1999) ³	Korea	—					✓	—	✓	✓	✓		—					
Hovareshti et al. (2007)	Iran	Holstein	✓					7–14				✓	7–14		✓			✓
Jimenez et al. (2008) ³	Spain	—	✓					60		✓			7					
Lopez-Benavides et al. (2009)	New Zealand	—			✓			Starting at 21		✓			14		✓			✓
Lopez-Benavides et al. (2006)	New Zealand	—			✓			Starting at 21		✓			14					✓
Matthews et al. (1988)	US	Mixed		✓				14		✓			8		✓			✓
McCarthy et al. (2005) ^{3,4}	US	—	✓					21		✓			5					

Continued

Table 1 (Continued). Study characteristics of the 62 included studies¹

Reference	Country	Heifer breed	Intervention type					Time of intervention application (d before calving)	Outcome type				Outcome measurement time (DIM)	Pathogens studied ²		
			AM	TSE	TD	Other	VAC		COM	CM	IMI	SCM		CR	Cont.	CNS
McDougall et al. (2008)	New Zealand		✓	✓				✓		✓			10	✓	✓	✓
McDougall et al. (2005) ³	New Zealand	Mixed	✓	✓				✓		✓			4 for IMI; 14 for CM			
Middleton et al. (2005)	US	Holstein	✓					10–14		✓		✓	7–28	✓	✓	✓
Newton and Chambers (2014) ³	New Zealand	—		✓				28	✓				—			
Lopez-Benavides et al. (2006)	New Zealand	—			✓			Starting at 21		✓			14			✓
Edinger et al. (2000)	Germany	Holstein			✓			Starting at 15	✓	✓			4	✓	✓	✓
Galphin and Patrick (1996) ³	US	Holstein				✓		300	✓				0			
Gentilini et al. (2012)	Brazil	Mixed					✓	60	✓	✓			7 for IMI; 100 for CM	✓		✓
Gilbert (2014) ³	Wales	—		✓				60	✓	✓			30			
Giraud et al. (1997)	Argentina	Holstein					✓	56	✓	✓	✓		15	✓		
Han and Park (1999) ³	Korea	—					✓	—	✓	✓	✓		—			
Hovareshti et al. (2007)	Iran	Holstein	✓					7–14				✓	7–14	✓		✓
Jimenez et al. (2008) ³	Spain		✓					60		✓		✓	7			
Lopez-Benavides et al. (2009)	New Zealand	—			✓			Starting at 21		✓			14	✓		✓
Lopez-Benavides et al. (2006)	New Zealand	—			✓			Starting at 21		✓			14			✓
Matthews et al. (1988)	US	Mixed		✓				14		✓			8	✓	✓	✓
McCarthy et al. (2005) ^{3,4}	US		✓					21		✓			5			
McDougall et al. (2008)	New Zealand		✓	✓				21		✓			10	✓		✓
McDougall et al. (2005) ³	New Zealand	Mixed	✓	✓				40	✓	✓			4 for IMI; 14 for CM			
Middleton et al. (2005)	US	Holstein	✓					10–14		✓		✓	7–28	✓	✓	✓
Newton and Chambers (2014) ³	New Zealand	—		✓				28	✓				—			
Nickerson and Boddie (1990) ^{3,4}	US	—				✓		—		✓			—	✓		✓
Nickerson et al. (1994) ^{3,4}	US	—				✓		—		✓			—	✓	✓	✓
Nickerson et al. (1999)	US	Jersey					✓	540		✓			0	✓	✓	✓
Nickerson et al. (2008)	US	Holstein				✓		365		✓			0	✓	✓	✓
Norman et al. (2003) ^{3,4}	US	Mixed	✓					14		✓			14	✓	✓	✓

Continued

Table 1 (Continued). Study characteristics of the 62 included studies¹

Reference			Country			Heifer breed	Intervention type					Time of intervention application (d before calving)	Outcome type				Outcome measurement time (DIM)		Pathogens studied ²		
							AM	TSE	TD	Other	VAC		COM	CM	IMI	SCM	CR	Cont.	CNS	Env.	
Oliver et al. (1992)	US	Jersey	✓							7	✓				7	✓	✓	✓			
Oliver et al. (1997a)	US	Jersey	✓							14	✓				3	✓		✓			
Oliver et al. (1997b) ^{3,4}	US	Mixed	✓							0	✓				0						
Oliver et al. (2003)	US	Jersey	✓							7	✓				3	✓	✓	✓			
Oliver et al. (2004)	US	Mixed	✓							14	✓				7, 14, 30	✓	✓	✓			
Oliver et al. (2007)	US	Mixed	✓							0	✓				30	✓	✓	✓			
Owens et al. (2001)	US	Jersey	✓							1st, 2nd, and 3rd trimesters	✓				0	✓					
Parker et al. (2007b)	New Zealand	—		✓					✓	30	✓				4	✓	✓	✓			
Parker et al. (2008)	New Zealand	—	✓	✓					✓	30	✓				5	✓	✓	✓			
Passchyn et al. (2013)	Belgium	—	✓							14	✓				3	✓	✓	✓			
Pellegrino et al. (2008)	Argentina	Holando Argentino					✓			Prepregnancy	✓				51	✓					
Pellegrino et al. (2010)	Argentina	Holando Argentino					✓			30	✓				21	✓					
Pol et al. (2015) ³	Argentina	—	✓							15	✓				50			✓			
Robertson et al. (2017) ⁴	New Zealand	—		✓						30	✓	✓	✓	✓	1 for IMI; 30 for CM; 56 for SCM	✓	✓				
Roger and Jiménez (2013) ^{3,4}	Spain	Holstein					✓			45	✓				—						
Roy et al. (2007)	Canada	Holstein	✓							9	✓				5	✓	✓	✓			
Sampimon et al. (2009)	Netherlands	Holstein	✓							63	✓	✓			10	✓	✓				
Santos et al. (2004)	US	Holstein					✓			15	✓	✓			28 for IMI; 135 for CM	✓	✓	✓			
Tenhagen et al. (2001)	Germany	Holstein					✓			35	✓	✓			21–28 for IMI; 90 for CM	✓	✓				
Timms et al. (1997) ^{3,4}	US	—			✓					12	✓				3	✓					
Tousová et al. (2011)	Czech Republic	Holstein					✓			45	✓				—						
Trinidad and Nickerson (1989) ^{3,4}	US	—	✓							180–90					0	✓	✓				
Trinidad et al. (1990) ³	—	Jersey	✓							—					0	✓	✓				
Vargas et al. (2016)	Brazil	Holstein	✓				✓		✓	60	✓				190						

¹Type of intervention studied: AM = antimicrobial; TSE = teat sealant; TD = teat dips/sprays; VAC = vaccine; COM = combination of multiple types. Intended application time for intervention was based on expected parturition date; actual time relative to calving was variable. Outcomes reported on the following: CM = clinical mastitis; SCM = subclinical mastitis; CR = cure rate of existing infections.

²Presented if the study differentiated infectious pathogens. Cont. = contagious; Env. = environmental.

³Inclusion and analysis based on abstract or conference proceedings.

⁴Not included in meta-analysis.

Table 2. Risk of bias as assessed by Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) guidelines (Hooijmans et al., 2013) from full-text manuscripts included in systematic review (n = 40)¹

Reference	Selection bias			Detection and performance bias			Attrition bias	Reporting bias
	Allocation concealment	Allocation implementation	Sequence generation	Baseline characteristics	Blinding	Random housing assessment	Incomplete outcome data	Selective outcome reporting
Atace et al. (2009)		✓		✓				✓
Barto and Bush (1978)		✓		✓				✓
Bastan et al. (2010)		✓		✓			✓	✓
Borm et al. (2006)		✓	✓	✓			✓	✓
Bowers et al. (2006)		✓		✓				✓
Bryan and Friton (2005)								✓
Bryan and Taylor (2009)		✓		✓				✓
Bush et al. (1977)		✓		✓			✓	✓
Compton et al. (2014)		✓	✓	✓			✓	✓
Contreras et al. (2013)		✓		✓			✓	✓
Edinger et al. (2000)		✓		✓			✓	✓
Gentilini et al. (2012)		✓		✓				✓
Giraud et al. (1997)		✓		✓				✓
Hovareshti et al. (2007)		✓		✓			✓	✓
Lopez-Benavides et al. (2009)		✓		✓				✓
Lopez-Benavides et al. (2006)		✓		✓			✓	✓
Matthews et al. (1988)		✓		✓				✓
McDougall et al. (2008)		✓		✓		✓		✓
McDougall et al. (2005)		✓		✓				✓
Middleton et al. (2005)		✓	✓	✓				✓
Nickerson et al. (1999)	✓							✓
Nickerson et al. (2008)							✓	✓
Oliver et al. (1992)	✓	✓		✓				✓
Oliver et al. (1997a)		✓		✓				✓
Oliver et al. (2003)		✓		✓				✓
Oliver et al. (2004)		✓		✓			✓	✓
Oliver et al. (2007)		✓		✓			✓	✓
Owens et al. (2001)		✓		✓			✓	✓
Parker et al. (2007b)		✓		✓			✓	✓
Parker et al. (2008)		✓		✓	✓			✓
Passchyn et al. (2013)	✓	✓		✓				✓
Pellegrino et al. (2008)	✓	✓		✓				✓
Pellegrino et al. (2010)			✓	✓				✓
Robertson et al. (2017)				✓				✓
Roy et al. (2007)	✓	✓		✓				✓
Sampimon et al. (2009)	✓	✓		✓				✓
Santos et al. (2004)	✓	✓		✓				✓
Tenhagen et al. (2001)			✓					✓
Toušová et al. (2011)					✓			✓
Vargas et al. (2016)	✓						✓	✓

¹A check mark indicates that the feature was thoroughly described and minimized risk of bias in that area.

($P = 0.07$), but tended to be higher than those for SCM ($P = 0.06$; Table 4). For subsequent meta-analyses and regressions, all outcomes other than cure rate were combined through a random-effects term included for outcome type within studies to account for any within-study correlation. Risk of cure of existing IMI following precalving treatment with antimicrobials was higher than spontaneous cure with no treatment [RR = 1.63 (95% CI: 1.19 to 2.25); $P = 0.003$].

Effect Size by Therapy Type and Pathogen Type. A meta-regression on treatment type using CM, SCM, and IMI as outcomes resulted in a difference in effectiveness among treatment types (Table 5). Teat

sealants and combination therapies were the most effective treatments ($P < 0.0001$), although antimicrobials, vaccines, and other treatments still reduced risk of CM, SCM, or IMI compared with untreated controls ($P < 0.0001$). Teat disinfection before calving did not reduce udder disease compared with untreated controls. Records not included in the meta-analysis also reported similar patterns—antimicrobials were effective at preventing CM and IMI, as well as curing existing IMI. Farms observed longitudinally had decreasing prevalence of IMI and incidence of CM around calving after incorporating precalving antimicrobial treatment into heifer management (Acuña and Chertcoff, 2003).

Table 3. Outcome definitions in full-text manuscripts included in the systematic review (n = 40)

Reference	Outcomes defined ¹	Outcome definition ²
Ataee et al. (2009)		Culturing protocol only [National Mastitis Council (NMC) guidelines]
Barto and Bush (1978)		Culturing protocol only (NMC guidelines)
Bastan et al. (2010)		Culturing protocol only (NMC guidelines)
Borm et al. (2006)		Culturing protocol only (NMC guidelines)
Bowers et al. (2006)	T	MAS-D-TEC test for mastitis (SCC via conductivity)
Bryan and Friton (2005)	P	Clinical mastitis—specific signs not described
Bryan and Taylor (2009)	T	Clinical mastitis—described signs and symptoms
Bush et al. (1977)		Culturing protocol only (NMC guidelines)
Compton et al. (2014)	T	Culturing protocol (NMC guidelines), >200 cfu/mL for IMI
Contreras et al. (2013)	T	Culturing protocol (NMC guidelines), >500 cfu/mL for IMI
Edinger et al. (2000)	P	Culturing protocol provided for IMI; changes in secretion and veterinary treatment for CM
Gentilini et al. (2012)	P	Culturing protocol (NMC guidelines) for IMI; SCC >250,000 cells/mL for SCM; clinical signs for CM
Giraud et al. (1997)	T	Primary pathogen cultured and SCC, >250,000 cells/mL for IMI
Hovareshti et al. (2007)		Culturing protocol defined for <i>Staphylococcus aureus</i>
Lopez-Benavides et al. (2006)	P	Culturing protocol for <i>Streptococcus uberis</i> and CNS; clinical signs for CM
Lopez-Benavides et al. (2009)	T	Culturing protocol defined for <i>S. uberis</i> , ≥100 cfu/mL for IMI
Matthews et al. (1988)	T	Duplicate quarter samples with same pathogen for IMI; clinical signs for CM
McDougall et al. (2005)		Clinical signs for CM, but signs/symptoms not described
McDougall et al. (2008)	T	Same pathogen isolated from sequential or parallel milk samples for IMI
Middleton et al. (2005)	T	≤2 species cultured, and same species in 2 sequential samples for IMI; SCC reported; California Mastitis Test used for SCM
Nickerson et al. (1999)		Culturing protocol only (NMC guidelines)
Nickerson et al. (2008)		Culturing protocol only
Oliver et al. (1992)	T	Same pathogen isolated at 2 sequential samplings
Oliver et al. (1997a)		Culturing protocol only (NMC guidelines)
Oliver et al. (2003)		Culturing protocol only (NMC guidelines)
Oliver et al. (2004)	T	Culturing protocol (NMC guidelines), ≥100 cfu/mL for IMI
Oliver et al. (2007)	T	Culturing protocol (NMC guidelines), ≥100 cfu/mL for IMI
Owens et al. (2001)		Culturing protocol only
Parker et al. (2007b)	T	≥300 cfu/mL of same species in sequential samples for IMI
Parker et al. (2008)	T	≥300 cfu/mL of same species in parallel samples for IMI
Passchyn et al. (2013)	T	≥100 cfu/mL for IMI
Pellegrino et al. (2008)		Culturing protocol only (NMC guidelines)
Pellegrino et al. (2010)	T	Presented cfu from samples
Robertson et al. (2017)	T	≥500 cfu/mL for IMI; ≥100 cfu/mL and clinical signs for CM; ≥120,000 cells/mL for SCM
Roy et al. (2007)	T	Different IMI definitions by species, very thoroughly detailed
Sampimon et al. (2009)	T	≥100 cfu/mL for major pathogen IMI, ≥500 cfu/mL for major pathogen IMI
Santos et al. (2004)		Culturing protocol only (NMC guidelines)
Tenhagen et al. (2001)	P	Culturing protocol only for IMI; clinical changes in secretion for CM; reported SCC
Toušová et al. (2011)	P	Clinical mastitis, specific signs not described
Vargas et al. (2016)	T	≤2 species cultured, ≥100 cfu/mL for <i>S. aureus</i> and <i>Streptococcus agalactiae</i> IMI, ≥1,000 cfu/mL for other species

¹T = definitions were thoroughly described; P = definitions were partially described.

²SCM = subclinical mastitis; CM = clinical mastitis.

Other effective management strategies were the use of physical intramammary devices (Nickerson and Boddie, 1990), implementing fly control for heifers on pasture (Nickerson et al., 1994), and earlier removal of calves after calving to prevent suckling (Compton and McDougall, 2008).

Although studies differentiated culture results to varying levels of detail, pathogens were grouped into 3 categories: contagious (*Staphylococcus aureus*, *Streptococcus agalactiae*), environmental (*Escherichia coli*, non-*agalactiae* streptococci), and CNS. Subgroup analyses including meta-regression on treatment type were

conducted using the 3 categories as outcomes (Table 6). Antimicrobials, teat sealants, and vaccines all presented similar reductions (RR = 0.51, 0.40, and 0.56, respectively) in contagious pathogen CM and IMI compared with untreated controls. Only 1 study reported on teat dips/sprays (Edinger et al., 2000) and 1 on combination therapies (McDougall et al., 2008); in both studies, the 2 therapies did not change occurrence of CM or IMI compared with controls. Manuscripts not included in the pooled estimate also reported a reduced incidence of CM and IMI by contagious pathogens when heifers received precalving treatment with antimicrobials, and

Figure 2. Forest plot of effect size estimates from all ($n = 48$) studies included in the meta-analysis assessing effectiveness of precalving therapy at improving postcalving udder health in dairy heifers. Size of boxes reflects relative size of effect estimate; diamonds represent pooled estimates within strata. CM = clinical mastitis; new IMI = IMI that was not present precalving; SCM = subclinical mastitis (defined as elevated SCC).

Table 4. Meta-regression of records included in a systematic review precalving therapies for improving udder health in primiparous dairy heifers on the outcome used to assess udder health

Outcome type	Risk ratio ¹ (95% CI)	No. of records	Heterogeneity ²
Clinical mastitis	0.50 (0.39–0.64)	15	$I^2 = 0.85$
IMI	0.61 (0.51–0.74)	34	$Q = 343.5$
Subclinical mastitis ³	0.30 (0.18–0.50)	2	$P < 0.0001$

¹Ratio of risk of infection in treated heifers to risk of infection in untreated controls.

²Heterogeneity refers to residual heterogeneity from meta-regression model attributed to differences among studies. I^2 = statistic to quantify the amount of variation due to study heterogeneity; Q = Cochran Q statistic for significance.

³Subclinical mastitis defined as a heifer having an elevated SCC (measured by electrical conductivity of milk, the California mastitis test or a quantified SCC >120,000 or 250,000 cells/mL).

also when fly control was implemented for precalving heifers on pasture.

Teat sealants and combination therapies reduced occurrence of CM and IMI with environment pathogens over untreated controls (RR = 0.27 and 0.25, respectively; Table 6). Antimicrobials, teat disinfection, and vaccines did not have any benefit compared with untreated controls, but 2 studies not included in the meta-analysis (Nickerson et al., 1994; Tenhagen et al., 2001) reported a reduction of clinical signs during *E. coli* IMI following precalving vaccination. Fly control for heifers on pasture reduced *Streptococcus* spp. IMI. Studies reporting on CM and IMI where CNS were isolated reported a reduction compared with controls in heifers treated with antimicrobials, teat-sealants, and combination therapies, with combination therapies having the greatest reduction (RR = 0.34), and similar conclusions were drawn from studies not included in the meta-analysis. Vaccines did not reduce CM and IMI with CNS occurrence compared with untreated controls. Due to only single studies assessing other therapies and teat dips/sprays, pooling was not possible, but neither

demonstrated a reduction in occurrence of CM and IMI with CNS compared with untreated controls.

Meta-Analysis Statistics

Heterogeneity. Significant heterogeneity was present in all analyses. Mixed effects meta-analysis using all records with random effects for outcome type and intervention type had an overall $I^2 = 0.87$ ($Q = 439.28$, $P < 0.0001$). Introducing outcome type as a moderator did not reduce heterogeneity noticeably; $I^2 = 0.85$ ($Q = 343.54$, $P < 0.0001$), nor did conducting a meta-regression using outcome type affect heterogeneity; $I^2 = 0.85$ ($Q = 369.78$, $P < 0.0001$). Sub-group analyses by pathogen type led to reductions in residual heterogeneity, except for the CNS analysis: contagious pathogen analysis $I^2 = 0.29$ ($Q = 39.36$, $P = 0.03$); environmental pathogen analysis $I^2 = 0.57$ ($Q = 71.77$, $P < 0.0001$); CNS analysis $I^2 = 0.88$ ($Q = 212.71$, $P < 0.0001$).

Publication Bias. Based on visual assessment of the funnel plot of standard error compared with the effect size estimate (Figure 3) from each study, we inferred

Table 5. Meta-regression of records included in a systematic review of precalving treatment for improving udder health in primiparous dairy heifers on the type of treatment assessed in the study

Intervention type ¹	Risk ratio ² (95% CI)	No. of records	Heterogeneity ³
Antimicrobial	0.58 (0.46–0.74)	27	
Teat sealant	0.40 ⁴ (0.30–0.52)	7	$I^2 = 0.85$
Teat dips/spray	1.50 ⁴ (0.74–3.1)	3	$Q = 369.78$
Vaccine	0.55 (0.35–0.86)	12	$P < 0.0001$
Combination	0.34 (0.25–0.45)	4	
Other	0.50 (0.11–1.1)	4	

¹Antimicrobial includes antibiotics as well as antiseptic compounds such as chlorhexidine. Combinations in included studies consisted of teat sealants and antimicrobials; vaccines and antimicrobials; teat sealants, vaccines and antimicrobials. Other includes insecticides, and management practices such as prepartum milking (Appendix Table A1).

²Ratio of risk of infection in treated heifers to risk of infection in untreated controls.

³Residual heterogeneity from meta-regression model attributed to differences in studies. I^2 = statistic to quantify the amount of variation due to study heterogeneity; Q = Cochran Q statistic for significance.

⁴Risk ratio different ($P < 0.05$) from studies assessing antimicrobial effectiveness.

that some asymmetry due to missing studies showing an increased risk following treatment with small sample sizes (bottom right quadrant). Egger's regression small study effects indicated a possibility of publication bias as an increasing standard error was associated with a larger effect size estimate ($P < 0.0001$; Figure 4).

DISCUSSION

Management of mastitis on dairy farms often starts after the heifer's first calving, too often resulting in less than optimal udder health for first lactation heifers (De Vlieghe et al., 2012). Many housing and management practices associated with udder health of older cows are also associated with improved udder health in primiparous heifers (De Vlieghe et al., 2012), but the level of implementation in nonlactating heifers is unknown. Prophylactic treatments of the udder before calving (e.g., antimicrobial dry cow therapy or using internal teat sealants) are widely adopted for use in the lactating herd (e.g., Olde Riekerink et al., 2010), but these treatments are less commonly used in primipa-

rous heifers. This systematic review and meta-analysis of studies assessing effectiveness of precalving therapies for improving postcalving udder health in heifers demonstrated an overall benefit to udder health in heifers when using any kind of precalving prophylactic treatment. Treatments varied in both cost to purchase as well as costs and labor associated with application, so an understanding of the effectiveness of treatments is important to inform management decisions. Effectiveness of treatments varied considerably and was also dependent on the type of bacteria causing the IMI.

This is the first review to meta-analyze results from studies of multiple types of precalving therapies in primiparous heifers. Recent reviews have considered heifer mastitis as a whole (De Vlieghe et al., 2012), antimicrobial treatment to control heifer mastitis (Nickerson, 2009), vaccination efficacy against staphylococcal infections in all lactating cattle (Middleton et al., 2009), and effectiveness of treatments other than conventional antimicrobials against CM in all lactating cows (Francoz et al., 2017). The distribution of pathogens in IMI of heifers differs from older cows (Piepers et al., 2009;

Table 6. Meta-regression of records included in a systematic review of precalving treatment for improving udder health in primiparous dairy heifers on the type of treatment assessed in the study, stratified by type of pathogen

Pathogen and intervention type ^{1,2}	Risk ratio ³ (95% CI)	No. of records	Heterogeneity ⁴
Contagious			
Antimicrobial	0.41 (0.34–0.77)	15	
Teat sealant	0.40 (0.22–0.72)	4	$I^2 = 0.29$
Teat dips/spray	1.48 ⁵ (0.52–4.27)	1	$Q = 39.36$
Vaccine	0.56 (0.34–0.92)	8	$P = 0.025$
Combination	0.93 ⁵ (0.33–1.26)	1	
Environmental			
Antimicrobial	0.58 (0.46–0.74)	18	
Teat sealant	0.27 ⁶ (0.15–0.49)	5	$I^2 = 0.57$
Teat dips/spray	0.56 (0.17–1.8)	3	$Q = 71.77$
Vaccine	0.78 (0.20–2.98)	4	$P < 0.0001$
Combination	0.25 ⁶ (0.13–0.50)	2	
CNS			
Antimicrobial	0.51 (0.35–0.76)	15	
Teat sealant	0.46 (0.29–0.73)	4	$I^2 = 0.89$
Teat dips/spray	1.48 (0.31–7.03)	2	$Q = 212.71$
Vaccine	0.89 (0.38–2.07)	4	$P < 0.0001$
Combination	0.34 ⁶ (0.21–0.56)	2	
Other	0.80 ⁵ (0.19–3.34)	1	

¹Contagious pathogens include *Staphylococcus aureus* and *Streptococcus agalactiae*. Environmental pathogens include *Escherichia coli* and non-*agalactiae* streptococci.

²Antimicrobial includes antibiotics as well as antiseptic compounds such as chlorhexidine. Combinations in included studies consisted of teat sealants and antimicrobials; vaccines and antimicrobials; and teat sealants, vaccines, and antimicrobials. Other includes insecticides and management practices such as prepartum milking (Appendix Table A1).

³Ratio of risk of infection in treated heifers to risk of infection in untreated controls.

⁴Residual heterogeneity from meta-regression model attributed to differences in studies. I^2 = statistic to quantify the amount of variation due to study heterogeneity; Q = Cochrane Q statistic for significance.

⁵Intervention type within pathogen type stratum did not have enough records for meta-analysis. Risk ratio presented was calculated from the single record within that stratum.

⁶Risk ratio was different ($P < 0.05$) from studies assessing antimicrobial effectiveness.

Supré et al., 2011; Naqvi et al., 2018), as well as their immune response to infection (de Haas et al., 2002, 2004), so results from studies on older lactating cattle may not be generalizable to heifers. A synthesis of studies aimed at heifers in particular is therefore necessary when trying to implement more targeted management strategies. Findings from this review were in line with most individual studies and reviews on lactating cows that demonstrated a net positive benefit of any type of treatment for improving udder health. Previous reviews have demonstrated differential effectiveness of antimicrobials (Barkema et al., 2006; Nickerson, 2009) and teat sealants (Crispie et al., 2004), based on the pathogen (when used in lactating cows), whereas vaccine development tends to focus on a specific type of pathogen such as *S. aureus* (Middleton et al., 2009) or *E. coli* (Enviracor and StartVac). Compared with the present review focusing on heifers specifically, variation in treatment effectiveness by pathogen type was similar to previous reports from studies focusing on older cows.

As mentioned above, management of nonlactating heifers is less than ideal, with practices such as comingling with older cattle and moving cows close to calving and increasing stress being common on most dairy

farms (Parker et al., 2007a; McDougall et al., 2009). This could be an important reason why treatments were effective in improving udder health. It is therefore likely that effectiveness of treatments, particularly antimicrobial treatment, will be less on farms that devote more attention to nonlactating heifers.

Although the effectiveness of antimicrobials was consistent across gram-positive pathogens, their use needs to be balanced with concerns about the rising prevalence of antimicrobial resistance (Barkema et al., 2015; Tang et al., 2017). To prevent overuse, the NMC's 10-point plan for heifer mastitis management recommends prepartum antibiotic treatment only under very specific conditions. Other treatments can be just as effective, if not more so, than antimicrobials in heifers, and current research is beginning to focus on their effectiveness in older cows (Francoz et al., 2017; Ruiz et al., 2017). Antimicrobial treatment of nonlactating heifers improved udder health more than other treatments when contagious (gram-positive) bacteria caused udder health problems after calving. In contrast, teat sealants and combination therapies were more effective than antimicrobial treatment when environmental pathogens were involved, suggesting that antimicrobials

Figure 3. Egger's regression for small-study effects in a meta-analysis of studies assessing effectiveness of precalving therapies at improving udder health in dairy heifers plotted as the natural log of the risk ratio from a given study against the estimate of variance from the same study. Vertical bars indicate 95% confidence intervals at the intercept (variance = 0; largest possible studies).

are not always the best option. It is therefore important when udder health problems after calving are an issue on a farm and antimicrobial treatment is considered, to culture quarter milk of heifers after calving.

In addition to selecting the best treatment for each farm, personnel must also be properly trained in its application. For example, improper application of intramammary treatments, either causing trauma by inserting the cannula too far or using poor hygiene, can actually increase a quarter's risk of infection (du Preez, 2000). It is difficult to quantify the effect of iatrogenic infections in studies included in this review. Although some studies mentioned disinfection/preparatory procedures, no studies detailed training for personnel in treatment application. Safety of treatment application and ease of application may also vary by treatment

type and should be considered when selecting a treatment. For example, feed supplements are much easier and safer to administer compared with prepartum intramammary infusions of antimicrobials. A comparison of systemic antibiotic treatment with those applied intramammarily could further inform decisions if they differ in effectiveness or ease of application. This comparison was not possible for the present review because intramammary infusions of antibiotics ($n = 31$) greatly outnumbered systemically applied antimicrobials ($n = 6$), resulting in a lack of power due to the unbalanced groups. Similarly, a comparison of antimicrobial treatments based on classes may have resulted in differences in effectiveness; however, because the vast majority of antibiotic products used in the included studies were macrolides or macrolide combinations, lack of power

Figure 4. Funnel plot to assess asymmetry and publication bias in meta-analysis of studies assessing effectiveness of precalving therapies at improving udder health in dairy heifers. Relative risk = 1 indicates equal risk of udder disease in treated and untreated animals (no effect of treatment). Darkest triangle indicates area of 90% confidence, light gray triangle indicates area of 95% confidence, and white triangle indicates area of 99% confidence.

was again an issue. Nickerson (2009) provided a more comprehensive and in-depth review specific to antimicrobial treatments for heifers.

In comparing all types of treatments for heifers, this review faced the challenge of significant between-study variability, not only in treatments used, but also in other aspects of study design and setting. Although we tried to explore heterogeneity through subgroup analyses, many other study characteristics still varied considerably to allow for valid subgroup analyses (e.g., timing of treatment application, study location, timing of data collection, and geographic location). As a result, significant residual heterogeneity was present even after stratification. Although the significant between-study variability may cast some doubt on the precision of the pooled results, the overall message remains unchanged, as the direction of the effect remained consistent among studies, even if the effect size did not. Specifically, precalving treatment of heifers improved udder health after calving, and the degree of improvement varied by treatment type and pathogen. Variation in characteristics such as breed, geographic location, and sampling season or timing of treatment application and specific treatment are inherently linked, making it difficult to be consistent across studies from various countries and studying a variety of treatments.

An assessment of the risk of bias focuses on characteristics of study design and reporting in a way that is less dependent on factors outside of the researchers' control. The assessment using the SYRCLE tool (Hooijmans et al., 2014) highlighted some areas with significant risk of bias. Although randomization of housing and outcome assessment order are less likely to contribute to detection or performance bias in livestock studies (SYRCLE was designed for laboratory trials using animals), blinding of both treatment allocator or outcome assessor was not mentioned in most of the studies, nor was allocation concealment. For some outcomes (e.g., SCM or IMI), blinding may be inherent, because milk samples are collected by farm personnel and the outcome (SCC or bacterial culture) is assessed by laboratory technicians. Despite the possible inherent blinding of outcome assessors, lack of blinding of the treatment allocator could still contribute to detection bias, and both types of blinding should be reported, with justification provided if blinding was not done. Similarly, randomization and allocation sequence generation were only described in 6 studies, and presented a possible source of selection bias. Clear outcome definitions, especially for IMI, were also inconsistently reported; therefore, different definitions could contribute to residual heterogeneity. Although just defining IMI did not change effect size from studies that did not, looking at specific definitions would likely

provide different results because each would have its own operating characteristics (Dohoo et al., 2011a,b). This likely is not a reflection of study quality, but more on inconsistency in reporting standards for studies on livestock. The Reporting Guidelines for Randomized Controlled Trials for Livestock (**REFLECT**; O'Connor et al., 2010) were developed to address this issue, but are still not widely implemented. Although this review reports on a substantial number of studies, an updated review in the future once the REFLECT statement is more widely implemented would likely detect more consistency among studies, leading to more precise pooled estimates of protective effect size.

As the first review to focus on the effectiveness of all existing precalving therapies at improving udder health in primiparous dairy heifers, the present study effectively synthesized results from a large number of studies. Heterogeneity was explored through subgroup analyses and meta-regression, focusing primarily on the main goals of the study: understanding variation in therapy success by treatment type and pathogen. Despite this exploration of heterogeneity, even stratification by both treatment type and pathogen still yielded significant residuals and unexplained study variability. As discussed, this was likely a reflection of differences in study settings, design characteristics, and reporting. Although this degree of study variability affected precision of pooled estimates, based on the direction of the effect, there was a positive effect on udder health when precalving treatments were applied. Publication bias in a review is another aspect that could affect the reliability of findings, and Egger's regression indicated that there were significant small-study effects in the review. Based on examination of the funnel plot, no studies had a negative effect of precalving treatment and used a small sample size. Although this may be a reflection of publication bias, in this case it is more clearly reflective of the true heterogeneity in the included studies, attributed primarily to treatment intensity and differences in underlying risk (Egger et al., 2008). Treatments varied considerably in timing of application and dosage, both of which would increase variability. Differences in geographical location, affecting housing, nutrition and other management factors, and animal breed could have contributed to differences in regional risks for mastitis, or differences in susceptibilities of heifers to disease. Asymmetry in the funnel plot may also be an artifact (Egger et al., 2008), as the majority of interventions under study were very unlikely to increase the risk of udder diseases over untreated heifers, making it very unlikely that small studies would show harm to udder health by precalving treatments. Although statistical evidence suggested publication bias, this was more

likely a reflection of study variability as quantified by the residual heterogeneity rather than a bias in study selection.

CONCLUSIONS

In this first review of precalving treatments for improving udder health in primiparous dairy heifers, we assessed the findings of 60 studies, 48 of which were meta-analyzed for pooled estimates. Overall, any type of precalving therapy improved udder health by reducing occurrence of IMI, CM, and SCM compared with untreated controls. Variation in success by therapy type demonstrated that studies assessing antimicrobials, teat sealants, vaccines, and combination therapies demonstrated a significant reduction in the risk of udder disease, whereas those using teat dips/sprays or other types of therapy did not reduce risk compared with untreated heifers. There was also significant variation by pathogens that the studies examined: antimicrobials, teat sealants, vaccines, and combination therapies were effective at reducing risk of udder disease caused by contagious pathogens; antimicrobials, teat sealants, and vaccines were effective at reducing risk of udder disease caused by environmental pathogens; and finally, antimicrobials, teat sealants, vaccines, and combinations were effective at reducing udder disease caused by CNS. Once rigid reporting guidelines are more widely implemented by journals and authors, an updated literature review on udder health of dairy heifers should yield more precise pooled estimates, with decreased between-study variability.

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APPENDIX

The following search terms were used in database searches. The MeSH search is integrated in the Web of Science, so the same terms were employed there.

The search terms used were separated into 3 separate components corresponding to the study population, intervention, and outcome type. Each of these was combined with the Boolean operator AND. Subject heading searches were conducted separately from the text search as the available subject headings were not comprehensive.

- 1) To identify studies with a study population looking at heifers and not just all cows grouped together, the search was split into 2 parts (to ensure that the age of animals in the study was appropriate, as well as restrict our search to dairy cows only), which were combined using the Boolean operator AND:
 - a. The terms “dairy” and “milk*” combined with the Boolean operator OR. The corresponding subject headings were “dairy herds,” “dairy cows,” “dairy cattle,” and “milking” combined with the operator OR.
 - b. The terms “heifer*,” “primiparous,” “primigravid,” “nulliparous” combined with the Boolean operator OR. The corresponding subject headings were “heifers” and “bred heifers” combined with the Boolean operator OR.
- 2) To identify studies with the relevant interventions, the search was again split into 2 parts (timing of intervention had to be precalving, as well as specify all types of intervention), which were again combined using the Boolean operator AND:
 - a. The terms “precalving,” “prepartum,” and “calving” combined with the Boolean operator OR. The corresponding subject heading search terms were “calving” and “prepartum period” combined using the operator OR.
 - b. The terms “therap*,” “treat*,” “antimicrob*,” “antibiotic,” and “seal*” combined using the operator OR. The corresponding subject headings were “drug therapy,” “therapy,” “treatment,” and “combination therapy” combined using the Boolean operator OR.
- 3) To identify all possibly relevant outcomes the search terms used were “mastit*,” “infect*,” “clinical mastit*,” “bacteri*,” “inflam*,” “pathogen,” and “microorganism” combined using the Boolean operator OR. The corresponding subject headings used were “mastitis,” “summer mastitis,” “subclinical mastitis,” “mycotic mastitis,” “bovine mastitis,” and “inflammation” combined using the Boolean operator OR.

Table A1. Details on product used, primary therapeutic ingredient(s), method of application, and categorization of intervention for each of the 62 studies¹

Reference	Product(s) used (intervention category)
Acuña and Chertcoff (2003)	Intramammary cloxacillin benzathine (AM)
Acuña et al. (2015)	Enemast dry cow (Merial Argentina), intramammary cloxacillin benzathine and ampicillin trihydrate (AM)
Acuña Pasqualina et al. (2006)	Dry-off (WestfaliaSurge Argentina), external teat sealant (TSE)
Alaniz et al. (2013)	Enviracor J-5 (Pfizer Animal Health), <i>Escherichia coli</i> vaccine (VAC)
Ataee et al. (2009)	Tyloject (Razak Co., Tehran, Iran), intramuscular tylosin (AM)
Barto and Bush (1978)	Cefquinome (Cobactan; Intervet, Boxmeer, the Netherlands), intramuscular cefquinome (AM)
Bassel et al. (2003)	Benzathine cephalirin (Bristol Laboratories, Syracuse, NY), intramammary (AM)
Bastan et al. (2010)	Observational study, comparison of farms using and not using precalving teat dipping (TD)
Borm et al. (2006)	Rilexine 500 (Virbac, France), intramammary cephalixin monohydrate and neomycin (AM)
Bowers et al. (2006)	Cefa-Lak (Fort Dodge Animal Health, Fort Dodge, IA), intramammary cephalirin sodium (AM)
Bray et al. (1989)	Prepartum milking twice daily beginning 3 wk prepartum (other)
Bryan and Friton (2005)	Intramammary penicillin and dihydrostreptomycin (AM)
Bryan and Taylor (2009)	Masticillin (Stockguard Laboratories Ltd., Hamilton, New Zealand), intramuscular procaine penicillin (AM)
Bush et al. (1977)	Mamyzin (Boehringer Ingelheim Vetmedica GmbH), parenteral penethemate hydroiodide (AM)
Compton and McDougall (2008)	Iodine teat dip (TD)
Compton et al. (2014)	Intercept (Bayer, New Zealand Ltd., Manukau City, New Zealand), bismuth subnitrate and chlorhexidine internal teat sealant (COM)
Contreras et al. (2013)	Reducing interval from calving to first milking (other);
Edinger et al. (2000)	SelPor (Ancare, New Zealand), precalving, pour-on selenium feed supplementation (other)
Galphin and Patrick (1996)	Tylosin (Elanco, Greenfield, IN), intramuscular (AM)
Gentilini et al. (2012)	Gelstadip (Pfizer GmbH, Karlsruhe, Germany), iodine teat dip (TD)
Gilbert (2014)	Insecticide impregnated tail tag (other)
Giraud et al. (1997)	Rotatec-J5 <i>E. coli</i> vaccine (VAC)
Han and Park (1999)	Orbeseal (Zoetis Animal Health), internal teat sealant (TSE)
Hovareshti et al. (2007)	Proprietary <i>Staphylococcus aureus</i> vaccine (VAC)
Jimenez et al. (2008)	Proprietary <i>S. aureus</i> vaccine (VAC)
Lopez-Benavides et al. (2009)	Nafpenzal DC (Intervet International, Holland), intramammary sodium nafcillin, procaine benzyl penicillin and dihydrostreptomycin (AM);
Lopez-Benavides et al. (2006)	Tylan (Elanco Animal Health, Eli Lilly), intramammary tylosin (AM);
Matthews et al. (1988)	Baytril (Bayer, Bayer Leverkusen, Germany), subcutaneous enrofloxacin (AM)
McCarthy et al. (2005)	Cepravin VS-intramammary cephalonium (AM)
McDougall et al. (2005)	Teatguard Plus (Ecolab, Hamilton, New Zealand), iodine teat spray (TD)
McDougall et al. (2008)	Teatguard Plus (Ecolab), iodine teat spray (TD)
Middleton et al. (2005)	Latex external teat sealant with germicide (TSE)
Newton and Chambers (2014)	Albadry Plus (Pfizer Inc.), intramammary novobiocin, penicillin G (AM);
Nickerson and Boddie (1990)	Orbeseal (Pfizer Inc.), internal teat sealant (TSE);
Nickerson et al. (1994)	T-Hexx (Hydromer), teat dip (TD)
Nickerson et al. (1999)	Teatseal (Pfizer Animal Health, Auckland, New Zealand), bismuth subnitrate internal teat sealant (TSE);
Nickerson et al. (2008)	Tylan 200 (Elanco Animal Health, Manukau City, New Zealand), intramuscular tylosin (AM)
Norman et al. (2003)	DryFlex (DeLaval, Hamilton, New Zealand), external teat sealant (TSE);
Oliver et al. (1992)	Rumensin ABC (Elanco Animal Health, Manukau City, New Zealand), parenteral monensin (AM)
Oliver et al. (1997a)	Pirsue (Pfizer Inc., Kalamazoo MI), intramammary pirlimycin hydrochloride (AM)
Oliver et al. (1997b)	Teat Seal (Zoetis), internal teat sealant (TSE)
Oliver et al. (2003)	5 intramammary device models (other)
Oliver et al. (2004)	Fly control (other); dry cow antibiotic (AM)
Oliver et al. (2007)	Lysigin (Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO), <i>S. aureus</i> vaccine (VAC)
	Lysigin (Boehringer Ingelheim Vetmedica Inc.), <i>S. aureus</i> vaccine (VAC)
	Today (Bristol Meyers, Evansville, IN), intramammary cephalirin sodium (AM);
	Cefalak, intramammary cephalirin sodium (AM)
	Intramammary sodium cloxacillin; intramammary cephalirin sodium (AM)
	Today (Bristol Meyers), intramammary cephalirin sodium (AM)
	Pirsue (Upjohn Co., Kalamazoo, MI), intramammary pirlimycin hydrochloride (AM);
	Albacillin (Upjohn Ltd., West Sussex, England), intramammary penicillin procaine and novobiocin sodium (AM)
	Intramammary sodium cloxacillin (AM); intramammary cephalirin sodium (AM)
	Albacillin (Pfizer Animal Health, Kalamazoo, MI), intramammary novobiocin sodium and penicillin procaine (AM);
	Pirsue (Pfizer Inc.), intramammary pirlimycin hydrochloride (AM)
	Albacillin (Pfizer Animal Health), intramammary novobiocin sodium and penicillin procaine (AM);
	Pirsue (Pfizer Inc.), intramammary pirlimycin hydrochloride (AM)

Continued

Table A1 (Continued). Details on product used, primary therapeutic ingredient(s), method of application, and categorization of intervention for each of the 62 studies¹

Reference	Product(s) used (intervention category)
Owens et al. (2001)	Tomorrow (Fort Dodge Animal Health), intramammary cephalirin benzathine (AM); Quartermaster (Pharmacia Upjohn, Kalamazoo, MI), intramammary penicillin and streptomycin (AM); Albadry Plus (Pharmacia Upjohn), intramammary penicillin and novobiocin (AM); Micotil (Eli Lilly and Co., Indianapolis, IN), intramammary tilmicosin (AM) Cefasafe (Intervet, Boxmeer, the Netherlands), intramammary cephalonium (AM)
Parker et al. (2007b)	Teat Seal (Pfizer Animal Health, New Zealand Ltd., Auckland, New Zealand), internal bismuth subnitrate teat sealant (TSE)
Parker et al. (2008)	Teat Seal (Pfizer Animal Health, New Zealand Ltd.), internal bismuth subnitrate teat sealant (TSE)
Passchyn et al. (2013)	Tylan 200 (Elanco Animal Health, Manukau City, New Zealand), intramammary tylosin (AM) Mamyzin Stop M (Boehringer Ingelheim GmbH, Ingelheim, Germany), intramuscular penethamate hydroiodide (AM)
Pellegrino et al. (2008)	Proprietary <i>S. aureus</i> vaccine (VAC)
Pellegrino et al. (2010)	Proprietary <i>S. aureus</i> vaccine (VAC)
Pol et al. (2015)	Advocin 180 (Zoetis), parenteral danofloxacin (AM)
Robertson et al. (2017)	TeatSeal (Zoetis, Auckland, New Zealand), internal teat sealant (TSE)
Roger and Jiménez (2013)	StartVac, <i>E. coli</i> and <i>S. aureus</i> vaccine (VAC)
Roy et al. (2007)	Pirsue (Pfizer Animal Health, Kirkland, QC, Canada), intramammary pirlimycin hydrochloride (AM)
Sampimon et al. (2009)	Orbenin Extra Dry Cow (Pfizer, Capelle aan de IJssel, the Netherlands), intramammary cloxacillin (AM)
Santos et al. (2004)	Prepartum milking (other)
Tenhagen et al. (2001)	Proprietary <i>S. aureus</i> vaccine (VAC)
Timms et al. (1997)	Persistent barrier teat dip (TD)
Toušová et al. (2011)	StartVac, <i>E. coli</i> and <i>S. aureus</i> vaccine (VAC)
Trinidad and Nickerson (1989)	Intramammary penicillin and streptomycin (AM)
Trinidad et al. (1990)	Intramammary penicillin and streptomycin (AM)
Vargas et al. (2016)	Albadry Plus (Pfizer Animal Health), novobiocin sodium and procaine penicillin (AM); Enviracor J5 (Pfizer Animal Health), <i>E. coli</i> vaccine (VAC)

¹AM = antimicrobial; TSE = teat sealant; TD = teat dips/sprays; VAC = vaccine; COM = combination of multiple types.