The effect of internal teat sealant products (Teatseal and Orbeseal) on intramammary infection, clinical mastitis, and somatic cell counts in lactating dairy cows: A meta-analysis

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ABSTRACT

The objective of this study was to assess the efficacy of internal teat sealant products containing bismuth subnitrate (Teatseal and Orbeseal; Pfizer Animal Health, West Ryde, Australia) when used alone, or in the presence of antibiotic dry cow therapy (ADCT), before or at drying off on the incidence of new intramammary infections (IMI), clinical mastitis, and milk somatic cell count (SCC) during lactation. The literature search identified 18 English-language publications on the use of Teatseal in dairy cattle. A total of 12 studies with 17 subtrials or comparisons including 13 positive control subtrials (internal teat sealant and ADCT vs. ADCT) and 4 negative control subtrials (internal teat sealant vs. untreated) examining IMI were included in the analysis. Internal teat sealants, alone or in the presence of ADCT, reduced the risk of acquiring new IMI after calving by 25% [risk ratio (RR) = 0.75; 95% confidence interval (CI): 0.67 to 0.83]. Internal teat sealants reduced the risk of IMI by 73% compared with untreated cows (RR = 0.27; 95% CI: 0.13 to 0.55). The results of both meta-analyses of IMI, with positive and negative controls, were heterogeneous [I^2 (a statistic that describes the proportion of total variation in study effect estimates that is due to heterogeneity) = 65.4 and 92.1%]. No farm or cow factors studied significantly contributed to the heterogeneity of the results. A total of 16 studies (21 subtrials), including 14 positive control subtrials and 7 negative control subtrials, examining clinical mastitis were included in the analysis. Internal teat sealants alone and in the presence of ADCT reduced the risk of clinical mastitis after calving in lactating cows by 29% (RR = 0.71; 95% CI: 0.62 to 0.82), and 48% (RR = 0.52; 95% CI: 0.37 to 0.75), respectively. The results of the meta-analysis on clinical mastitis with positive controls were homogeneous (I^2 = 33.6%), whereas the results of studies with negative controls were heterogeneous (I^2 = 60.4%). No farm or

cow factors studied that had sufficient data to evaluate significantly contributed to the heterogeneity of the results. The estimated linear score (LS) of milk SCC after calving in published studies ($n = 3$) and for studies that provided raw data ($n = 2$), was significantly lower for cattle treated with internal teat sealants and ADCT in 3 studies than for cattle treated with internal teat sealants only. The estimated LS of pooled raw data of 3 studies from 32 herds showed that the LS of cows treated with internal teat sealant and ADCT was not significantly different than those treated with ADCT only. This study found that the application of internal teat sealants in the presence of ADCT or the use of internal teat sealants alone at dry off significantly reduced the incidence of IMI and clinical mastitis in lactating dairy cows compared with respective control groups. Further studies are needed to investigate the effect of internal teat sealants on postpartum milk SCC in lactating dairy cows.

Key words: antibiotic dry cow therapy, mastitis, meta-analysis, Teatseal

INTRODUCTION

Clinical and subclinical mastitis are major concerns for dairy producers and cause significant economic losses to the dairy industry. The losses include antibiotic treatment, lower milk yield, decreased fertility, and increased culling or death (Bradley, 2002). Although environmental and host factors are critical to the pathogenesis of mastitis, more than 137 different organisms have been identified as causes of clinical mastitis (Watts, 1988). This diversity of agents is reflected in treatment difficulties and limits the efficacy of prevention programs. Interventions that provide means of reducing the risk of infection and the sequelae to infection, such as elevated SCC, independent of causal organisms, are of great potential benefit.

Treatment of cows with long-acting antibiotics at dry off has been an important element of therapeutic and preventive measures in dairy farms (Bramley and Dodd, 1984). Dry cow therapy has been used successfully for

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the treatment of subclinical mastitis at drying off and to protect the udder against new infections during the early dry period (Browning et al., 1990; Williamson et al., 1995). However, milk loss as a result of withholding periods and concerns over the use of antibiotics that may contribute to an increased risk of antibiotic residues and bacterial resistance (WHO, 1994; Berry and Hillerton, 2002b; Oliver and Murinda, 2012; Ruegg, 2012) are matters that suggest the need for alternate approaches to mastitis control.

The use of internal teat sealants as a preventive measure provides an alternate approach to traditional antibiotic programs. The use of internal teat sealants at drying off mimics the physiological mechanisms that close the teat canal (Meaney, 1977). After drying off, a keratin plug forms naturally in the teat canal and acts as an important defense mechanism against IMI. However, the rate of closure of teat canal varies among cows, and it has been suggested that 50% of teats may still be open 10 d after drying off (Williamson et al., 1995). Methods of achieving an artificial closure have been examined for the prevention of IMI during the past 30 yr (Meaney, 1977; Williamson et al., 1995; Woolford et al., 1998). A new teat sealant containing bismuth subnitrate was developed by Meaney (1977), who showed that this was effective at preventing new dry period infections in an artificial challenge study. A commercial form of a bismuth subnitrate-based product with the presence of short-acting antibiotic was further developed in Ireland (Osmonds Teat Seal; Cross Vetpharm Group Ltd., Dublin, Ireland). Subsequently, Woolford et al. (1998) experimented with a reformulated version of the teat sealant (Teatseal; Bimeda Ltd., Auckland, New Zealand) to assess its potential for the prevention of new IMI and clinical mastitis during the dry period and subsequent lactation.

Both challenge (Meaney, 1977) and field (Woolford et al., 1998) studies reported a significant reduction in the incidence of IMI and clinical mastitis in cows treated with internal teat sealant products containing bismuth subnitrate and in the presence of antibiotic dry cow therapy (**ADCT**) during dry off and lactation (Woolford et al., 1998). Similar studies in the United Kingdom (Huxley et al., 2002), Australia (Runciman et al., 2010), New Zealand (Parker et al., 2007, 2008; Laven and Lawrence, 2008), United States (Godden et al., 2003; Cook et al., 2005), and Canada (Sanford et al., 2006b; Baillargeon and LeBlanc, 2010) demonstrated that the use of internal teat sealants at dry off can be effective in preventing IMI or new IMI and clinical mastitis in the subsequent lactation. Crispie et al. (2004) stated that if internal teat sealants are not used hygienically and according to the manufacturer's instructions, this could lead to mastitis. Therefore, the

incorporation of antibiotics into internal teat sealants may prevent inadvertent contamination during infusion and improves product safety.

The objectives of this study were to assess the efficacy of an internal teat sealant containing bismuth subnitrate, sold as Orbeseal and Teatseal (Pfizer Animal Health, West Ryde, Australia), when used alone or in the presence of ADCT before or at drying off on the incidence of the cumulative number of new IMI and clinical mastitis cases from the time of dry off to 100 to 150 d of lactation, and milk SCC in a period from calving to the first 3 wk of lactation. The term internal teat sealant refers to Orbeseal and Teatseal throughout this paper.

MATERIALS AND METHODS

Literature Search

The literature search used PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>), Google Scholar (<http://scholar.google.com/>), ScienceDirect (<http://www.sciencedirect.com/>), and CAB (<http://www.cabi.org>), contact with workers in the field, records of Pfizer Animal Health, and investigation of references in papers that were identified. The search strategy was to identify papers that contained data on prospective, randomized, controlled experiments examining effects of internal teat sealants, either alone or in the presence of ADCT on the incidence of new IMI and clinical mastitis during the dry period and lactation. Papers identified included 18 English-language publications on the use of internal teat sealants (Teatseal Bimeda, NZ; and Teatseal and Orbeseal, Pfizer Animal Health) in dairy cattle.

The following key words were used in different combinations for our search: internal teat sealant, teat sealer, Teat seal, Teatseal, Orbeseal, intramammary infection, clinical mastitis, postpartum milk SCC, subclinical mastitis, cattle, and cow. The most commonly reported outcomes were IMI and clinical mastitis during the lactation. The definitions used in these studies are listed in Appendix Table A1. In almost all cases, clinical mastitis was diagnosed by dairy managers. Subsequently milk samples were collected by the farm staff or study personnel for bacteriological examination.

Inclusion and Exclusion Criteria

Studies were included or excluded based on criteria developed by the authors. Quality assessment criteria included randomization of study groups and comparability of treatment groups at entry to each trial. Experiments were included in the analysis if they reported that the cattle used were lactating dairy cows and they

reported the data required on IMI and clinical mastitis (i.e., number of cases in each group) to determine the relative risk. Only 3 studies (Godden et al., 2003; Baillargeon and LeBlanc, 2010; Runciman et al., 2010) reported measures of milk SCC [i.e., linear score (**LS**) of SCC]. We were also able to obtain the raw data for milk SCC from 4 investigators.

A total of 21 studies and comparisons were excluded from the analysis. Studies that failed to meet the selection criteria included reviews ($n = 2$), experimentally infected cows ($n = 6$), studies that compared internal teat sealant with lacticin 3147 or a combination of teat sealant with lacticin 3147 ($n = 3$), and studies published in non-English journals ($n = 6$). Comparisons and trials within studies that treated cows systemically with antibiotics (Parker et al., 2008; experiments 1, 3, and 4) or when internal teat sealant was used in both treatment and control groups (Bradley et al., 2010; experiment 2) were also excluded from analysis.

Data Extraction

A total of 18 field studies with 22 IMI and 27 clinical mastitis subtrials and comparisons were reviewed. Of this, 17 IMI and 21 clinical mastitis subtrials and comparisons met the eligibility criteria for meta-analysis. Data recorded included authors, year of publication, country, number of farms, breed, parity, unit of observation for treatment allocation and analysis, DIM, dry period, type of ADCT used, diagnostic methods of IMI and clinical mastitis, and farming systems. A summary of studies and variables that were measured is provided in Table 1 and Appendix Table A2). Information on the type and name of ADCT and teat sealant, number of cows and quarters, and number of cows with IMI and clinical mastitis in the control and treatment groups is provided in Table 2.

Statistical Analysis

A meta-analysis was conducted on the incidence of IMI and clinical mastitis using Stata (Intercooled Stata v.12; StataCorp, 2011). Fixed effect (Mantel and Haenszel, 1959) and random effects (DerSimonian and Laird, 1986) models were used to assess the efficacy of internal teat sealants on the risk of IMI and clinical mastitis. In this study, risk ratio (**RR**) is the risk of IMI or clinical mastitis in cows that were treated with internal teat sealant commercial products or in the presence of ADCT (e.g., cloxacillin or cephalonium) compared with cows in control groups: either negative controls (untreated controls) or positive controls that were treated with ADCT. A total of 17 IMI and 21 clinical mastitis trials and comparisons were eligible

for the meta-analyses. Studies conducted to assess the effect of therapeutic dry cow treatments on mastitis in dairy cattle needed to have adequate randomization among mammary quarters or cows.

The statistical methods of meta-analytic procedures that were used in this paper have been previously published (Lean et al., 2012; Rabiee et al., 2012). Briefly, a fixed-effect model is based on the assumption that every study evaluates a common treatment effect. Specifically, the fixed-effect assumption means that the hypothetical effect of treatment is the same in all studies. The summary treatment effect estimate resulting from this method of meta-analysis is a fixed treatment effect, and the confidence interval provided describes how certain we are about the pooled estimate.

A Mantel-Haenszel risk ratio (RR_{M-H}) is calculated using the following formula (Rothman, 1986), which is generated from a contingency 2×2 table:

$$RR_{M-H} = \frac{\sum (a_j N_{0j} / T_j)}{\sum (b_j N_{1j} / T_j)}, \quad [1]$$

where a_j is the number of IMI or clinical mastitis cases (cow or quarter) in the treatment group for trial j , b_j is the number of IMI or clinical mastitis cases (cow or quarter) in the control group for trial j , and N_{0j} and N_{1j} represent the number of cows in the treated and control groups, respectively. The numerator and denominator are summed over j , the number of trials.

The random effects model assumes that the true treatment effects in individual studies may differ from each other. The true treatment difference in each trial is also assumed to be a realization of the random variable. The random variable is usually assumed to be normally distributed. The standard error of each trial estimate is increased and reflects the within- and between-trial variation. A pooled DerSimonian and Laird (1986) risk ratio (RR_{D+L}) is calculated with the following formula:

$$RR_{D+L} = \frac{\sum (W_i RR_i)}{\sum (W_i)}; \quad [2a]$$

$$95\% \text{ CI} = \theta - [1.96 \times \text{SE}(\theta)] \text{ to } \theta + [1.96 \times \text{SE}(\theta)]; \quad [2b]$$

$$\text{Predictive interval} = t(\text{df}) \times \sqrt{(\text{SE}^2 + \tau^2)}, \quad [2c]$$

where W_i is the weight (inverse variance) of individual studies and RR_i is the risk ratio of each subtrial or comparison (i). The 95% confidence interval for the overall estimate θ was estimated using Equation 2b,

Table 1. Studies that investigated the effect of teat sealant products (Teatseal and Orbeseal; Pfizer Animal Health, West Ryde, Australia) on the incidence of IMI and clinical mastitis during the dry period and after calving

Study	Breed	Parity	Unit of treatment	Unit of analysis	DIM	Dry period ¹ (d)	SCC at enrollment
Bradley et al. (2010)	Guernsey Holstein-Friesian	Multiparous	Quarter, within cow	Quarter	100	61 (uninfected); 70 (infected)	Uninfected: clinical history and SCC <200 of the last 3 mo; infected: SCC >200 Bulk milk SCC <300
Bradley et al. (2011)	Holstein-Friesian	Multiparous	Cow, quarters between cows	Quarter	100	62.3 (CDC); 64.3 (COMBO); 62.7 (OEDC)	Bulk milk SCC <300
Runciman et al. (2010)	—	Multiparous	Cow, quarters between cows	Cow	98.5; 98.6	63.6; 64.4	SCC <250; SCC >250
Woolford et al. (1998)	Holstein-Friesian and Friesian × Jersey	Multiparous and primiparous	Quarter, within cow	Quarter	150		SCC <200
Godden et al. (2003)		Multiparous	Quarter, within cow	Quarter	60	28 to 100	
Berry and Hillerton (2002a)			Cow, quarters between cows	Quarter and cow	100		SCC <200
Berry et al. (2003)			Cow, quarters between cows	Quarter and cow	100	56 to 70	
Newton et al. (2008)			Quarter, within cow	Quarter	100		SCC >200
Cook et al. (2005)			Cow, quarters between cows	Quarter	100		
Huxley et al. (2002)			Cow, quarters between cows	Quarter and cow	100	>51	Herd selection: bulk SCC < 200; cow selection: SCC < 200 SCC >150; SCC <150
Laven and Lawrence (2008)	Friesian and Friesian × Jersey		Cow and heifer	Cow and heifer	85		
Parker et al. (2007)	Friesian, cross-bred, and Jersey	Primiparous	Quarter, within heifer	Heifer	14		
Parker et al. (2008)		Primiparous	Heifer	Heifer	14		
Sanford et al. (2006b)	Holstein-Friesian	Multiparous	Quarter, within cow	Quarter	60	30 to 90	
Izak et al. (2011; abstract)		Multiparous	Cow	Cow	100	At least 45 d	SCC <250 and no clinical mastitis
Baillargeon and LeBlanc (2010)	Holstein-Friesian	Multiparous	Cow	Cow	105	28 to 120 d (mean: 62)	

¹CDC = cephalosporin cefquinome; COMBO = combination treatment comprising narrow-spectrum cloxacillin and an internal teat sealant; OEDC = narrow-spectrum antibiotic treatment comprising cloxacillin alone.

Table 2. Number of herds, animals and quarters, products used in control and treatment groups, and number of cases with IMI and clinical mastitis

Study	No. of herds	No. of animals	No. of quarters	Criteria	Control	Treatment	IMI at quarter or cow levels ¹		Clinical mastitis after calving (quarter/cows) ²	
							Control	Treatment	Control	Treatment
Bradley et al. (2010)	6	419	1,604	High SCC infected (>250)	250 mg of cephalonium ³	250 mg of cephalonium + Orbeseal ⁴	330/830	271/831	84/862	59/862
		391	1,632	Low SCC uninfected (<250)	250 mg of cephalonium + Orbeseal	Orbeseal	256/779	288/777	41/816	44/816
Bradley et al. (2011)	8	305	1,220	Bulk milk SCC <300	150 mg of cephalosporin cefquinome ⁵	600 mg of cloxacillin + Orbeseal	207/577	187/613	25/151	31/154
		306	1,236		600 mg of cloxacillin	600 mg of cloxacillin + Orbeseal	232/588	187/613	37/155	31/154
Runciman et al. (2010) ⁷	6	886		SSC >250	600 mg of cloxacillin	Teatseal ⁸ + 600 mg of cloxacillin	156/2,147	79/2,069	129/299	81/265
Woolford et al. (1998)	3	1,127	1,033	SSC <250	600 mg of cloxacillin	Teatseal			82/331	54/312
		528			Negative control	Teatseal ⁹	85/528	13/505	22/143	6/121
					250 mg of cephalonium	Teatseal	14/528	13/505	10/143	6/121
					Negative control	600 mg of cloxacillin	85/528	10/505	22/143	11/121
Godden et al. (2003)	2	419		Dry period	250 mg of cephalonium	600 mg of cloxacillin + Teatseal	14/528	10/505	10/143	11/121
				1 to 3 DIM	600 mg of cloxacillin	600 mg of cloxacillin + Orbeseal				
				6 and 8 DIM	600 mg of cloxacillin	600 mg of cloxacillin + Orbeseal	236/812	187/821		
				60 DIM	600 mg of cloxacillin	600 mg of cloxacillin + Orbeseal	210/811	167/809		
Berry and Hillerton (2002a)	7	401	1,596	Dry period	Negative control	Osmonds Teat Seal ¹⁰			66/862	51/862
		398	1,583	At calving	Negative control	Osmonds Teat Seal ¹⁰				
Berry and Hillerton (2007)	1	1,127		At calving	Cephalonium	Cephalonium + Orbeseal	39/531	22/596	93/799; 62/201	27/784; 21/197
Newton et al. (2008)	10	283	Total: 1,924; After calving enrolled: 313		600 mg of cloxacillin	600 mg of cloxacillin + Orbeseal	181/566	152/566	15/133	4/150
Cook et al. (2005)	3	528	2,112	1–3 DIM	Penicillin-dihydrostreptomycin ¹¹	Orbeseal penicillin-dihydrostreptomycin	160/971	81/1,009	50/141	23/142
Huxley et al. (2002)	16	IMI: 467; mastitis: 479	2,016	After calving	250 mg of cephalonium	Teatseal	369/940	321/923	31/242	25/237
Laven and Lawrence (2008)	1	249 Infected		After calving (first 11 wk after calving)	600 mg of cloxacillin	Teatseal			17/114	11/135
		217 Noninfected			Negative control	Teatseal			11/108	7/109
Parker et al. (2007)	5	206 Heifers	1,020	IMI within 4 DIM	Negative control	Teatseal			18/102	6/104
		255 Heifers			Negative control	Teatseal	66/505	49/501	34/501	21/501

TEATSEAL IN DAIRY CATTLE

Continued

Table 2 (Continued). Number of herds, animals and quarters, products used in control and treatment groups, and number of cases with IMI and clinical mastitis

Study	No. of herds	No. of animals	No. of quarters	Criteria	Control	Treatment	IMI at quarter or cow levels ¹		Clinical mastitis after calving (quarter/cows) ²	
							Control	Treatment	Control	Treatment
Parker et al. (2008)	30	1,067	4,146	IMI within 5 DIM	Tylosin ¹² (i.m.)	Teatseal	259/1,028	77/1,028	74/268	60/268
					Negative control	Teatseal	255/1,036	77/1,028	69/265	60/268
					Tylosin (i.m.)	Tylosin (i.m.) + Teatseal	259/1,028	97/1,052	74/268	104/266
					Negative control	Tylosin (i.m.) + Teatseal	255/1,036	97/1,052	69/268	104/266
Sanford et al. (2006b)	16	408 cows with negative milk culture	1,562	Negative milk culture 14 d before dry off	500 mg of cloxacillin	Orbeseal	54/788	48/774		
		326 cows with positive milk culture	1,209	Positive milk culture 14 d before dry off	500 mg of cloxacillin	500 mg of cloxacillin + Orbeseal	49/605	28/604		
Izak et al. (2011)	2	78		SCC <250 and with no previous IMI	600 mg of cloxacillin	Teatseal			2/36	10/42
Baillargeon and LeBlanc (2010)	12	1,334			Antimicrobial dry-cow therapy (ADCT)	Orbeseal + ADCT			125/669	97/665

¹New IMI after calving includes number of acquired IMI during the dry period and at or after calving and diagnosed after calving.

²Number of clinical quarters over total number of quarters; number of clinical quarters over total number of cows; number of clinical cows over total number of cows.

³Cepravin Dry Cow (Intervet Schering-Plough Animal Health, Milton Keynes, UK).

⁴Orbeseal (Pfizer Animal Health, Sandwich, UK).

⁵Cephaguard Dry Cow (Virbac Ltd., Bury St. Edmunds, UK).

⁶Orbenin Extra Dry Cow (Pfizer Animal Health, Sandwich, UK).

⁷Environmental pathogens.

⁸Teatseal (Pfizer Animal Health).

⁹Teatseal [Bimeda (NZ) Ltd., Auckland, New Zealand].

¹⁰Osmonds Teat Seal (Cross Vetpharm Group, Tallaght, Dublin, Ireland).

¹¹Quartermaster (Pfizer Animal Health).

¹²Tylan (Elanco Animal Health, Manukau City, New Zealand).

Table 3. Relative risk and probability of IMI and clinical mastitis in dairy cows treated with internal teat sealants (Teatseal and Orbesal; Pfizer Animal Health, West Ryde, Australia) alone or in the presence of antibiotic dry cow treatments (ADCT), number needed to treat (NNT), and heterogeneity among trials

Variable	Subgroup (no. of comparisons)	Relative risk and 95% CI		NNT	Heterogeneity ¹		
		Random effect (DerSimonian and Laird, 1986)	P-value		Q statistics (chi-squared)	I ² [% (95% CI)]	P-value
IMI	Positive control (n = 13)	0.75 (0.67, 0.83)	<0.001	20	34.71	65.4 (38, 81)	<0.001
	Negative control (n = 4)	0.27 (0.13, 0.55)	<0.001	7	37.79	92.1 (83, 96)	<0.001
Clinical mastitis	Positive control (n = 14)	0.71 (0.62, 0.82)	<0.001	21	19.58	33.6 (0, 65)	0.11
	Negative control (n = 7)	0.52 (0.37, 0.75)	<0.001	13	15.15	60.4 (9, 83)	0.019

¹I² = a statistic that describes the proportion of total variation in study effect estimates that is due to heterogeneity.

where θ is the log RR and SE is the standard error of log RR (Egger et al., 2001). The confidence interval of the approximate predictive distribution of a future trial was estimated based on the extent of heterogeneity. This incorporated uncertainty in the location and spread of the random effects distribution used Equation 2c, where t is the t -distribution with $k - 2$ degrees of freedom (where k is the number of experiments), SE^2 is the squared standard error, and τ^2 is the heterogeneity statistic (Bradburn et al., 1998).

The number needed to treat (NNT) is the number of individuals that need to be treated to expect that 1 case of mastitis would be prevented (<http://handbook.cochrane.org/>). The NNT is equal to $1/(\text{control group event risk} - \text{treatment group event risk})$ (Bradburn, 2003). The NNT was calculated in this study to provide several cows that needed to be treated with internal teat sealant to prevent 1 additional IMI or clinical case of mastitis after calving in lactating dairy cows is provided in Table 3. The NNT was calculated with Stata using the methods of Bradburn (2003).

Forest Plots

The effects of internal teat sealant on the risk of IMI and clinical mastitis in lactating dairy cows are provided in the forest plots (Figures 1 and 2). Points to the left of the line at $RR = 1$ represent a reduction in the risk of IMI or clinical mastitis, whereas points to the right of the line indicate an increase in the risk of IMI or clinical mastitis. Each square represents the RR for that study or subtrial. The upper and lower limit of the line connected to the square represents the upper and lower 95% confidence interval for the RR. The size of the square reflects the relative weighting of the study to the overall RR estimate with larger squares representing greater weight. The dotted vertical line represents the overall RR estimate. The diamonds at the bottom represent the 95% confidence interval for the overall estimates of fixed effect (Mantel and Haenszel, 1959) and random effects (DerSimonian and Laird, 1986) models.

The lines that are horizontally stretched on D+L diamond shapes are the predicted intervals and represent a conservative estimate of a predicted response to future treatment. The solid vertical line with RR of 1 is provided to indicate no net effect of intervention.

The SCC data for cows after calving in 3 studies (Cook et al., 2005; Sanford et al., 2006b; Baillargeon and LeBlanc, 2010) containing 32 herds were extracted and analyzed to compare the effect of internal teat sealant in the presence of ADCT versus ADCT treatment alone. The LS of postpartum milk SCC were analyzed using a mixed-effects linear model in which treatment, parity, and LS at drying off were the fixed effect terms and herd was the random effects term:

$$y_{ij} = \beta_0 + \beta_1 X_{1ij} + \beta_2 X_{2ij} + \beta_3 X_{3ij} + \mu_j + \varepsilon_{ij}, \quad [3]$$

where y_{ij} is the response variable (LS in lactation), β_0 (intercept) is the overall mean of LS, and β_1 to β_3 are the fixed-effect coefficients of internal teat sealant, parity, and LS at drying off, respectively. In this model, X_{1ij} to X_{3ij} are the fixed-effect terms (internal teat sealant, parity, and LS at drying off), μ_j is the random-effect of herd, and ε_{ij} is the residuals (error term). The interaction between internal teat sealant and herd was also examined.

Assessment of Heterogeneity

Heterogeneity among the estimated RR for studies included in the meta-analysis were assessed using a chi-squared (χ^2) test of heterogeneity. In this case, the null hypothesis was that the effect of treatment was the same across j trials and the null hypothesis was rejected if the heterogeneity test statistic was greater than a critical value that separated the upper 10% of a χ^2 distribution with $(j - 1)$ degrees of freedom. We used an α level of 0.10 because of the relatively poor power of the χ^2 test to detect heterogeneity among a small number of experiments (Egger and Davey Smith, 2001). Heterogeneity of results among experiments was

quantified using the I^2 statistic (Higgins and Thompson, 2002; Higgins et al., 2003), which describes the proportion of total variation in study effect estimates that is due to heterogeneity.

Higgins and Thompson (2002) developed measures of the impact of heterogeneity in a meta-analysis from mathematical criteria that are independent of the number of studies and the treatment effect metric. The I^2 is described as a percentage of the total variation that is due to heterogeneity rather than chance:

$$I^2 (\%) = \frac{[Q - (k - 1)]}{Q} \times 100; \quad [4a]$$

$$95\% \text{ UI} = \sqrt{\frac{1}{k-1} [Q \pm 1.96\sqrt{\text{Var}(Q)}]}, \quad [4b]$$

where Q is the chi-squared (χ^2) heterogeneity statistic, k is the number of experiments, and I^2 was calculated according to Thompson and Sharp (1999) and Higgins et al. (2003). Uncertainty intervals (UI) for I^2 (dependent on Q and k) were calculated using the method described by Higgins and Thompson (2002) using Equation 4b. Negative values of I^2 are equal to zero; consequently, I^2 lies between 0 and 100%. A value higher than 50% may be considered substantial heterogeneity (Higgins et al., 2003).

Meta-Regression

Meta-regression analyses were used to explore the source of heterogeneity of response using the logarithm of individual RR (log RR) for each experiment as the outcome and the associated standard error as the measure of variance. We have previously described the meta-regression procedures (Lean et al., 2012; Rabiee et al., 2012).

Publication Bias

No definitive means exists to assess the presence of publication bias in meta-analysis (<http://handbook.cochrane.org>). Several statistical tests exist for detecting funnel plot asymmetry [for example, the rank correlation test of Begg and Mazumdar (1994) and the linear regression test of Egger et al. (1997)], but these do not have considerable power and are rarely used. Funnel plots can be used visually to demonstrate the presence or absence of small-study effect as publication bias.

A funnel plot (as shown in Figures 3 and 4) is a simple scatter plot of the intervention effect estimates from individual studies against a measure of each study's size or precision. It is commonly presented as a

plot of the effect estimates on the horizontal scale, and the measure of variance on the vertical axis. The name funnel plot arises because the precision of the estimated intervention effect increases as the size of the experiment increases. Effect estimates from small experiments will, therefore, scatter more widely at the bottom of the graph, with the spread narrowing among larger experiments. In the absence of bias, the plot should approximately resemble a symmetrical (inverted) funnel. If bias exists (for example, because smaller experiments without statistically significant effects remain unpublished), this will lead to an asymmetrical appearance of the funnel plot, with observations (trials and subtrials) missing from the bottom corners of the graph. In this situation, the effect calculated in a meta-analysis will tend to overestimate the intervention effect (Egger et al., 1997). The more pronounced the asymmetry, the more likely it is that the bias will be substantial. The dashed lines provide the pseudo-confidence intervals for the RR estimates within the funnel plot. The vertical line is where the RR = 1.

Contour-Enhanced Funnel Plots

Contour-enhanced funnel plots have been proposed by Peters et al. (2008) to include contour lines corresponding to the statistical significance ($P = 0.01, 0.05$, and 0.1). This approach allows reviewers to differentiate among the trials that are highly significant from trials that are perceived to be missing. The contour-enhanced funnel plots may help to differentiate asymmetry caused by publication bias from that due to other factors. For example, if experiments appear to be missing in areas of statistical nonsignificance, then this adds credence to the possibility that the asymmetry is caused by publication bias. Conversely, if putative missing studies are in areas of higher statistical significance, this suggests that the observed asymmetry may more likely be due to factors other than publication bias.

Milk SCC

We were able to obtain the raw milk SCC data from 4 investigators (Cook et al., 2005; Sanford et al., 2006b; Parker et al., 2007; Baillargeon and LeBlanc, 2010) and estimated the LS of milk SCC after calving. Data from Parker et al. (2007) were not used in this report because these samples were collected immediately after calving and may have contained colostrum (K. I. Parker, Animal Health Centre, Morrinsville, New Zealand, personal communication). We analyzed data that were provided by Cook et al. (2005) and Sanford et al. (2006b), but not published in the original papers, and estimated the LS of milk SCC after calving for each

Table 4. Milk SCC (linear score) of cows treated with internal teat sealants (Teatseal and Orbeseal; Pfizer Animal Health, West Ryde, Australia) and antibiotic dry cow treatments (ADCT) during the first 3 wk after calving

Study	Experiment/ period	Source	DIM	Control	Treatment	Statistical model and variables accounted for	Linear score of SCC; no. of cows (mean $\pm$ SE)		P-value
							Control	Treatment	
Godden et al. (2003; United States)	Period 1	Paper	1–3	600 mg of cloxacillin	600 mg of cloxacillin + Orbeseal	Multivariate linear regression (PROC MIXED ¹ ; not specified)	NA ² ; 5.40 $\pm$ 0.17	NA; 5.10 $\pm$ 0.17	<0.001
Runciman et al. (2010; Australia)	Period 2	Paper	6 and 8 7	600 mg of cloxacillin	Teatseal + 600 mg of cloxacillin	Multivariate linear regression (accounted for age, milk yield, SCC in previous lactation, herd)	NA; 3.10 $\pm$ 0.18 NA; 4.72 $\pm$ 0.05	NA; 2.80 $\pm$ 0.18 NA; 4.41 $\pm$ 0.05	<0.001
Baillargeon and LeBlanc (2010; Canada)		Paper	>5	ADCT	Orbeseal + ADCT	Mixed linear regression [accounted for parity, season of calving and herd (random)]	1,334 2.50 $\pm$ 0.18	2.44 $\pm$ 0.18	0.11
Cook et al. (2005)		Data	15–18	Penicillin- dihydrostreptomycin	Orbeseal + penicillin- dihydrostreptomycin	Mixed linear regression [accounted for age, FCM, SCC in previous lactation and herd (random)]	306; 2.71 $\pm$ 0.10	292; 2.32 $\pm$ 0.10	0.004
Sanford et al. (2006b)	Experiment 1	Data	7	500 mg of cloxacillin	Orbeseal	Mixed linear regression [accounted for age, SCC in previous lactation and herd (random)]	1,510; 2.59 $\pm$ 0.10	815; 2.53 $\pm$ 0.12	0.57
	Experiment 2				500 mg of cloxacillin + Orbeseal		1,510; 2.59 $\pm$ 0.10	679; 2.74 $\pm$ 0.13	0.18

¹SAS Institute Inc., Cary, NC.²NA = not available.

Table 5. Analysis of linear score (LS) of individual cows in early lactation using a mixed effects linear regression model after adjusting for herd effects (random effects), parity, and LS at dry off (fixed effects)¹

Item	Without LS at dry off (n = 4,023)		With LS at dry off (n = 2,506)	
	Coefficient ± SE	P-value	Coefficient ± SE	P-value
Treatment	−0.086 ± 0.070	0.22	−0.034 ± 0.090	0.72
Parity				
2	0.175 ± 0.09	<0.058	0.0309 ± 0.11	0.78
3	0.747 ± 0.11	<0.001	0.684 ± 0.15	<0.001
4	0.817 ± 0.13	<0.001	0.844 ± 0.18	<0.001
≥5	1.270 ± 0.13	<0.001	1.222 ± 0.18	<0.001
LS at dry off	NA	NA	0.058 ± 0.021	0.005
	Variance and 95% CI	Log-likelihood	Variance and 95% CI	Log-likelihood
Random effect (herd)	0.129 (0.062, 0.269)	−8,776.317	0.052 (0.010, 0.254)	−5,528.2007
	Predicted mean for LS (mean ± SE)		Predicted mean for LS (mean ± SE)	
ADCT ²	2.75 ± 0.08		2.63 ± 0.08	
Teatseal ³ + ADCT	2.67 ± 0.09		2.60 ± 0.10	

¹Predicted means are detailed.²ADCT = antibiotic dry cow treatments.³Pfizer Animal Health, West Ryde, Australia. **Figure 1.** Forest plot of studies examining the risk of acquiring new IMI at the quarter level in lactating dairy cows treated with internal teat sealant compared with positive control (top) and negative control (bottom). Box sizes are proportional to the inverse variance of the estimates. Summary estimates of treatment effects (diamond shapes) are shown using (1) a fixed effects approach [M-H specifies a fixed effect model using the Mantel and Haenszel (1959) method], (2) a random effects approach [D+L specifies a random effects model using the method of DerSimonian and Laird (1986)], and (3) the predicted interval of a future trial, with the estimate of heterogeneity being taken from the inverse variance fixed effect model. RR = risk ratio; I² = a statistic that describes the proportion of total variation in study effect estimates that is due to heterogeneity. Color version available in the online PDF.

study separately (Table 4). The results were not significant, but the internal teat sealants + ADCT reduced the LS of milk SCC in 2 trials, but not in Sanford et al. (2006b; experiment 2). The LS of postpartum milk SCC data of 3 studies (Cook et al., 2005; Sanford et al., 2006b; Baillargeon and LeBlanc, 2010) were then pooled as 1 data set and analyzed using a mixed-effects linear model, in which herds within studies were used as a random effects term, to explore the effect of internal teat sealant in the presence of ADCT on LS (Table 5).

RESULTS AND DISCUSSION

Sufficient studies of suitable quality were identified to allow a meta-analysis to be conducted (Tables 1 and 2). Most experiments included in the published papers were conducted in multiple herds and several factors that could act to confound estimates of the effect of treatment were investigated by qualitative review and meta-regression to determine if the outcomes of these studies (IMI and clinical mastitis) may have been influenced by some of these factors.

Random Allocation of Treatment Among Front and Rear Quarters Within Cow

The probability of risk of infection among front and rear glands should be considered during the random allocation of treatments to different quarters within cow, as Adkinson et al. (1993) and Barkema et al. (1997) suggested that the risk of clinical mastitis is higher in the rear than front glands. If treatments were not applied equally across the front and rear glands or not accounted for, this could lead to a bias in assessing the efficacy of treatments. Berry et al. (2003), however, found no statistical differences in infection status at calving with respect to front or rear, or left or right quarters when cows were treated with ADCT or internal teat sealant (Orbeseal; Pfizer Ltd., Tadworth, UK). In all studies ($n = 6$) that quarter within cows were the unit of observation and each quarter was treated with a different treatment regimen, the treatments were randomly allocated among the front and rear glands to avoid a potential bias.

Between-Quarter Interdependence Within Cows

Several studies ($n = 6$) existed in which the quarters within a cow were allocated to different treatment regimens, such as ADCT, untreated (control), and treated with internal teat sealants with or without ADCT (Table 2). Concerns existed about the interdependence of quarters in studies that used different treatments at the quarter level within cow, as failure to account

for interdependence of quarters within a cow may lead to inaccurate variance estimates and errors in assessing treatment effects. Berry et al. (2003) demonstrated that quarters that were untreated during the dry period were independent from treated quarters of the same cow with respect to the risk of an IMI at calving. Sanford et al. (2006a) studied whether intramammary half-udder infusion of cloxacillin resulted in transfer of cloxacillin from treated to untreated mammary gland quarters within nonlactating cows. This study also investigated the concentrations of antibiotic in quarters, and whether selection of ipsilateral versus diagonal-contralateral quarters for treatment affected cloxacillin transfer among quarters. Cloxacillin was detected in 25% of all untreated mammary gland quarters (Sanford et al., 2006a), but concentrations of cloxacillin in untreated quarters were below MIC for targeted mastitis pathogens. Their study also found significant differences in cloxacillin concentrations in the ipsilateral or diagonal treatment groups. The results of their study suggest that within-cow half-udder comparison trials are valid for antimicrobial trials in nonlactating cows, although transfer of antimicrobials does occur in trace concentrations (Sanford et al., 2006a).

Based on understandings derived from Berry et al. (2003) and Sanford et al. (2006a) regarding the random allocation and interdependence of quarters, we believe that the papers included in this meta-analysis have adequately addressed factors that may influence the validity of study designs.

IMI

A total of 12 studies with 17 subtrials, including 13 positive control subtrials and 4 negative control subtrials, on IMI were included in the analysis (Figure 1). Based on the considerations outlined above, the results are presented at the quarter level. The incidence of IMI reported includes the infected quarters that may have occurred during the dry period as well as those arising during and after calving. The use of internal teat sealants alone and in the presence of ADCT reduced the risk of acquiring new IMI after calving in lactating cows by 25% compared with the ADCT alone ($RR = 0.75$; 95% CI: 0.67 to 0.83).

Treatment of cows with internal teat sealant alone [except in the study of Woolford et al. (1998), which was a combined treatment] reduced the risk of IMI by 73% of those in the untreated (negative control) cows ($RR = 0.23$; 95% CI: 0.13 to 0.55). The predictive interval estimates included 1.0 for the negative control studies (Figure 1), indicating that some null or negative responses to treatment with teat sealants may be observed in future studies.

Figure 2. Forest plot of studies examining the risk of clinical mastitis up to 150 DIM at the cow and quarter levels in lactating dairy cows treated with internal teat sealant compared with positive control (top) and negative control (bottom). Box sizes are proportional to the inverse variance of the estimates. Summary estimates of treatment effects (diamond shapes) are shown using (1) a fixed effects approach [M-H specifies a fixed effect model using the Mantel and Haenszel (1959) method], (2) a random effects approach [D+L specifies a random effects model using the method of DerSimonian and Laird (1986)], and (3) the predicted interval of a future trial, with the estimate of heterogeneity being taken from the inverse variance fixed effect model. RR = risk ratio; I^2 = a statistic that describes the proportion of total variation in study effect estimates that is due to heterogeneity. Color version available in the online PDF.

Overall pooling of the study approaches (i.e., use of internal teat sealants alone or in the presence of ADCT) reduced the risk of IMI by 39% compared with cows treated with ADCT (positive control) and untreated (negative control) cows (RR = 0.61; 95% CI: 0.51 to 0.72). However, given the marked differences in the pooled RR estimates between studies using teat sealants in the presence of ADCT and those without, we consider that the estimates of effect used in estimates of efficacy should be specific to whether teat sealant treatment is combined with ADCT or not.

The results of both meta-analyses of IMI, with positive and negative controls, were heterogeneous ($I^2 = 65.4\%$ and 92.1% ; $P < 0.001$; Table 3). The sources of heterogeneity were explored, using the methods of Knapp and Hartung (2003) and Harbord and Higgins (2008), using variables for which we had sufficient data, including presence or absence of antibiotic in the control group, type of antibiotics used in control groups,

infection status of cows before treatment, and location (country) of study. None of these factors significantly contributed to the heterogeneity of the results. We were unable to explore the effect of housing and farming systems on the IMI outcomes because the individual studies that were included in the analysis had pooled data from different farming and feeding systems into a single point estimate. The heterogeneity of results is not of great concern because all these studies showed the same direction toward a reduction in IMI (RR < 1). It is likely that differences in treatment protocols and the procedures used to detect the presence of IMI among studies contributed to the heterogeneity.

The NNT for each cow to benefit from the intervention is considered a useful, clinically meaningful way of reporting binary outcomes from randomized trials. For every 20 cows that were treated with the internal teat sealants in the presence of ADCT, 1 additional cow responded positively (i.e., did not have an IMI) to the

Figure 3. Contour-enhanced funnel plots of risk of IMI for lactating dairy cows treated with internal teat sealants with and without antibiotic. Legend: levels of significance for studies (●) within the broken lines are 0.01, 0.05, and 0.10; FE = fixed effect.

combined treatment compared with ADCT treatment alone, whereas for every 7 cows that were treated with internal teat sealant in the presence of ADCT, the occurrence of 1 IMI was prevented compared with the untreated (negative control) cows.

Clinical Mastitis

A total of 16 studies with 21 subtrials, including 14 positive control subtrials and 7 negative control subtrials, on clinical mastitis up to 150 DIM were included in the analysis (Figure 2). The results were presented at both the quarter and cow levels. The incidence of

Figure 4. Contour-enhanced funnel plots of risk of clinical mastitis for lactating dairy cows treated with internal teat sealants with and without antibiotic. Legend: levels of significant for studies (●) within the broken lines are 0.01, 0.05, and 0.10; FE = fixed effect.

clinical mastitis includes clinical cases that occurred at calving and during the first 100 to 150 DIM.

The application of internal teat sealant products alone and in the presence of ADCT significantly reduced the risk of clinical mastitis after calving in lactating dairy cows (Table 3; $P < 0.001$). The use of internal teat sealants alone and in the presence of ADCT reduced the risk of clinical mastitis after calving in lactating cows by 29% compared with cows treated with ADCT alone ($RR = 0.71$; 95% CI: 0.62 to 0.82). Treatment of cows with internal teat sealants alone (Figure 3) reduced the risk of clinical mastitis by 48% ($RR = 0.52$; 95% CI: 0.37 to 0.75) compared with the untreated cows (negative control). Overall, the use of internal teat sealants alone or in the presence of ADCT reduced the risk of clinical mastitis after calving by 34% ($RR = 0.66$; 95% CI: 0.58 to 0.76; Table 3). Again, marked differences existed in the pooled RR estimates between studies using teat sealants in the presence of ADCT and those without. Therefore, estimates of effect used should be specific to whether teat sealant treatment is combined with ADCT or not. Both analyses (i.e., the negative and the studies combined with ADCT) of the effects of teat sealants on clinical mastitis had the predictive intervals that included 1.0 (Figure 2), indicating that some null or negative responses to treatment with teat sealants may be observed in future studies.

The results of meta-analysis on clinical mastitis with positive controls were homogeneous ($I^2 = 33.6\%$; $P < 0.106$), whereas the results of studies with negative controls were heterogeneous ($I^2 = 60.4\%$; $P < 0.019$), but the overall results were heterogeneous ($I^2 = 46.5\%$; $P < 0.001$). We explored the source of heterogeneity, using the methods of Knapp and Hartung (2003) and Harbord and Higgins (2008), with the variables for which we had adequate data, including DIM, presence or absence of antibiotic in the control group, unit of treatment (quarters within cow vs. quarters between cows), type of antibiotic used in control groups, infection status of cows before treatment, and the location (country) where the study was conducted. None of these factors significantly contributed to the heterogeneity of the results.

Two studies (Woolford et al., 1998; Bradley et al., 2011) differed from other studies in the direction of effect. These studies compared the internal teat sealants in the presence of cloxacillin versus cows treated with cefquinome or cloxacillin (Bradley et al., 2011) and the risk of clinical mastitis in the internal teat sealants in the presence of cloxacillin was greater than the cefquinome (Figure 3). Bradley et al. (2011) suggested that cows in the cefquinome group were less likely to develop clinical mastitis than those in the cloxacillin-treated group. In Woolford et al. (1998), the unit of treatment was quarter within cow and the infected quarters with-

in cows were allocated to the ADCT and combination of internal teat sealants and ADCT groups, whereas healthy quarters were allocated to negative and positive controls or internal teat sealants in the presence of ADCT. This difference in study design may explain the differences between the outcomes of their study compared with other studies. A sensitivity analysis was conducted by removing subtrial 2 of Woolford et al. (1998), but little change occurred in the overall point estimate (RR = 0.65; 95% CI: 0.57, 0.75) and the results remained heterogeneous ($I^2 = 45.9\%$; $P = 0.014$).

The NNT analysis indicated that we need to treat 21 cows with internal teat sealant + ADCT to prevent 1 case of clinical mastitis compared with ADCT alone. When comparing the internal teat sealants + ADCT versus negative control (untreated), 13 cows needed to be treated to prevent an additional case of mastitis.

Sources of heterogeneity in these studies may include differences in the definition and diagnostic methods used for identifying clinical mastitis. Again, we could not explore the effects of housing and farming systems on clinical mastitis, because individual studies pooled data from different farming and feeding systems into a single point estimate.

Although no robust statistical test exists to precisely quantify the presence or absence of publication bias in a meta-analysis, funnel plots are used to evaluate the presence or absence of publication bias. We used contour-enhanced plots to differentiate between the highly significant trails and the other studies as proposed by Peters et al. (2008). The contour-enhanced funnel plots of IMI and clinical mastitis showed some evidence of publication bias (Figures 3 and 4). The probability of publication of studies with results showing a reduction in the number clinical mastitis cows in treatment group was greater than studies that reported an increase in the number of clinically mastitic cows. The point estimates on the very left side of the funnel plot may indicate highly significant reductions ($P < 0.01$) in the probability of IMI and clinical mastitis in dairy cows treated with teat sealant, rather than publication bias.

Milk SCC

Three studies reported LS of milk SCC data after calving (Godden et al., 2003; Baillargeon and LeBlanc, 2010; Runciman et al., 2010). Godden et al. (2003) demonstrated that quarters treated with Orbesal and cloxacillin had a significantly lower mean LS at both 1 to 3 DIM and 6 to 8 DIM than control, cloxacillin only, quarters (Table 4; $P < 0.001$). An Australian study (Runciman et al., 2010) also showed that the mean LS for the cows treated with Teatseal and cloxacillin was significantly lower compared with cows treated with

cloxacillin (Table 5; $P < 0.001$) after accounting for herd, age, milk production and SCC status in previous lactation. However, Baillargeon and LeBlanc (2010) found no significant reduction between ADCT and Teatseal and ADCT combined groups (Table 4; $P = 0.11$) across several herds.

The analysis of pooled LS data, before and after controlling for LS at drying off, showed that no significant differences in LS existed between cows treated with internal teat sealants + ADCT versus ADCT alone. The interaction between LS at drying off and treatments was also not statistically significant. These limited data suggest that the effect of internal teat sealants + ADCT on postpartum milk SCC deserves further study.

CONCLUSIONS

The application of internal teat sealants with or without ADCT at dry off significantly reduced the incidence of IMI and clinical mastitis in lactating dairy cows. Further studies are needed to investigate the effect of internal teat sealants on milk SCC in lactating dairy cows after calving. Further clinical trials may provide further evidence of effects of treatment on this outcome.

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APPENDIX

Table A1. Definitions of IMI and clinical mastitis (CM) used by the study investigators

Reference	Definitions of IMI and CM
Bradley et al. (2010, 2011)	IMI: isolation of an organism was considered indicative of an IMI CM: farm personnel trained in the detection and sampling of CM throughout the study period (≤ 150 DIM)
Bradley et al. (2011)	IMI: new IMI was defined as the presence of a pathogen in the postcalving sample that had not been present at sampling at drying off CM: farm personnel trained in the detection and sampling of CM throughout the study period (≤ 150 DIM)
Runciman et al. (2010)	IMI: presence of clotted or discolored milk beyond the first 3 squirts of milk when the suspected quarter was hand stripped, or a hot or swollen quarter, or both
Woolford et al. (1998)	IMI: during the lactation (last 7 d) and after calving, milk samples were collected for bacteriological examination and detection of IMI CM: during the dry period, udder examination was conducted, which involved palpation of individual quarters to detect any difference in tissue firmness between adjacent quarters and hence the presence of clinical IMI; quarters determined to be clinically infected by palpation were sampled for bacteriological culture up to 150 DIM
Godden et al. (2003)	Not provided
Berry and Hillerton (2002a, 2007)	IMI: pathogen is isolated in 2 consecutive samples (2 out of 3 samples), or a pathogen obtained from 1 milk sample with elevated SCC in comparison with other quarter SCC CM: CM cases were detected by farm staff or at routine sampling time. These were defined when visible changes in the milk, such as watery milk, clots or flakes, or changes in the udder such as swelling or heat, were observed
Newton et al. (2008)	CM: CM was defined as the presence of an abnormal secretion or udder changes, or both (heat pain or swelling, or both) as diagnosed by the herds person Clinical cases of mastitis were identified by herds persons and milk samples were collected for bacteriological examination A minor pathogen was defined as either a coagulase-negative <i>Staphylococcus</i> species or a <i>Corynebacterium</i> species
Cook et al. (2005)	IMI: new dry period infection: a significant isolate of an organism found in a quarter at the 1 to 3 DIM sample that was not present in the quarter sample at dry off Dry period cure: absence of an organism in a quarter at the 1 to 3 DIM sample that was present at dry off CM: quarter case of mastitis: a mastitis event in a single quarter; repeat cases occurring in the same quarter were considered a new clinical quarter case if the interval between events exceeded 7 d CM: cow case of mastitis: a cow suffering mastitis in 1 or more quarters at the same time; repeat cow cases were considered a new cow case if the interval between events exceeded 7 d
Huxley et al. (2002)	Diagnosis, grading, and sampling of CM was conducted by trained herds persons CM: CM quarter case: a single recorded occurrence of CM in a quarter, including all multiple quarter and recurrent cases; recurrent cases were classified as cases recurring in the same quarter greater than 5 d after the end of the last clinical episode and caused by the same pathogen CM: CM cow case: a cow experiencing a mastitis event, regardless of the number of quarters affected or the number of times each quarter was affected (if caused by the same pathogen)
Laven and Lawrence (2008)	CM: diagnosis of CM was performed by a farmer and was based on the presence of clots in the milk, or of swelling, heat, or pain of the udder
Parker et al. (2007)	CM: CM was defined as the presence of grossly visible changes (e.g., clots or blood) in the milk composition or swelling, pain, or both, in the gland; herd owners collected duplicate milk samples from glands diagnosed with CM
Parker et al. (2008)	CM: CM was diagnosed by the herd owner based on the presence of visible changes (e.g., clots or discoloration) in the milk or swelling/pain in the gland
Sanford et al. (2006b)	IMI: IMI was defined as isolation of any major mastitis-causing organism in conjunction with an SCC $>200,000$ cells/mL, or isolation of CNS (>10 cfu/0.01 mL of milk) in conjunction with an SCC $>200,000$ cells/mL CM: CM was defined as abnormal milk with or without signs of heat, redness, or swelling in the quarter and with or without clinical signs of systemic disease
Izak et al. (2011)	CM: CM was defined as the presence of visibly abnormal milk and (or) abnormal quarter or signs of systemic illness; isolates were identified according to the procedures of the National Mastitis Council (Izak and Ackermann, 2010)
Baillargeon and LeBlanc (2010)	CM: CM was defined as the presence of visibly abnormal milk (e.g., presence of clots, watery secretion) beyond the third forestripping, with or without signs of abnormality in the quarter or signs of systemic disease; following the clinical diagnosis, milk samples from the affected quarters were collected by producers

Table A2. Studies that investigated the effect of internal teat sealants (Teatseal and Orbeseeal; Pfizer Animal Health, West Ryde, Australia) on the incidence of IMI and clinical mastitis during the dry period and after calving

Study	Country	Milk sampling interval	Diagnosis description and procedures	Housing/farming
Bradley et al. (2010)	United Kingdom	Prior to final milking and postcalving	Bacteriological examination postcalving; clinical cases until 150 DIM	Freestalls, covered straw yards, pasture
Bradley et al. (2011)	United Kingdom	Prior to final milking and postcalving	Bacteriological examination 2 wk precalving; clinical cases until 150 DIM	Freestalls, covered straw yards, pasture (combination of all)
Runciman et al. (2010)	Australia	Postcalving milk samples (d 21, 30, and 100)	Clinical examination and then bacteriological tests of quarters	Pasture (n = 6)
Woolford et al. (1998)	New Zealand	Within 7 d of last lactation and within 24 h after calving	Clinical examination and bacteriological tests	Pasture (n = 3)
Godden et al. (2003)	United States	Immediately before final milking, 1 to 3 DIM and 6 to 8 DIM	Bacteriological tests at dry off, dry off and 1 to 3 DIM, dry off and 6 and 8 DIM, and then up to 60 DIM	Paddock (grazing) before dry off, and then freestall during experiment (n = 2)
Berry and Hillerton (2002a)	United Kingdom	One week before drying off, at drying off, at calving, and 1 wk after calving	Bacteriological tests at 1 wk before dry off, at dry off, at calving, 7 DIM, and clinical observation by farm staff until 150 DIM	Organic farms (n = 2) and research farms (n = 2)
Berry et al. (2003)	United Kingdom	One week before drying off, at drying off, at calving, and 1 wk after calving	Bacteriological tests at 1 wk before dry off, at dry off, at calving, 7 DIM, and clinical observation by farm staff until 150 DIM	Research farm (n = 1)
Newton et al. (2008)	United Kingdom	Two days before drying off, 1 d before drying off, at drying off, 4 d after calving, and 8 to 11 d after calving	Bacteriological tests at 2 d before drying off, 1 d before drying off, on the day of drying off, 4 DIM, and between 8 and 10 DIM	
Cook et al. (2005)	USA	Prior to drying off and within 3 d of calving	Bacteriological tests at dry off, within 3 d of calving, and on clinical cases up to 150 DIM	Freestall (rubber matt = 1; sand bedded = 1; straw bedded = 1)
Huxley et al. (2002)	United Kingdom	Prior to drying off and after calving before the first machine milking	Bacteriological tests at dry off, after calving, and before first milking	Pasture (summer) and covered stalls (winter)
Laven and Lawrence (2008)	New Zealand	Not provided	Clinical diagnosis by farmer	Grazing (n = 1)
Parker et al. (2007)	New Zealand	Prior to treatment and within 4 d of calving	Bacteriological tests at 31 d before calving start date, 4 DIM, and clinical diagnosis from calving to 14 DIM	Grazing (n = 5)
Parker et al. (2008)	New Zealand	Prior to treatment and within 5 d of calving	Bacteriological tests at 31 d before calving start date, and clinical diagnosis from calving to 14 DIM	Grazing (n = 30)
Sanford et al. (2006b)	Canada	Two weeks before calving and within 8 d after calving	Bacteriological tests at 14 d before dry off, end of lactation, within 8 DIM, and 60 DIM on cows with clinical signs	Freestall (n = 8); tie-stall (n = 8)
Izak et al. (2011; abstract)	Argentina	Not provided	Bacteriological tests from calving to 10 DIM and at 150 DIM	
Baillargeon and LeBlanc (2010)	Canada	Postcalving mastitic cows were sampled	Bacteriological tests on clinical mastitis cases up to 105 DIM	Freestall (n = 7); tie-stall (n = 5)