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Multiplex polymerase chain reaction as a mastitis screening test for *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis* in bulk milk samples

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Effective diagnostic tools for screening herds for mastitis pathogens are important in development and monitoring of mastitis control programmes. A multiplex polymerase chain reaction (PCR) assay for simultaneous detection of *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis* was used in preliminary studies to assess its applicability as an alternative method for monitoring mastitis caused by these organisms at the herd level. PCR was used to detect the presence of these organisms in bulk milk samples. Correlations with bulk milk somatic cell counts (BMCC), total bacteria counts and thermoduric bacteria counts were evaluated. A total of 176 bulk milk samples were collected from 42 herds on five consecutive occasions at approx. 10-d intervals. *Str. uberis* was the most common organism in these bulk milk samples. There was no relationship between presence of either *Staph. aureus*, *Str. dysgalactiae* or *Str. uberis* and BMCC, total bacteria counts or thermoduric bacteria counts. However, presence of *Str. agalactiae* was associated with high BMCC and total bacteria counts. The results of this study show that regular analysis of bulk milk using this multiplex PCR assay may be a useful tool for monitoring herd status with respect to *Str. agalactiae*, but is of less value for monitoring occurrence of *Staph. aureus*, *Str. dysgalactiae* and *Str. uberis*. Further investigations are needed to clarify the relationship between positive PCR results and the prevalence of infected cows in the herd.

Keywords: Bovine, cell count, bacteria count, thermoduric.

Regular monitoring of bulk tank milk is a vital part of dairy herd health programmes to control mastitis. Analysis of bulk milk can provide information on the extent of subclinical infection, the pathogens involved, and the level of milking hygiene and equipment sanitation in a herd (Guterbock & Blackmer, 1984).

Bulk milk tests that primarily relate to mastitis are bulk milk somatic cell counts (BMCC) and bulk milk cultures. A persistently high BMCC indicates a mastitis problem in a herd. It has been estimated that for each 100 000 cells/ml, approximately 10% of cows in a herd have mastitis (Ryan, 1991). This test provides no information, however, about types of pathogens responsible. Such information is important for implementation of appropriate mastitis control programmes.

Bulk milk culture has been used as a screening test for the presence of mastitis pathogens within a herd. This method reduces the cost and overcomes the problems of collecting and processing large numbers of samples, as is necessary when individual quarters or individual cows are cultured on a repeated basis as a standard procedure.

There are a number of limitations associated with bulk milk culture. No standardized methods have been established for the collection and culture of bulk milk samples (Godkin & Leslie, 1993). Methods used differ with respect to location within the tank from which samples are collected, volume of milk collected, number of samples used and frequency of sampling. Various bacteriological techniques have also been used in diagnostic laboratories to isolate and identify organisms in bulk milk samples, including the use of differential and selective media. These

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techniques can be complex and time consuming. Bulk milk culture is also reported to have a low sensitivity for determining the presence of major bacterial mastitis pathogens (Godkin & Leslie, 1993).

Given that PCR is a rapid and sensitive method to identify bacteria, it was used in this preliminary study to assess its applicability as an alternative tool for monitoring mastitis caused by *Staph. aureus*, *Str. agalactiae*, *Str. dysgalactiae* and *Str. uberis* at the herd level. Correlations between the results using this method and BMCC, total bacteria counts and thermoduric bacteria counts were also evaluated.

Materials and Methods

Bulk milk sample collection

Bulk milk samples were provided by a large milk processing co-operative operating in south-eastern Australia, drawing milk from farms using pasture-based dairying systems. Samples provided were those collected from supplier herds for routine milk quality analysis of butterfat and protein content, cell count and bacteria count. Herds were selected to represent those with low, medium or high rolling average BMCC. Five consecutive milk samples were collected from most of the herds at approx. 10-d intervals. A total of 176 bulk milk samples from 42 herds were forwarded by the factory laboratory immediately after the completion of routine processing.

Herds

Based on the geometric mean BMCC ($BMCC_{gm}$) of up to 5 values, the 42 herds were divided into three groups. Group 1 consisted of 14 herds that had low $BMCC_{gm}$ (<200 000 cells/ml). Thirteen herds with medium $BMCC_{gm}$ (200 000–400 000 cells/ml) were allocated to group 2 and 15 herds with high $BMCC_{gm}$ (>400 000 cells/ml) were allocated to group 3.

Data obtained from the milk factory

Bulk milk somatic cell counts were determined using a Fossomatic cell counter (FOSS A/S, Hillerød, Denmark) and routinely recorded by the milk factory. Bacteria counts, as determined using Bactoscan (FOSS A/S, Hillerød, Denmark), and thermoduric bacteria counts were also provided by the milk factory.

Sample preparation for PCR

DNA was extracted from milk samples using the Rneasy (Qiagen Pty Ltd, PO Box 25, Clifton Hill 3068, Victoria, Australia) spin column method. One millilitre of milk was inoculated into 9 ml Streptosel (Becton Dickinson, Franklin Lakes, NJ 07417 USA) and the culture incubated for 8–12 h prior to extraction. One hundred microlitres of broth culture was taken and mixed with 350 µl RLT buffer (Qiagen) containing 1% 2-mercaptoethanol. After mixing vigorously, 300 µl of 100% ethanol was added, and 15 µl Qiaex II matrix (Qiagen) was then added. The suspension was loaded onto a spin column (Axygen Inc, Hayward California, USA). Columns were then centrifuged at 9000 ***g*** for 30 s and the flow-through discarded. Columns were washed once with 600 µl RLT buffer and then washed twice with 500 µl RPE buffer (Qiagen). For each wash, columns were centrifuged at 9000 ***g*** for 30 s. After the final wash, columns were centrifuged at 16 000 ***g*** for an additional 90 s to remove any excess ethanol. Thirty microlitres of Rnase-free water was placed directly on the matrix. DNA was then eluted from the matrix by centrifugation at 9000 ***g*** for 1 min and 5 µl of the extract was subjected to PCR.

Oligonucleotide primers

Oligonucleotide primers were derived from published sequences (Forsman et al. 1997), with primer sequences for *Str. agalactiae*, *Str. dysgalactiae* and *Str. uberis* adjusted from the published sequences to have similar T_m values (Phuektes et al. 2001).

PCR amplification

Multiplex PCR was performed with 2·5 U *Taq* DNA polymerase (Roche Diagnostics Pty Ltd, Castle Hill, NSW 2154) in 50-µl reactions containing 500 ng of extracted DNA in 10 mM-Tris–HCl (pH 8·3), 2·5 mM-$MgCl_2$, 50 mM-KCl, 200 µM each of dATP, dTTP, dCTP and dGTP, 0·4 µM each of the *Str. agalactiae* and *Str. dysgalactiae* primers, 0·1 µM of the *Str. uberis* primers and 0·3 µM each of the *Staph. aureus* primers. Reactions were incubated at 95 °C for 5 min, then 36 cycles of 95 °C for 1 min, 59 °C for 30 s and 72 °C for 30 s and finally incubated at 72 °C for 7 min. A tube containing the PCR mixture without template DNA was routinely included as a negative control.

Analysis of PCR products

Amplification products were analysed by electrophoresis through 2% agarose gels or 20% polyacrylamide gels. Twelve microlitres of amplified sample was electrophoresed in a 2% agarose gel containing ethidium bromide at 0·1 µg/ml. Gels were run at 6 V/cm for 45 min and PCR products were visualized by u.v. transillumination. Seven microlitres of amplified product was used for analysis by polyacrylamide gel electrophoresis. Gels were run at 6 V/cm for 2 h and then stained with silver (Herring et al. 1982). Fragments from a *Hae*III restriction endonuclease digest of plasmid pUC18 were used as molecular weight markers.

Statistical analysis

Log-transformations of the BMCC, total bacteria counts (TBC) and thermoduric bacteria counts were used because

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Table 1. PCR results on five consecutive bulk-milk samples and geometric means of BMCC, total bacteria counts and thermoduric bacteria counts of herds classified as having low BMCC

	Geometric mean			PCR result				
Herd	BMCC ($\times 10^{-3}$)	Total bacteria ($\times 10^{-3}$ cells/ml)	Thermoduric bacteria (cells/ml)	Week 1	Week 2	Week 3	Week 4	Week 5
1	48·7	20·4	868·5	Su, Sau	Su	—	Su	Sd
2	59·2	28·9	1290·5	Sa	Sa	—	—	—
3	61·2	18·3	510·4	Su	—	—	—	†
4	66·6	18·9	310·7	Su	Su	—	Su	†
5	66·9	35·3	975·7	Su	Su	†	—	†
6	75·1	28·3	763·4	—	Su	—	—	Sd, Su
7	82·8	15·5	1190·6	Sd, Su	Sd, Su	—	Sd	—
8	86·9	35·8	740·6	Su	Su	†	Su	Sd, Su
9	87·7	17·9	1206·8	Su	—	—	—	Su
10	88·1	31·3	594·7	Su	Su	—	Su, Sau	Su, Sau
11	90·0	42·2	743·6	Su, Sau	Su, Sau	—	Su, Sau	Su
12	93·3	18·6	827·1	Sd	Sd, Su	†	Sd	—
13	98·4	29·4	958·4	—	—	—	Su	†
14	112·8	34·3	894·8	Su	—	—	Su	—

Abbreviations: Sa = *Streptococcus agalactiae*; Sd = *Streptococcus dysgalactiae*; Su = *Streptococcus uberis*; Sau = *Staphylococcus aureus*; — = negative; † = no sample

Table 2. PCR results on five consecutive bulk milk samples and geometric means of BMCC, total bacteria counts and thermoduric bacteria counts of herds classified as having medium BMCC

	Geometric mean			PCR result				
Herd	BMCC ($\times 10^{-3}$)	Total bacteria ($\times 10^{-3}$ cells/ml)	Thermoduric bacteria (cells/ml)	Week 1	Week 2	Week 3	Week 4	Week 5
15	211·6	25·7	538·6	Sau	—	—	Su	Su
16	269·9	32·2	763·5	Sa	Sa	Sa, Su	Sa	Sa, Su
17	271·3	26·5	1163·6	Sd, Su	Su	Su, Sau	Su, Sau	Su
18	291·0	63·5	255·1	Su	Su	—	Su	Su
19	295·4	26·5	392·0	—	Sd	—	†	†
20	303·1	34·5	1364·7	Su	Sd, Su	Su, Sau	Sd, Su Sau	Sd, Sau
21	332·9	58·1	389·4	Sd, Su, Sau	†	Su	Sd	Sd, Su, Sau
22	348·4	52·3	1403·1	—	Su	Su	Su	Su
23	349·4	48·9	679·0	Su	Sd, Su	—	Su	Su
24	371·4	55·7	862·0	Su, Sau	Sd, Su	—	—	Sd, Su
25	378·7	34·0	955·9	Sau	Sd, Su, Sau	Su	Su	Sd, Su, Sau
26	378·9	56·4	1619·4	Sd	Sd, Su	Sd, Su	†	†
27	379·8	37·6	734·1	Sau	Sd, Sau	—	—	Sd, Su, Sau

Abbreviations: Sa = *Streptococcus agalactiae*; Sd = *Streptococcus dysgalactiae*; Su = *Streptococcus uberis*; Sau = *Staphylococcus aureus*; — = negative; † = no sample

the raw data were not normally distributed. The geometric means (gm) of BMCC, TBC and thermoduric bacteria counts of sequential samples collected from each herd were determined. One-way analysis of variance was used for comparing mean TBC and mean thermoduric bacteria counts between groups of herds. Fisher's exact test was used to examine the relationship between the presence of specific organisms in the first sample for each herd and either the BMCC, TBC or thermoduric bacteria count for that sample. Two categories of TBC were used to calculate Fisher's exact test, using an arbitrary threshold of 50 000 bacteria/ml. Thermoduric bacteria counts were also divided into two categories, using 1000 bacteria/ml as the arbitrary threshold. Statistical significance was defined as $P<0{\cdot}05$. Agreement between the detection of specific organisms in multiple samples from the same herd was analysed using the kappa statistic (Fleiss, 1981). A kappa value >0·8 is regarded as very good agreement, 0·61–0·8 good, 0·41–0·6 moderate, 0·21–0·4 fair, and <0·2 poor (Altman, 1991).

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Table 3. PCR results on five consecutive bulk milk samples and geometric means of BMCC, total bacteria counts and thermoduric bacteria counts of herds classified as having high BMCC

	Geometric mean			PCR result				
Herd	BMCC ($\times 10^{-3}$)	Total bacteria ($\times 10^{-3}$ cells/ml)	Thermoduric bacteria (cells/ml)	Week 1	Week 2	Week 3	Week 4	Week 5
28	467·0	89·1	1023·4	—	Sd, Sau	†	†	†
29	485·0	41·4	754·7	—	—	—	—	—
30	488·5	85·4	2534·1	Sa, Sd, Su	†	†	Sa, Su	Sa, Su
31	495·1	80·1	3260·4	Sd	†	†	Sd, Su	Sd, Su
32	499·6	297·0	501·4	Sa	Sa	Sa	†	†
33	520·9	123·0	968·8	Sd	Sd	Sd, Su	Sd, Su	†
34	551·5	35·8	1099·5	Sa, Sd, Su, Sau	Sa, Sd	Sa, Sd, Su	Sd, Sau	†
35	555·5	48·7	1401·9	Sau	Sa, Sd, Su, Sau	Sa, Sd, Su	Sa, Su, Sau	Sa, Su
36	576·6	58·5	1356·0	Sa, Su	Sa, Su	Sa, Su	Sa, Su	Sa
37	583·6	97·6	1627·8	Sd, Su	†	†	Sd	Sd, Su
38	607·0	63·4	1084·5	Sd	Sd, Su, Sau	†	†	Sd, Su
39	610·3	54·2	1827·7	Sa, Su	Sa	Sa, Su	Sa, Sd, Su	Sa
40	624·4	41·7	838·8	Sa, Su	†	†	Sa, Su	Sa
41	659·7	156·3	736·0	Sa	Sa	Sa	†	†
42	716·0	430·5	748·9	Sa	Sa	†	†	†

Abbreviations: Sa = *Streptococcus agalactiae*; Sd = *Streptococcus dysgalactiae*; Su = *Streptococcus uberis*; Sau = *Staphylococcus aureus*; — = negative; † = no sample

Results

PCR results

PCR results on bulk milk samples collected from 42 herds are presented in Tables 1–3. Of the 176 bulk milk samples, 43 samples were negative for all four bacterial species, 72 samples were positive for one species and 61 samples were positive for more than one species. Of the samples negative by PCR, 60% (26/43) were from herds with low BMCC; 26% (11/43) of such samples were from herds with medium BMCC and 13% (6/43) were from herds with high BMCC. Of the samples positive for more than one species, 82% (50/61) were from herds with medium and high BMCC. The species most frequently present in bulk milk was *Str. uberis*, being found in 78 (44%) samples. *Str. dysgalactiae*, *Str. agalactiae* and *Staph. aureus* were present in 49 (28%), 38 (22%) and 28 (16%) samples respectively. *Str. uberis* was found in at least one sample from 35 herds. *Str. dysgalactiae* was detected in 23 herds. *Staph. aureus* was detected in 14 herds and *Str. agalactiae* was detected in 11 herds.

Consistency of detection of *Staph. aureus*, *Str. agalactiae*, *Str. dysgalactiae* and *Str. uberis* in sequential samples was examined by using kappa statistics (Table 4). Good agreement for the presence of these organisms in multiple samples was found only for *Str. agalactiae*. Where *Str. agalactiae* was detected by PCR, it was consistently detected in repeated samples collected from herds. *Str. dysgalactiae* was found to be more consistently detected in herds with high BMCC than in herds with low or medium BMCC (Table 5), but there was no relationship between consistency of detection and BMCC with the other three pathogens.

BMCC, TBC and thermoduric bacteria counts

BMCC of samples from each herd were relatively stable during the study period. Mean $BMCC_{gm}$ of samples from herds in groups 1, 2 and 3 were 79 831, 321 697 and 562 714 cells/ml respectively. Relationships between BMCC and TBC and thermoduric bacteria counts were also investigated (Table 6). Mean TBC_{gm} in samples from group 3 (113 510 bacteria/ml) was significantly higher than mean TBC_{gm} in samples from group 1 (26 800 bacteria/ml) and group 2 (42 460 bacteria/ml) ($P<0{\cdot}01$). Similarly, mean thermoduric bacteria counts were significantly higher in group 3 herds (1317 cfu/ml) than in either group 1 or group 2 herds (848 and 855 cfu/ml respectively, $P<0{\cdot}05$).

Association between the presence of pathogens in bulk milk samples and BMCC

There were no obvious relationships between the presence or absence of *Staph. aureus*, *Str. dysgalactiae* or *Str. uberis* and BMCC in herds. Prevalence of each of the three pathogens was not significantly different between the three groups of herds ($P=0{\cdot}197$, $0{\cdot}277$ and $0{\cdot}206$ respectively). However, a significant relationship was found between prevalence of *Str. agalactiae* and BMCC ($P<0{\cdot}01$). *Str. agalactiae* was more commonly identified in samples from herds in group 3 (8/15) than in samples from herds in groups 1 (1/14) and 2 (1/13).

Association between the presence of pathogens in bulk milk samples and total bacterial counts

There were no significant differences between the prevalence of *Staph. aureus*, *Str. dysgalactiae* or *Str. uberis* in

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Table 4. Agreement between PCR assays for *Staph. aureus, Str. agalactiae, Str. dysgalactiae and Str. uberis* on repeated bulk-milk samples from 42 herds

	PCR results (n=number of herds)				
Organism	Negative†	Variable results‡	Positive§	kappa value	SE‖
Staph. aureus	28	14	0	0·23	0·06
Str. agalactiae	31	3	8	0·88	0·06
Str. dysgalactiae	18	18	6	0·42	0·06
Str. uberis	7	32	3	0·13	0·06

† Negative by PCR in all samples collected from a herd
‡ Positive by PCR in at least one sample, but not all
§ Positive by PCR in all samples collected from a herd
‖ Standard error of kappa

Table 5. Agreement between PCR assays for *Str. dysgalactiae* in repeated bulk-milk samples in herds with low, medium and high BMCC

		PCR results (n=number of herds)				
BMCC groups ($\times 10^{-3}$ cells/ml)	No. of herds	Negative†	Variable results‡	Positive§	kappa value	SE‖
1 (<200)	14	8	6	0	0·23	0·094
2 (200–400)	13	4	8	1	0·21	0·096
3 (>400)	15	6	4	5	0·67	0·12

† Negative for *Str. dysgalactiae* in all samples collected from a herd
‡ Positive for *Str. dysgalactiae* in at least one sample, but not all
§ Positive for *Str. dysgalactiae* in all samples collected from a herd
‖ Standard error of kappa

Table 6. Distribution of total bacteria counts and thermoduric bacteria counts in different BMCC groups

	Total bacteria counts ($\times 10^{-3}$ cells/ml)				Thermoduric bacteria counts (cells/ml)			
BMCC groups ($\times 10^{-3}$ cells/ml)	$Mean_{gm}$†	SD	Minimum	Maximum	$Mean_{gm}$	SD	Minimum	Maximum
1 (<200)	26·80*	8·45	15·47	42·20	848·28	273·14	310·67	1290·45
2 (200–400)	42·46*	13·71	25·66	63·54	855·41	427·69	255·1	1619·41
3 (>400)	113·51	109·71	35·82	430·50	1317·58	746·58	501·4	3260·35

* Significantly different (P<0·05) from the high BMCC group
† $Mean_{gm}$=Mean of the geometric means for each herd

samples from herds with TBC >50 000 cells/ml and samples from herds with TBC <50 000 cells/ml (P=0·51, 0·53 and 0·40 respectively).

A relationship between prevalence of *Str. agalactiae* and TBC was only found in samples from herds that were consistently positive for *Str. agalactiae*. *Str. agalactiae* was more consistently found in samples from herds with TBC >50 000 cells/ml than in samples from herds with TBC <50 000 cells/ml (P=0·045). The three herds that were inconsistently positive for *Str. agalactiae* had an average TBC <50 000 cells/ml. When the results of these three herds were included in the statistical analyses to compare the differences between TBC in samples from herds that were negative for *Str. agalactiae* and in samples from herds that were positive for *Str. agalactiae*, there was no relationship between high TBC and the presence of *Str. agalactiae* (P=0·28).

Association between the presence of pathogens in bulk milk samples and thermoduric bacterial counts

No relationship was found between the presence of *Staph. aureus, Str. agalactiae, Str. dysgalactiae* or *Str. uberis* and numbers of thermoduric bacteria in bulk milk samples (P=0·74, 1·0, 0·058 and 0·68 respectively). Prevalence of each organism in samples from herds with thermoduric bacteria counts >1000 cells/ml was not significantly different from that in herds with thermoduric bacteria counts <1000 cells/ml.

Discussion

The multiplex PCR assay used in this study was an easy and rapid method to detect the four major mastitis pathogens, *Staph. aureus, Str. agalactiae, Str. dysgalactiae* and

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Str. uberis, in bulk milk. PCR required little time to process and results were easier to interpret than bulk milk cultures, which are subject to contamination with bacteria from the environment.

Str. uberis and *Str. dysgalactiae* were the most prevalent species in bulk milk samples. As found in previous studies (Bramley et al. 1984; Hogan et al. 1986), their presence in bulk milk was not significantly associated with higher BMCC or higher numbers of bacteria in bulk milk. As these species are ubiquitous in the cow's environment, it is more likely that these organisms enter the bulk milk from non-specific contamination rather than in milk from infected cows. The inconsistency of the detection of these organisms in sequential samples also suggests sporadic contamination of the bulk milk samples by bacteria from the environment. Nevertheless, the consistent detection of *Str. dysgalactiae* in a herd with high BMCC might suggest the presence of *Str. dysgalactiae* mastitis in the herd (Herds 4, 6, 7 and 10 in Group 3). Clinical mastitis due to *Str. dysgalactiae* has been found to be more common in herds with high BMCC (Barkema et al. 1998).

A considerably higher prevalence and incidence of clinical mastitis due to *Staph. aureus* has been observed by analysis of quarter milk samples in herds with high BMCC (Erskine et al. 1988; Barkema et al. 1998). There was no significant association, however, between presence of *Staph. aureus* in bulk samples and BMCC in the present study. Although it has been proposed that presence of *Staph. aureus* in bulk milk is a reliable indicator of infection of the herd due to this organism (Gonzalez et al. 1986; Godkin & Leslie, 1993), interpretation of the detection of *Staph. aureus* in bulk milk is not as reliable as interpretation of the detection of *Str. agalactiae*. *Staph. aureus* may enter bulk milk from non-milk sources as there is evidence of contamination of both composite and bulk milk by *Staph. aureus* strains from milkers (Adesiyun et al. 1997). The method used to collect samples in this study did not allow the origin of the *Staph. aureus* to be ascertained, and thus the possibility that *Staph. aureus* came from the environment rather than from infected cows cannot be excluded.

There was a close correlation between detection of *Str. agalactiae* and either high BMCC or total bacteria counts, which concurs with previous reports (Ward & Shultz, 1972; Hogan et al. 1986; Erskine et al. 1987; Keefe, 1997). It was found that most herds consistently positive for *Str. agalactiae* had high total bacteria counts, suggesting either a relatively high prevalence of *Str. agalactiae* infections in these herds or that the infections in these herds were in a phase of high shedding. However, none of the herds from which *Str. agalactiae* was inconsistently detected had average total bacterial counts of more than 50 000 cells/ml. Intermittent recovery of *Str. agalactiae* in these herds may suggest that prevalence of *Str. agalactiae* infections in these herds was low. It is also possible that there was a change of the herd profile during the study period, with infected cows being treated or being removed from this herd or being milked separately, such that infected milk from these cows did not get into the vat.

Str. agalactiae is an obligate parasite of the udder and its presence in bulk milk is a conclusive demonstration of shedding from infected quarters (Bartlett, 1991). Hence, identification of *Str. agalactiae* in bulk milk is indicative of the presence of *Str. agalactiae* mastitis in the herd. Further investigation is necessary to determine the sensitivity of the PCR assay for detection of the presence of *Str. agalactiae* infection in the herd. Sensitivity of bulk milk cultures for detecting *Str. agalactiae* varies from 20% to 84% (Postle, 1968; Gonzales et al. 1986; Bartlett, 1991; Keefe, 1997). The variation is thought to be due to differences in methods used as well as to prevalence of infected cows within the herd and rate of bacterial shedding, which is related to the stage of infection (Keefe, 1997). It has been reported that at least 5% of the quarters in a herd must be infected with *Str. agalactiae* before the organism can be detected in bulk milk cultures (Postle, 1968). The use of enrichment before DNA extraction and the high sensitivity of PCR itself might be expected to yield a higher sensitivity for detection of *Str. agalactiae*. The good agreement seen with repeated samples from the same herds might be an indication of the high sensitivity of the assay.

The results indicated that most herds with low BMCC were free of *Str. agalactiae* infections, as *Str. agalactiae* was found in only a single herd with a low BMCC and then in only the first two samples. Conversely, prevalence of *Str. agalactiae* in samples from high BMCC herds was high, accounting for 60% of the high BMCC herds evaluated. Although *Str. agalactiae* can be readily eliminated (Keefe, 1997), the results indicate that this organism remains a significant cause of mastitis in many herds in Australia.

There was no difference in the number of thermoduric bacteria in samples from herds with different BMCC and there was no relationship between the presence of each of the mastitis pathogens and thermoduric bacteria counts. This would be expected as the mastitis pathogens cannot survive pasteurization and the presence of thermoduric bacteria reflects inadequate milking machine hygiene rather than the mastitis status of the herd.

Regular analysis of bulk milk by PCR may be a useful tool for determining the herd status with regard to *Str. agalactiae*, but appears to be of less value for monitoring the occurrence of mastitis caused by *Staph. aureus*, *Str. dysgalactiae* and *Str. uberis*. Use of PCR in conjunction with BMCC could be a rapid and convenient means to determine if *Str. agalactiae* has been eradicated from infected herds after treatment and whether appropriate control programmes have been implemented. Alternatively, PCR could be used to detect if infected animals have been introduced into herds that were free of this organism. It may also be used to evaluate the efficacy of control practices adopted for herds. Relationship between

positive PCR results and prevalence of infected cows in the herd requires further clarification.

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