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## Diagnosis and treatment of subclinical mastitis in early lactation in dairy goats

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### ABSTRACT

The objectives of the study were to define the sensitivity and specificity of the California Mastitis Test (CMT) in determining the presence of intramammary infection in postpartum dairy goats and to determine whether antibiotic therapy increased bacteriological cure rate and lowered somatic cell count (SCC) compared with untreated controls. A CMT was performed and milk samples were collected for bacteriology from 211 glands of 106 does between 0 and 10 d after kidding. From a population of 3,239 glands from goats in 4 commercial herds, goats with one or both glands with a CMT score of >1 and from which bacteria were isolated were either assigned to be treated with 3 intramammary infusions at 12-h intervals of 75 mg of sodium ampicillin and 250 mg of sodium cloxacillin ( $n = 57$  glands) or left as untreated controls ( $n = 49$  glands). Milk samples were collected again  $14 \pm 3$  and  $21 \pm 3$  d later for bacteriology and SCC determination. Composite milk yield, goat SCC, length of lactation, and survival data were collected. A partial budget was constructed to assess the cost effectiveness of treatment. At a cut point of greater than trace, the sensitivity, specificity, and positive and negative predictive values of the CMT were 0.74, 0.74, 0.42, and 0.92, respectively. Treatment increased the bacteriological cure rate compared with no treatment [30/57 (53%) vs. 6/49 (12%)], but there was a pathogen by treatment interaction whereby treatment increased cure proportion in glands infected with minor, but not major, pathogens. Treatment reduced the foremilk gland-level SCC [1,595 (95% CI = 1,106–2,300) vs. 3,028 (95% CI = 2,091–4,385) geometric mean ( $\times 1,000$ ) cells/mL] but not the SCC at goat level [1,596 (95% CI = 1,219–2,090) vs. 1,488 (95% CI = 1,132–1,955) geometric mean ( $\times 1,000$ ) cells/mL] compared with no treatment. Milk yield,

risk of removal from the herd, and length of lactation were not altered by treatment. Treatment resulted in a loss of NZ\$20.39/does. It was concluded that use of the CMT as a screening test resulted in a higher likelihood of finding a gland that would be infected than selecting a gland at random. Treatment increased bacteriological cure rate and reduced SCC at gland level compared with no treatment. However, at goat level, milk yield, SCC, and survival were not altered, resulting in no economic benefit of treatment.

**Key words:** goat, subclinical mastitis, diagnosis, therapy

### INTRODUCTION

The prevalence of subclinical mastitis in dairy goats ranges between 5 and 30% (Contreras et al., 2007), with CNS being the most prevalent isolates (Kalogridou-Vassiliadou, 1991; White and Hinckley, 1999; Contreras et al., 2007). *Staphylococcus aureus* is also commonly isolated, but in the majority of studies its prevalence is lower than that of CNS (Contreras et al., 2007). However, *S. aureus* appears to induce a higher SCC than most of the CNS species (Deinhofer and Perntner, 1995) and is considered a major pathogen in goats.

Somatic cell count is commonly used as a milk quality standard at the individual animal and herd level. Somatic cell count is higher in doe glands from which bacteria are isolated (Contreras et al., 1996; White and Hinckley, 1999), and this is associated with an influx of neutrophils (Droke et al., 1993). However, SCC appears to increase in later lactation and in older goats, even in the absence of IMI (Wilson et al., 1995). Estrus (McDougall and Voermans, 2002), caprine arthritis encephalitis virus (Ryan et al., 1993), and stressors such as vaccination (Lerondelle et al., 1992) also increase SCC in goats.

Somatic cell count is inversely related with milk yield (Zeng and Escobar, 1995), and increased SCC affects milk composition in does (Raynal-Ljutovac et al., 2007). Increased SCC and California Mastitis Test (CMT) scores were associated with increased milk pH, and a

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CMT score  $>2.5$  resulted in lower cheese yields (Galina et al., 1996). Half-udder milk yield was approximately 35% lower and SCC was 4-fold higher following deliberate infection with CNS (Leitner et al., 2004). Using structural equation modeling, which allows for bidirectional effects of 2 variables on each other, as well as repeated measures associations, it was estimated that a 1-unit increase in log base 2 SCC was associated with a 16 to 20 g/gland per day decrease in doe milk yield (de los Campos et al., 2006).

The CMT score, an indirect measure of SCC, is well correlated with SCC in goats (Maisi, 1987; Contreras et al., 1996). Additionally, the CMT score is positively associated with the presence of an IMI; glands with a CMT score  $>0$  have sensitivity, specificity, positive predictive value, and negative predictive value of 0.79, 0.67, 0.38, and 0.93, respectively, when assessed at 6 wk after kidding (McDougall et al., 2001). In dairy cattle, a rapid decline in gland-level SCC occurs in the first 10 d postpartum along with an associated decrease in sensitivity and increase in specificity for detection of IMI using SCC. The effect of DIM on CMT score is less clear (Sargeant et al., 2001). The test characteristics of the CMT appear not to have been assessed in goats in the first week postpartum. The CMT test has the advantages of being undertaken beside the animal rapidly and with minimal cost. Thus, CMT may have a role as a screening test to identify goats for further examination (e.g., bacteriology) or to withhold them from supply to maintain an acceptable bulk milk SCC.

Intramammary infections with CNS may persist for extended periods of time, with the same isolate present for up to 7 mo (Contreras et al., 1997). Treatment of IMI may increase cure rate and result in a lower SCC and higher milk yields compared with no treatment. The objectives of this study were to define the sensitivity and specificity of the CMT in diagnosing IMI in the first 10 d after kidding and to assess the efficacy of treatment of IMI early in lactation in dairy goats.

## MATERIALS AND METHODS

These studies were undertaken with approval of the Animal Ethics Committee of AgResearch Ruakura (Hamilton, New Zealand). Spring-kidding dairy goat herds ( $n = 4$ ) in which herd owners undertook milk production recording (herd testing), maintained records of treatments of goats, and were willing to be involved were enrolled. To assess the test characteristics of CMT, foremilk samples were collected by technicians for microbiology, SCC, and CMT between 0 and 10 d after kidding from a subset of 106 goats (211 glands) from the 4 herds.

### **Effect of Treatment on Bacteriological Cure, SCC, and Production**

In a treatment study, the CMT score on a scale of 0, trace, 1, 2, or 3 was determined for each gland ( $n = 3,239$ ) of goats (from 4 herds) that had been kidded 1 to 4 d. Each gland was sampled at only 1 time point. From those with a CMT score  $>1$ , 1 foremilk sample ( $\sim 5$  mL) was collected for bacteriology and a second foremilk sample ( $\sim 25$  mL) was collected for SCC determination. When a gram-positive major mastitis pathogen (i.e., *S. aureus* or *Streptococcus* spp.) was isolated, the goat was either assigned to treatment or left as an untreated control. Additionally, a random sample of one of 2 sequential goats from which CNS or *Corynebacterium* spp. (minor pathogens) were isolated was either assigned to be treated or left as an untreated control as above. Treatment was assigned at the goat level, so where 2 glands from the same goat were enrolled, both were either treated or left untreated.

Treatment consisted of 3 intramammary infusions at 12-h intervals of 75 mg of sodium ampicillin and 250 mg of sodium cloxacillin (Lactaclox, Norbook Animal Health, Auckland, New Zealand). Milk was withheld for 108 h (9 milkings) after administration of the third infusion. Treatment (d 0) occurred 3 or 4 d after initial sample collection. At  $14 \pm 3$  and  $21 \pm 3$  d after initiation of treatment, foremilk samples were collected for bacteriology and SCC determination from each enrolled gland. Records including herd testing data, disease dates and reasons, removals (culls or deaths) with dates and reasons, final milking date, and subsequent kidding dates were recovered.

### **Laboratory Procedures**

Following gentle inversion of the milk sample, 10  $\mu$ L of milk was spread onto a quadrant of a 0.1% esculin, 5% sheep blood agar plate (Fort Richard, Auckland, New Zealand). All samples were incubated for 48 h at 37°C before assessment at 48 h. Any isolate present with  $>2$  colonies/plate was speciated per NMC (1999) recommendations. A sample was defined as contaminated when  $>3$  distinct colony types of bacteria were present. A gland was defined as cured where the pathogen isolated from the pretreatment sample was not isolated from either of the posttreatment samples. In the 2 glands in which there were 2 isolates pretreatment (in both cases a *Streptococcus uberis* and a CNS, with 1 gland from each treatment group), the gland was coded as infected with *Strep. uberis* for descriptive purposes, but cure was defined considering both isolates. In one case cure occurred (no bacteria were isolated from ei-

ther posttreatment sample), whereas in the other case *Strep. uberis* was isolated once posttreatment, so the gland was defined as uncured. Initially, all CNS were treated as 1 group, ignoring the species identity. However, following species identification (see below), cure for each gland infected with CNS was reassessed with knowledge of the specific CNS species. At goat level, cure was defined as occurring when both glands were cured if 2 glands were enrolled. A gland was defined as having acquired a new IMI if a bacterial species not isolated at enrollment was isolated in either of the post-treatment samples.

A total of 121 CNS isolates from both pre- ( $n = 57$ ) and posttreatment ( $n = 64$ ) from glands that were included in the treatment study were subcultured and DNA was extracted as described by Únal et al. (1992) after 24 h of growth. The CNS species were defined using transfer RNA-intergenic spacer PCR (tDNA-PCR) in combination with capillary electrophoresis, supplemented with sequencing of the *rpoB* housekeeping gene if needed (Supré et al., 2009). The SCC was determined by a fluoro-optic method (Foss 5000, Foss, Hillerød, Denmark). To demonstrate a 30% difference in bacteriological cure rate between treated and untreated glands, 45 cases/treatment group were required at  $\alpha$  ( $P$ -value) = 0.05 and  $\beta$  ( $1 - \text{power}$ ) = 0.2.

### Statistical Analysis

#### *Relationship Between IMI, SCC, and CMT.*

The relationship between the CMT score and the probability of an IMI was analyzed by chi-squared following dichotomization of the CMT scores as  $>0$ , greater than trace,  $>1$ , or  $>2$ . The sensitivity, specificity, positive predictive value, and negative predictive value were calculated using standard techniques and the 95% confidence intervals were estimated (Wilson, 1927). The relationship was further examined by creating receiver operator curves. The relationships between log base 2 SCC and CMT score, DIM (coded as 0–1, 2, 3, and  $\geq 4$  d), age (coded into quintiles of approximately similar group sizes: 1, 2–3, 4–7, and  $>7$  yr), and IMI were initially evaluated by 1-way ANOVA and all were significantly associated. However, because gland was clustered within goat and goat within herd, a linear mixed model was created with gland and goat as random effects and with the CMT score (coded as 0, trace, 1, and 2 and 3) and herd as fixed effects. A separate linear mixed model was run to examine the effect of DIM and IMI status (infected vs. not infected) on log base 2 SCC.

**Effect of Treatment on Cure, SCC, and Production.** Treatment group balance was assessed using ANOVA for continuous variables and chi-squared for categorical variables. The distribution of the CNS spe-

cies between treatment groups and among herds was compared using chi-squared.

Bacteriological cure rate was analyzed using Mantel-Haenszel analysis with treatment group as the main effect and stratified by pathogen type (i.e., minor vs. major). The analysis was initially undertaken using cure databased on CNS at genus level and then repeated with knowledge of the CNS species.

The SCC in foremilk on d 14 and 21 were log base 2 transformed for analysis. Variables associated at the univariate level ( $P < 0.2$ ) were included in a linear mixed model with herd, treatment, sample time, and IMI categorized as minor or major as fixed effects, gland within goat as a random effect, and sample time as a repeated measure.

The associations between survival (i.e., the goat was in the herd in the subsequent lactation) and treatment, herd, age (coded into quintiles of approximately similar group sizes:  $<3$ , 3–4, 5–7, and  $>7$  yr old), Julian kidding date (as a categorical variable), IMI (coded as minor or major), and number of glands within the goat enrolled (i.e., 1 or 2) were initially tested for bivariate association with cure. Variables found to be associated ( $P < 0.2$ ) were offered to reverse stepwise logistic regression model using likelihood ratio as the inclusion-exclusion criteria. First-order interactions between the treatment group and the other main effects left in the model were examined. The length of lactation was analyzed using Kaplan Meier survival analysis and the effect of treatment on lactation length was tested using the log rank test.

The goat-level log base 2 SCC and untransformed milk solids production (i.e., fat and protein; kg/goat per day) data from each of the 4 production recordings (herd tests) that occurred at 47 (SD = 15), 113 (SD = 19), 182 (SD = 13), and 240 (SD = 15) DIM were analyzed in linear mixed models with goat as a random effect and herd, treatment, age (coded as  $<3$ , 3–4, 5–7, and  $>7$  yr old), herd test number, and IMI pathogen type as fixed effects. Herd test number was treated as a repeated measure and an autoregressive 1 covariance structure was used.

Data from the above models are presented as estimated marginal means, SEM, and standard errors of the difference, from which geometric means and SEM were calculated as required (i.e., for the log-transformed SCC data). Pairwise comparisons between levels of included variables were undertaken using the Bonferroni adjustment for multiple comparisons.

A partial budget for treatment of the goats was developed. The current (2009) payment to producers in New Zealand is NZ\$12/kg of milk solids (i.e., fat, protein, and lactose). It was assumed that benefits would include a reduced log base 2 SCC during the 21

d after treatment, and this was adjusted by the average number of glands in enrolled does (1.4) as was found in the current study. Another assumption was that no compensatory increase in production would occur where 1 gland was unaffected. The average daily milk solids production (fat and protein yield as kg/doe per day) of all does from the 4 herds was 0.16 kg/doe per day in the current study. The total milk solids (i.e., fat, protein, and lactose) from these goats was then estimated using the milk composition data of Zeng and Escobar (1995), who found that fat and protein constituted about 63% of the total milk solids. Thus, the average daily milk solids production was 0.25 kg/doe per day. Each unit increase in the log base 2 SCC is reported to reduce daily milk yield by 16 g/doe per day (de los Campos et al., 2006). It was assumed that the treatment resulted in an additional 10 d of lactation (see below). Additional costs included the cost of treatment (3 treatments at NZ\$5/treatment), labor cost (NZ\$1/treatment), the cost of undertaking the CMT (NZ\$0.39/test), and the cost of microbiology (NZ\$8/sample). Reduced revenue was attributed to the milk discard period of 4.5 d. No reduced costs associated with reduced risk of clinical or new subclinical IMI or for reduced culling were assumed. The cost benefit was then calculated as (extra revenue + reduced costs) – (reduced revenue + increased costs). Statistical analyses were undertaken using SPSS (v. 18, SPSS Inc., Chicago, IL).

## RESULTS

### **Relationship Between IMI, SCC, and CMT**

The prevalence of IMI was 43/211 (20.4%) glands. Infections were with CNS (n = 17; 8%), *Corynebacterium* spp. (n = 23; 11%), *S. aureus* (n = 2; 1%), and *Strep. uberis* (n = 1; 0.5%).

The frequency (%) of CMT scores was 89 (42%), 46 (22%), 28 (13%), 43 (20%), and 6 (3%) for scores 0, trace, 1, 2, and 3, respectively. The sensitivity and negative predictive value increased, and the specificity and positive predictive value increased, with increasing CMT cut off above which the test was declared positive (Figure 1a and b). The test characteristics did not change by DIM ( $P = 0.32$ ; Figure 1c and d) and there was no DIM by CMT interaction ( $P = 0.11$ ) when a score >1 was used as the cut point. The area under the receiver operator curve was 0.80 (SE = 0.04) and the CMT was significantly better than no test at predicting the presence of an IMI ( $P < 0.001$ ; Figure 2). The highest sensitivity and specificity occurred at a cut point >1 where the sensitivity and specificity were 0.63 and 0.87, respectively, at which point Youden's index

(i.e., sensitivity + specificity – 1; Thrusfield, 2005) was maximal.

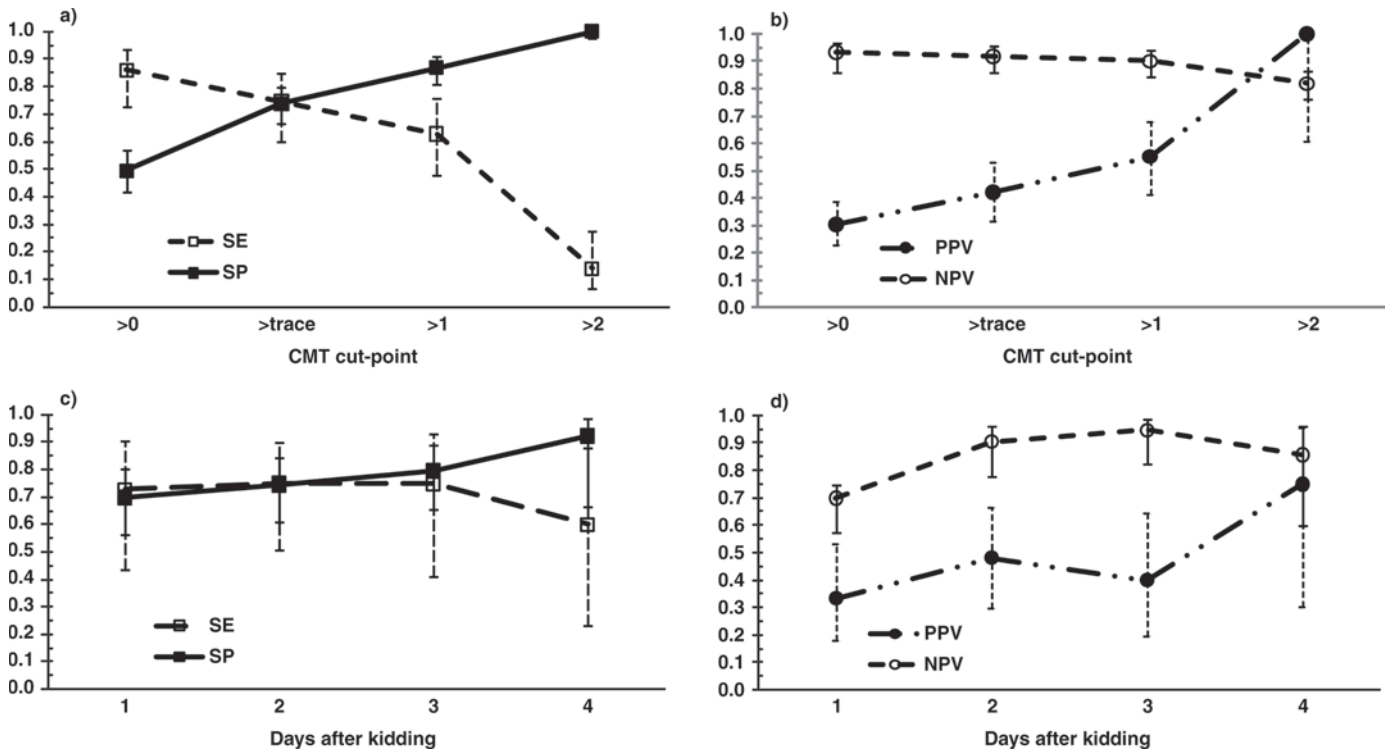
Log base 2 of foremilk SCC was positively associated with the CMT score ( $P < 0.001$ ; Figure 3a). The log base 2 SCC was higher in infected glands than in uninfected glands [2,006 (95% CI = 1,079–3,750) vs. 484 (95% CI = 346–3,676)  $\times$  1,000 cells/mL;  $P < 0.001$ ] and tended to decline with DIM ( $P = 0.07$ ; Figure 3b). There was no interaction of DIM with IMI status ( $P = 0.71$ ; Figure 3b), and herd was not significant ( $P = 0.10$ ).

### **Effect of Treatment on Bacteriological Cure, SCC, and Production**

Of the 3,239 glands examined, 512 (15.8%) had a CMT score >1; from these, 506 samples were collected for bacteriology. Of these, 233 (46%), 174 (34%), and 65 (13%) isolated no bacteria, CNS, and *Corynebacterium* spp., respectively (Table 1). A total of 57 of the pretreatment CNS were identified to species level as *Staphylococcus simulans* (55%), *Staphylococcus epidermidis* (13%), *Staphylococcus caprae* (12%), *Staphylococcus lugdunensis* (10%), *Staphylococcus hyicus* (3%), and *Staphylococcus cohnii* ssp. *urealyticus*, *Staphylococcus warneri*, and *Staphylococcus xylosus* (each 2%). No difference in distribution of the CNS species was found between the treatment groups ( $P = 0.61$ ). However, variation did exist among herds in the distribution of CNS species ( $P < 0.01$ ).

**Gland Level.** A total of 106 glands (49 in the control and 57 in the treatment group) met the final enrollment criteria and were resampled posttreatment (Table 2). No significant differences were found between the groups, but the treatment group tended ( $P = 0.07$ ) to be sampled earlier postkidding and to have more Saanens. The kidding date did not differ [July 16 (SEM = 1.7 d) vs. July 19 (SEM = 1.7 d) for the control and treatment groups, respectively;  $P = 0.26$ ] and there was no difference in the gland-level log<sub>10</sub> foremilk SCC before treatment [3.98 (SEM = 0.05) vs. 3.99 (SEM = 0.05) for the control and treatment groups, respectively;  $P = 0.88$ ].

The overall cure proportion was higher for treated glands than for control glands [30/57 (52.6%) vs. 6/49 (12.2%)]. However, a treatment group by pathogen type interaction ( $P < 0.001$ ) existed whereby treatment resulted in a higher cure proportion than controls for glands infected with a minor pathogen ( $P < 0.001$ ; Table 3), but no difference in cure proportion was found between treated and control glands for glands infected with a major pathogen ( $P = 0.10$ ; Table 3).



**Figure 1.** The sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV; 95% CI) for any intramammary infection for the California Mastitis Test (CMT) by (a, b) the cut-point and (c, d) DIM using greater than trace as the cut point.

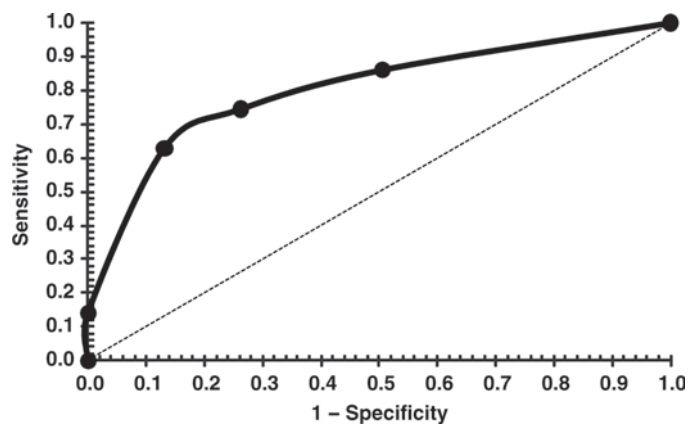
When the cure proportion was reanalyzed using the CNS species rather than the genus level designation, in only 1 case did the cure outcome change. In this case *S. caprae* was isolated pretreatment and *S. simulans* was isolated twice post treatment and the gland was reclassified as having cured.

New IMI were found in 7 of 106 (6.6%) glands. Six of the new IMI were attributed to CNS and 1 was attributed to yeast. Glands initially infected with a major pathogen tended to be more likely to acquire a new IMI (major or minor) than glands previously infected with a minor IMI [3/22 (13.6%) vs. 3/84 (3.54%);  $P = 0.07$ ]. Treatment did not alter the risk of a new IMI [4/49 (8.1%) vs. 2/57 (3.5%) for control and treatment, respectively;  $P = 0.30$ ].

The foremilk SCC were lower in the treated glands than in control glands [1,595 (95% CI = 1,106–2,300) vs. 3,028 (95% CI = 2,091–4,385) geometric mean ( $\times 1,000$ ) cells/mL for treated and control glands, respectively;  $P = 0.006$ ]. The difference in log base 2 terms was 0.925 between treatment and control. The gland-level foremilk SCC varied among herds ( $P = 0.04$ ), tended to vary among time periods ( $P = 0.053$ ), and was higher in glands with major compared with a minor IMI [4,317 (95% CI = 2,597–7,174) vs. 1,118 (95% CI = 856–1,462) geometric mean ( $\times 1,000$ ) cells/mL;  $P <$

0.001]. There were no interactions among the variables left in the model.

**Goat Level.** Eighty-eight goats were enrolled, 43 in the control group and 45 in the treated group. No difference in age, kidding date, breed distribution, number of enrolled goats with 2 glands enrolled, or distribution existed among herds in the treatment groups (all  $P > 0.1$ ).



**Figure 2.** Receiver operator curve for the California Mastitis Test (CMT) applied after kidding for predicting the presence of any intramammary infection.

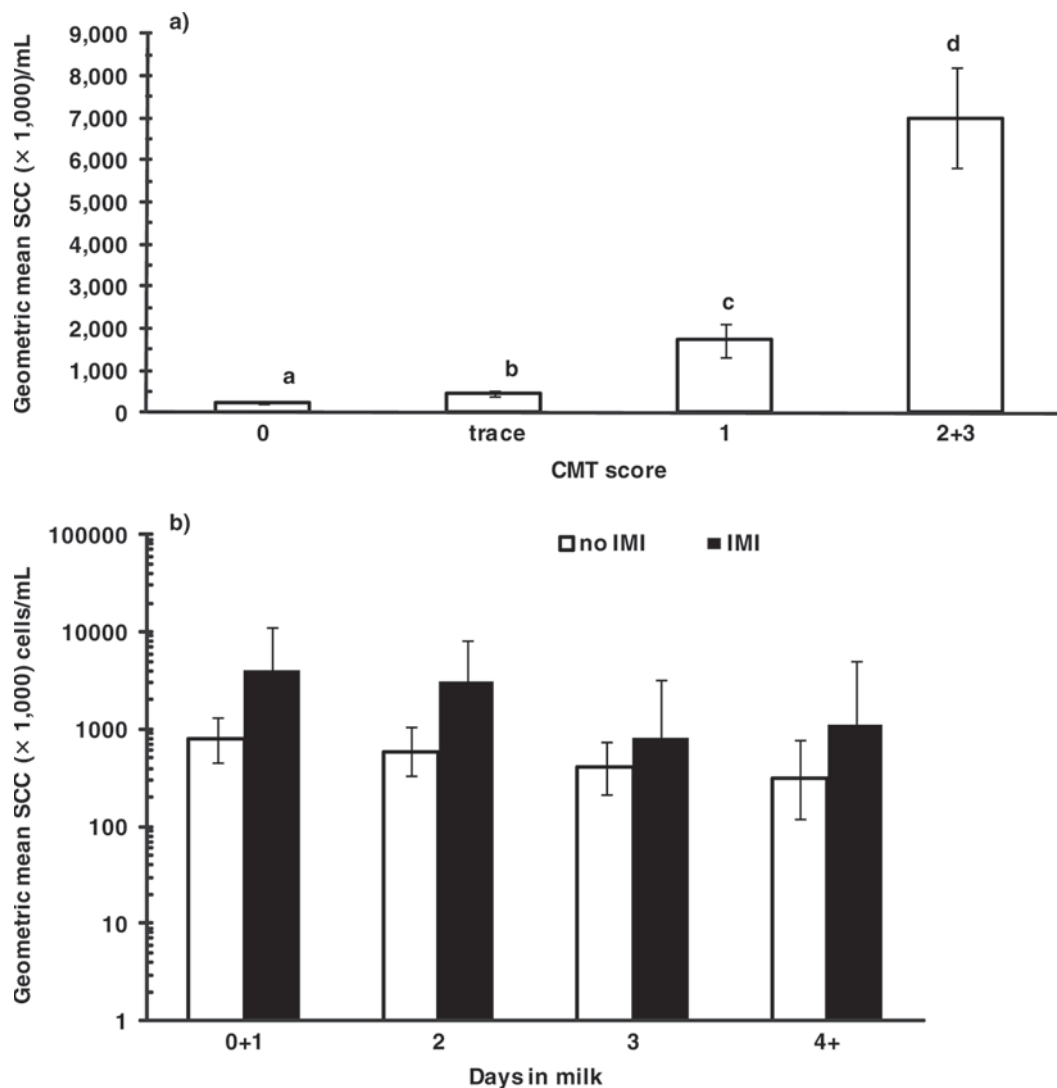
For bacteriological cure, an interaction between treatment group and pathogen type was observed whereby in goats with only minor pathogens isolated, treatment resulted in a higher cure proportion ( $P < 0.001$ ). In contrast, when a goat had a major pathogen, there was no difference in cure proportion between treated and control goats ( $P = 0.16$ ; Table 3).

A total of 41.1% of enrolled does were removed during or at the end of lactation. The proportion removed did not differ between groups [0.36 (SE = 0.08) vs. 0.47 (SE = 0.08) for control and treatment groups, respectively;  $P = 0.51$ ]. The proportion removed increased with age, with goats >4 yr old more likely to be removed than younger goats [0.29 (SE = 0.08), 0.15 (SE = 0.08), 0.53 (SE = 0.10), and 0.75 (SE = 0.11) for <3, 3–4, 5–7, and >7 yr old, respectively;  $P < 0.001$ ].

**Table 1.** The bacteriological results from glands with a California Mastitis Test (CMT) score of >1

Item	Samples, n	%
No growth	233	46.0
CNS	174	34.4
<i>Corynebacterium</i> spp.	65	12.8
<i>Escherichia coli</i>	2	0.4
<i>Streptococcus uberis</i>	3	0.6
<i>Staphylococcus aureus</i>	23	4.5
<i>Serratia</i> spp.	1	0.2
Yeast	3	0.6
<i>Bacillus</i> spp.	1	0.2
Contaminated <sup>1</sup>	1	0.2
Total	506	

<sup>1</sup>More than 3 distinct colony types identified.



**Figure 3.** Geometric mean (SEM) foremilk gland level SCC by (a) California Mastitis Test score and (b) DIM and infection status. White bars = uninfected glands; black bars = infected glands. Columns with different letters differ at  $P < 0.05$ .

**Table 2.** Descriptive data for the categorical variables of the 2 treatment groups<sup>1</sup>

Item	Control (n = 49)		Treatment (n = 57)		Total (n = 106)		P-value
	n	%	n	%	n	%	
<b>Bacteriology</b>							
CNS	25	51.0	29	50.9	54	50.9	0.84
<i>Corynebacterium</i> spp.	13	26.5	16	28.1	29	27.4	
<i>Escherichia coli</i>	0	0.0	1	1.8	1	0.9	
<i>Staphylococcus aureus</i>	9	18.4	10	17.5	19	17.9	
<i>Streptococcus uberis</i>	2	4.1	1	1.8	3	2.8	
<b>DIM</b>							
1	17	34.7	32	56.1	49	46.2	0.07
2	15	30.6	9	15.8	24	22.6	
3	13	26.5	9	15.8	22	20.8	
4+	4	8.2	7	12.3	11	10.4	
<b>Herd</b>							
1	17	34.7	27	47.4	44	41.5	0.31
2	10	20.4	5	8.8	15	14.2	
3	13	26.5	14	24.6	27	25.5	
4	9	18.4	11	19.3	20	18.9	
<b>Breed</b>							
Saanen	24	49.0	38	66.7	62	58.5	0.07
Cross or other	25	51.0	19	33.3	44	41.5	
<b>California Mastitis Test score</b>							
2	42	85.7	53	93.0	95	89.6	0.22
3	7	14.3	4	7.0	11	10.4	
<b>Glands enrolled, n</b>							
1	32	65.3	29	50.9	61	57.5	0.13
2	17	34.7	28	49.1	45	42.5	
<b>Age, yr</b>							
1	9	18.4	14	24.6	23	21.7	0.72
2-3	14	28.6	14	24.6	28	26.4	
4-5	11	22.4	8	14.0	19	17.9	
6-7	7	14.3	11	19.3	18	17.0	
>7	8	16.3	10	17.5	18	17.0	

<sup>1</sup>Control = untreated; treatment = 3 infusions at 12-h intervals of 75 mg of sodium ampicillin and 250 mg of sodium cloxacillin.

Only 6 goats (4 in the control group and 2 in the treatment group) were removed for mastitis-related reasons. Two of these goats (1 in each treatment group) died of mastitis. Length of lactation between groups did not differ [246 (SE = 13) vs. 236 (SE = 16) d for treatment and control, respectively;  $P = 0.87$ ; Figure 4].

Treatment did not affect the composite milk production recording SCC [1,596 (95% CI = 1,219–2,090) vs. 1,488 (95% CI = 1,132–1,955) geometric mean

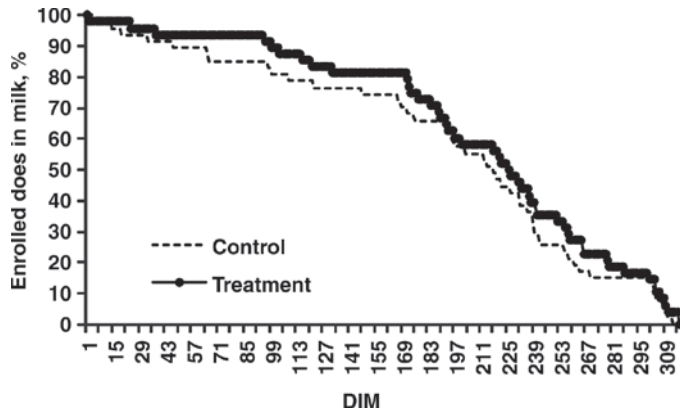
( $\times 1,000$ ) cells/mL for treated and control goats, respectively;  $P = 0.67$ ]. No milk production recording number by treatment interaction was detected ( $P > 0.2$ ; Figure 5a). The SCC varied among herds ( $P = 0.02$ ), was higher in goats with a major compared with a minor IMI ( $P = 0.003$ ), increased across lactation ( $P < 0.001$ ), and was higher in >7-yr-old goats compared with younger goats ( $P < 0.001$ ). No interactions were observed among the variables left in the model (all  $P$

**Table 3.** Number and percentage of glands and goats that underwent bacteriological cure by pathogen type and treatment group

Pathogen <sup>1</sup>	Group <sup>2</sup>	Gland level				Goat level			
		Cure, n		Total, n	%	Cure, n		Total, n	%
		No	Yes			No	Yes		
Minor	Control	35	4	39	10.3	29	4	33	12.1
	Treatment	15	30	45	66.7	15	21	36	58.3
	Total	50	34	84	40.5	44	25	69	36.2
Major	Control	9	2	10	18.2	8	2	10	20
	Treatment	12	0	12	0.0	9	0	9	0.0
	Total	21	2	23	8.7	17	2	19	10.5

<sup>1</sup>Minor = CNS or *Corynebacterium* spp.; major = *Staphylococcus aureus*, *Streptococcus uberis*, or *Escherichia coli*.

<sup>2</sup>Control = untreated; treatment = 3 infusions at 12-h intervals of 75 mg of sodium ampicillin and 250 mg of sodium cloxacillin.



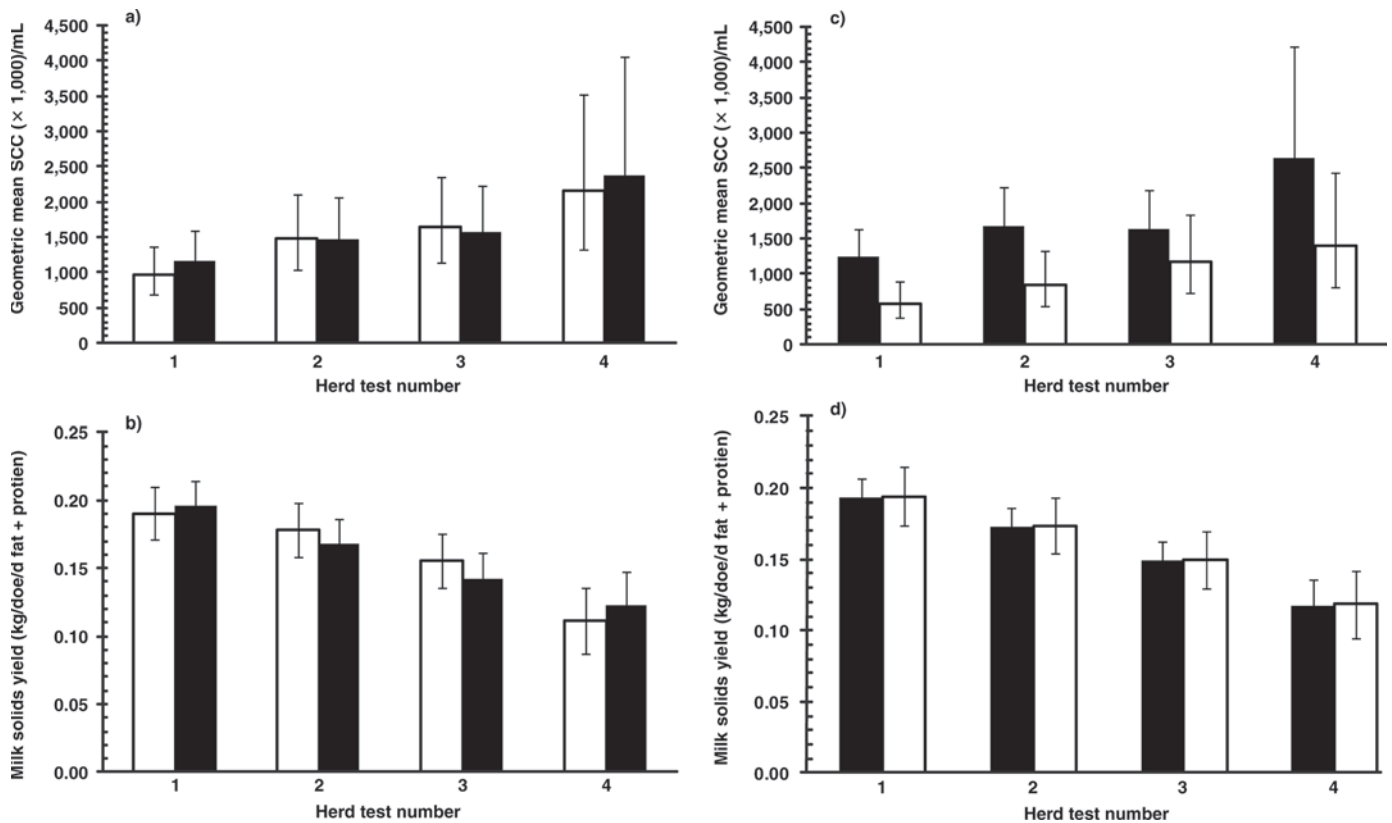
**Figure 4.** Percentage of enrolled does still in milk by DIM for does treated with a combination of ampicillin and cloxacillin (treatment) or left as untreated controls (control).

> 0.10). Goats that cured had lower SCC than those that did not [958 (95% CI = 664–1,382) vs. 1,615 (95% CI = 1,279–2,038) geometric mean ( $\times 1,000$ ) cells/mL for goats that did and did not cure, respectively;  $P = 0.005$ ; Figure 5c].

Treatment did not affect the milk solids production [0.16 (95% CI = 0.14–0.17) vs. 0.16 (95% CI = 0.14–0.18) kg/goat per day for treated and control goats, respectively;  $P = 0.97$ ]. No treatment by herd test interaction was observed ( $P = 0.13$ ; Figure 5b). The production varied among herds ( $P < 0.001$ ), was lower in goats <3 yr old compared with those that were 3 or 4 to 6 yr old ( $P < 0.001$ ), declined with stage of lactation ( $P < 0.001$ ), and was lower in goats treated in 2 glands than in those treated in 1 gland ( $P = 0.03$ ). First-order interactions between treatment group and age and with herd were not significant ( $P > 0.3$ ). Milk solids production did not differ between goats that did or did not cure [0.16 (95% CI = 0.14–0.18) vs. 0.16 (95% CI = 0.15–0.17) kg/goat per day for goats that cured and those that did not, respectively;  $P = 0.94$ ; Figure 5d]. The cost benefit was  $-\text{NZ}\$20.39/\text{treated doe}$  (Table 4).

## DISCUSSION

Knowledge of the CMT result increased the probability that a gland would have an IMI compared with not



**Figure 5.** Geometric mean milk production recording (a) SCC (95% CI) and (b) milk solids production (fat and protein; kg/doe per day; 95% CI) by milk production recording number for goats with subclinical mastitis that were treated with a combination of ampicillin and cloxacillin (black bars) or left as untreated controls (white bars), and (c) SCC (95% CI) and (d) milk solids production (fat and protein; kg/doe per day; 95% CI) by milk production recording number for goats that did not (black bars) or did (white bars) cure.



**Table 4.** Cost benefit of treatment of does with subclinical mastitis

Item	Value
Extra revenue	
Milk payment, NZ\$/kg of fat and protein	12.00
Log base 2 difference in SCC	0.925
Yield decline/unit log base 2 SCC, kg of milk/goat per day	0.016
Milk solids (fat, protein, and lactose), %	0.101
Total milk solids benefit, kg/d	0.002
Days over which SCC was lower	21
Total milk solids difference, kg/gland	0.03
Average no. of glands/doe	1.4
Total value of additional milk attributed to reduced SCC, NZ\$/doe	0.29
Additional DIM over lactation	10
Average milk solids, kg/d	0.25
Additional revenue from additional DIM, NZ\$/doe	30.47
Total additional revenue, NZ\$/treated doe	30.76
Reduced costs	
Reduced clinical mastitis incidence	0
Reduced incidence of new IMI	0
Reduced culling	0
Total reduced costs, NZ\$/doe	0
Reduced revenue	
Milk discard, d	4.5
Total reduced revenue, NZ\$/treated doe	13.71
Increased costs	
California Mastitis Test cost, NZ\$/doe	0.39
Microbiology, NZ\$/sample	8
Proportion CMT positive	0.42
Treatments, n	3
Cost/treatment, NZ\$	5
Labor cost/treatment, NZ\$	1
Total increased costs, NZ\$/doe	37.43
Net position, NZ\$/doe	-20.39

having undertaken the CMT. Treatment of subclinical IMI resulted in a higher cure proportion and a lower foremilk gland-level SCC but no difference in goat-level composite SCC, length of lactation, or risk of removal.

The CMT was shown to be useful in screening goats early after kidding for the presence of increased SCC and for the presence of an IMI. This result extends previous studies that had demonstrated that the CMT was a useful test later in lactation (Maisi, 1987; Contreras et al., 1996; McDougall et al., 2001). The positive predictive values (i.e., the proportion of glands that were CMT positive that had an IMI) were 0.30, 0.42, and 0.55 when the cut points were >0, greater than trace, and >1, respectively. Hence, 70, 58, and 45% of glands with a CMT score >0, greater than trace, and >1, respectively, did not have an IMI compared with 80% for a random sample drawn from the population (in which 80% were not infected and 20% were infected). However, increasing the cut point reduced the sensitivity, which resulted in more truly infected glands not being CMT test positive. Were CMT to be used as a screening test, increasing the cut point would result in fewer test-positive glands and an increased proportion of those tested having an IMI. However, at population level, more goats with an IMI would be

CMT test negative and hence be misclassified and assumed uninfected. The objective for use of the CMT in the treatment study was to reduce the costs associated with bacteriology. Given that bacteriology costs many times more than undertaking a CMT, using the CMT as a screening test may be cost effective. For example, in the treatment study only 36% of glands were CMT positive, with a resultant 64% reduction in bacteriology costs if all animals were sampled. However, the cost of misclassification errors is unknown. In the current study only those glands that were CMT and IMI positive were then enrolled in the treatment study. Thus, glands that were CMT test negative but did in fact have an IMI were not included in the study and, hence, the benefits of treatment were not quantified for this group. The negative predictive value of the CMT was >90% up to cut point of >1, indicating that <10% of infected glands would be misclassified as uninfected using the CMT. Because the CMT was positively correlated with SCC, the CMT could be used as a screening test of SCC and those does with an increased CMT could be withheld from supply to reduce the risk of increasing the bulk milk SCC. As previously reported in cattle (Sargeant et al., 2001), SCC declines rapidly after parturition but infected glands still have higher

SCC than uninfected glands, even in early lactation. However, in the current study CMT test characteristics were not affected by DIM, indicating the utility of this test at any stage of lactation.

Treatment increased the cure proportion over no treatment, but only in glands and goats in which a minor, but not major, IMI was present. The bacteriological cure rate for the minor pathogens was 67% at gland level in the current study. The bacteriological cure rates for CNS and other minor pathogens are reported to be between 20 and 90% in dairy cattle (Timms and Schultz, 1984; Deluyker et al., 2005; Pyörälä and Tapponen, 2009). The cure rate of the major pathogens, predominantly *S. aureus*, was not improved by treatment in the current study. Bacteriological cure of *S. aureus* is reported to range between 4 and 92% in dairy cows, but is commonly <30% (Barkema et al., 2006). An Italian study found no evidence of penicillin resistance in *S. caprae* or *S. epidermidis* of caprine origin (Moroni et al., 2005). The antimicrobial sensitivity of the isolates in the current study was not assessed. However, zone diffusion testing of 104 CNS and 7 *S. aureus* isolates from the same herds in the season preceding the study found that 29 and 3% of the CNS were penicillin G and oxacillin resistant, respectively, whereas none of the *S. aureus* isolates were resistant to either penicillin or oxacillin (S. McDougall; unpublished data). This suggests that the low cure rate of *S. aureus* may be unrelated to antimicrobial sensitivity. In the current study, the overall cure proportion was biased downward because all major gram-positive pathogens were by design enrolled compared with only 50% of the minor pathogens. Hence, if all glands with an IMI had been treated, a higher overall cure proportion would likely have resulted because of the greater cure rates of the minor pathogens.

Because of the heterogeneity of the CNS, speciation is required to advance the understanding of the effect, sources, transmission mechanisms, and control options for this group of bacteria (Zadoks and Watts, 2009). Identification to species level using phenotypic characteristics may result in lower typeability and accuracy than using genotypic techniques (Sampimon et al., 2009; Zadoks and Watts, 2009) with the resultant risk of misclassification errors. The current study is apparently the first to use a genotypic technique for CNS isolates of caprine origin. Because it is both accurate and relatively cheap, tDNA-PCR was used. The library used for this technique was updated recently for the identification of bovine CNS isolates from milk and teat apices (Supré et al., 2009). Because strain differences might occur between caprine and bovine CNS isolates, tDNA-PCR was supplemented with sequencing of the *rpoB* housekeeping gene in every case in which there

was any doubt. Indeed, strain differences between these hosts were seen in this study. There were some caprine CNS isolates of which the tDNA pattern seemed similar to that of *S. simulans*, but these formed a separate cluster. After sequencing, it was confirmed that these isolates belonged to *S. simulans* (>98% sequence similarity with GenBank entries). Eight different CNS species were isolated in the current study. All of these had been reported previously as having been isolated from goat milk using phenotypic techniques (Contreras et al., 2007). The distribution of CNS species varied among herds, with only *S. simulans*, the most prevalent pathogen, isolated from each herd in the current study. In contrast, *S. simulans* has generally been reported to be one of the less common CNS in previous studies (Contreras et al., 2007). However, previous studies have used phenotypic tests, such as the API Staph ID 32, which have been shown to correctly identify only 22% of *S. simulans* (Sampimon et al., 2009). The number of isolates of each species was too small to analyze the effect of CNS species on SCC or on bacteriological cure rate following treatment. The CNS species appear to vary in pathogenicity and persistence, and it has been hypothesized that differences in milking management techniques, teat antisepsis, and milk machine functionality may contribute to observed differences in prevalence of CNS species among herds (Contreras et al., 2007). Further studies are required to understand the epidemiology of the various CNS species in goats and, hence, to provide control methods for them.

Knowledge of CNS species did not alter the inferences drawn from the current study. It is feasible that one CNS species was present before treatment and cured but that the gland became reinfected with a different CNS species. In this case, if the CNS were not speciated, the treatment would have been defined as not effective. Six new infections with CNS were found in the current study, an incidence rate of 6 per 2,331 gland-days, indicating that transmission of CNS was occurring in these herds. However, from the 33 glands from which CNS species were isolated before and after treatment, we found only 1 example of a different CNS species being isolated after treatment. Other studies using phenotypic techniques have shown that CNS are persistent in goats, with the duration of infection averaging 1.3 mo for *S. caprae*, 3.4 mo for *S. epidermidis*, and 1.9 mo for other CNS spp. where no treatments were given (Moroni et al., 2005). Similarly several CNS species persisted for up to 7 mo (Contreras et al., 1997). However, these studies were performed using phenotypic methods, which lack accuracy; therefore, interpretation and comparison should be done with care. As is the limitation of almost all studies (using phenotypic as well as molecular methods), for species-level identifica-

tion, only 1 colony forming unit of every organism with the same growth characteristics on the esculin-blood agar plate was selected. If more than 1 CNS species with the same growth characteristics was present on the plate, this could have been missed. In addition, to draw accurate conclusions on persistency or reinfection after treatment, strain typing should be performed on all isolated bacteria.

Despite differences in gland-level SCC and cure proportion, no differences in milk production or in goat-level SCC between treatments were observed. The apparent discrepancy in SCC results between the gland-level and goat-level data may be attributed to the fact that 1) the gland-level samples were foremilk samples whereas the goat-level samples were composite of both glands across 2 whole milkings, 2) the gland-level SCC were assessed at 14 and 21 d posttreatment whereas the goat composite samples were collected at 4 time points across lactation, commencing at an average of 47 d after kidding (i.e., about 40 d after treatment), and 3) the individual gland SCC include only the infected, enrolled glands whereas the composite sample includes glands that were uninfected and unenrolled. Both data are presented because the gland-level assessment is the more precise way of assessing treatment effect, whereas the herd owners have access to only the composite herd test SCC data to judge treatment success. No measurable effect on milk production was found in cows with CNS clinical mastitis, whereas *S. aureus* infection depressed milk production for 8 wk after diagnosis (Gröhn et al., 2004). Additionally, it has been concluded that treatment of subclinical mastitis during lactation has no effect on subsequent milk production in dairy cows (McDermott et al., 1983; St Rose et al., 2003). However, milk yield is estimated to be reduced by between 16 and 20 g/gland per day with each increase in log base 2 SCC in goats (de los Campos et al., 2006). In that same study there was evidence of nonindependence of glands; that is, infection in one gland increased SCC in the other and potentially reduced production. However, increased milk yield by an uninfected gland in compensation for reduced production by an infected gland remains a possibility. It is also feasible that an IMI may have had irreversible effects on the mammary gland before treatment was instigated.

The partial budget suggested that it was not cost effective to undertake treatment of does with subclinical IMI in early lactation despite the increase in cure rate and the reduction in the gland-level foremilk SCC. However, not all potential benefits were measured in the current study. For example, in dairy cows, the probability that an infected cow infects another cow was an important factor in the cost benefit of treating subclinical mastitis. By successfully curing an existing

IMI, the risk of secondary infection and its associated costs is reduced (Swinkels et al., 2005). It was beyond the scope of the current study to assess the effect of reduced prevalence of infection on the incidence of new infections across the herds, and no data appear to exist quantifying the transmission of IMI within goat herds with or without treatment of existing IMI. Thus, any benefits associated with a reduced incidence of new infections across the whole herd as a result of lower prevalence of IMI could not be included in the current analysis.

It is concluded that use of the CMT within 10 d of kidding provided moderate sensitivity and specificity for detecting IMI. The CMT score was positively associated with the gland-level foremilk SCC, so it could be used as a screening test during the first few days of lactation to identify goats with high SCC that should not enter the supply. Treatment of subclinical mastitis resulted in an increased cure proportion compared with no treatment. However, this was only true for minor pathogens. Where a gland (or goat) was infected with a major pathogen, treatment had no benefit compared with no treatment. Treatment also reduced foremilk gland-level SCC but had no effect on subsequent composite goat-level SCC, production, or survival. Economic analysis suggests that treatment of subclinical mastitis may not be cost effective in dairy goats.

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