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Prevalence of Mastitis Pathogens in Ragusa, Sicily, from 2000 to 2006

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ABSTRACT

The objective of this study was to report the prevalence of intramammary infections (IMI) in Ragusa, Sicily, from milk samples (n = 18,711) collected between October 2000 and June 2006 from 101 dairy herds. Milk samples were collected at 9,747 cow sampling events from 5,285 individual cows. Samples were individual quarter (92.8%) or composite samples (7.2%) from an individual cow. Logistic regression was used to examine the prevalence of IMI at the level of milk sample and at the level of cow, controlling for year and season of collection, type of sample (individual quarter or composite), and type of housing and bedding of the cow at the time of collection. Bedding and housing types were as follows, respectively (number of herd groups): bedding: none (61), organic [51 (straw, 50; sawdust, 1)], and sand (3); housing: bedded pack (37), free stalls (57), tie stalls (4), and paddock (17). Raw prevalence of cow IMI for a sample event was as follows (percentage of cow samples): no growth, 47.4%; coagulase-negative staphylococci, 22.6%; *Staphylococcus aureus*, 20.6%; other *Streptococcus* spp., 11.1%; *Streptococcus agalactiae*, 2.3%; coliform bacteria, 2.9%; and other organisms, 5.8%. Prevalence of IMI differed by bedding type for *Staph. aureus* (none, 24.5%; organic, 12.7%; sand, 12.3%) and coagulase-negative staphylococci (none, 13.1%; organic, 27.4%; sand, 26.9%) but not for *Streptococcus* spp. or coliform bacteria. Prevalence of *Streptococcus* spp. IMI differed by housing type (tie stalls, 22.2%; bedded pack, 12.8%; free stalls, 8.4%; paddock, 7.1%). Housing was not associated with the prevalence of IMI for other bacteria. Herd monthly prevalence of *Staph. aureus* and *Streptococcus* spp. IMI was associated with decreased mean milk production (*Staph. aureus*, –1.42 kg/d per cow, SEM 0.51; *Streptococcus* spp., –1.31 kg/d per cow, SEM 0.64) and increased mean linear score (*Staph. aureus*, 1.01 units/d per cow, SEM 0.16; *Streptococcus* spp., 0.59 units/d per cow, SEM 0.22). Herds (n = 11) with a mean linear score (MLS) less than 3.3 units had the lowest prevalence of *Staph. aureus* IMI and monthly MLS and the greatest monthly mean milk production compared with other herds grouped by MLS [MLS 3.31 to 3.99 (n = 20), MLS 4.00 to 4.46 (n = 20), MLS >4.46 (n = 17), and MLS not available (n = 33)]. Implementation of a milk quality program to control gram-positive organisms is important for Ragusa.
Key words: mastitis prevalence, milk microbiology, housing and bedding, dairy cow

INTRODUCTION

Mastitis causes major economic losses in dairy herds because of its negative effects on milk production (Wilson et al., 1997), milk protein quality (Barbano et al., 1991), reproduction (Schrick et al., 2001), and animal longevity (Caraviello et al., 2005). Clinical mastitis alters the gross appearance of milk. Subclincal mastitis may not alter the gross appearance of milk but does increase the somatic cells in milk and reduce the quality. The most common cause of clinical and subclinical mastitis is IMI from several common bacterial species.

Mastitis pathogens are typically organized by type into environmental or contagious organisms (National Mastitis Council, 1987). Contagious organisms are further subcategorized into major and minor pathogens. Major contagious pathogens include *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Mycoplasma* spp. Coagulase-negative staphylococci and *Corynebacterium bovis* are considered minor contagious mastitis pathogens. Contagious pathogens survive in the udder of the cow; milk from infected cows is the main source of bacteria for uninfected cows, and new infections occur primarily during milking. Control measures for contagious pathogens focus on proper practices surrounding milking (Ruegg, 2003).

Environmental pathogens are commonly found in the cow resting environment. Environmental organisms include gram-negative species (coliform bacteria such as *Escherichia coli* and *Klebsiella* spp.), and *Streptococcus* spp. (*Streptococcus uberis*, *Streptococcus dysgalactiae*, and other *Streptococcus* spp.). *Streptococcus* spp. not identified as *Strep. agalactiae* are generally referred

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to as environmental streptococci. Moisture and fecal matter combine to increase the risk of teat-end exposure to environmental pathogens. Control of environmental mastitis depends on reducing exposure of the teat end to these organisms through maintenance of dry, clean bedding material in resting areas and diligent premilking udder preparation (Smith and Hogan, 1993). Other organisms may cause IMI, such as yeasts and algae, but these are not common pathogens (Wilson et al., 1997).

Control strategies differ for contagious and environmental organisms. Milk microbiological culture can be used to define the prevalence of contagious and environmental infections in a herd and aid in the development of a mastitis control program. Samples of milk for microbiological culture may be obtained from individual cows or from the bulk tank. A culture of bulk tank milk provides a useful screening tool to identify the presence of contagious mastitis organisms in a herd. However, microbiological culture of milk samples from individual cows remains the most effective strategy to identify causative mastitis organisms and to develop an effective control program (Ruegg, 2003). Milk samples may be collected from each individual functional mammary quarter (quarter sample), or mammary quarter samples may be combined into a single sample for each cow (composite sample).

Ragusa Province is the major milk-producing region in Sicily (Licitra et al., 1998). More than 350 dairy herds subscribe to the Associazione Regionale Allevatori–Ragusa record service (ARAR, equivalent to a DHIA organization). The Friesian breed comprises the majority of cows and herds. Mean herd size is 23 cows and production is 22.5 kg of milk/d per cow. Mean SCC per cow is 620,000 cells/mL; mean linear score (**MLS**) is 4.16 units per cow [linear score = $-3.621272 + 1.437903 \times$ natural log (SCC $\times$ 0.001)]. Many herds in Ragusa have not been able to meet the European standard for bulk milk SCC, which is based on a running 3-mo geometric mean bulk tank SCC below 400,000 cells/mL (Hillerton and Berry, 2004). Establishing a milk quality program is important for herds in Ragusa Province.

One of the goals of the CoRFiLaC organization is to present information and recommend practices to farm managers and service providers that improve net economic returns on dairy farms in Ragusa. This is accomplished through research and outreach, which document management programs that, when used by farm managers, may improve the competitiveness of Ragusa dairy herds (Licitra et al., 1998). Improvement in milk quality is a major management strategy that can enhance competitiveness and improve the quality of traditional milk products, such as Ragusana cheese.

Prevalence of mastitis organisms in Ragusa dairy herds has not been described. The purpose of this study was to describe the prevalence of major mastitis pathogens in milk samples collected from Ragusa dairy herds from October 2000 to June 2006. This information will be used to develop more effective extension programs to improve milk quality in the Ragusa region.

MATERIALS AND METHODS

Sample Collection

A milk quality program was begun in 2000 by the extension service from CoRFiLaC. The program involved evaluation of milking practices, hygiene of cows and resting areas, technical evaluation of the milking system, and collection of milk samples for bacteriological culture. Herds entered the program at the request of the producer. Cows were selected for culture based on the preference of the herd owner, signs of clinical mastitis, elevated SCC in milk from monthly test records, and suggestions from CoRFiLaC personnel. Milk samples were collected from individual mammary quarters or were composite samples of all functioning quarters.

Milk was collected into individual 5-mL sealable plastic containers by personnel from CoRFiLaC. Briefly, teat ends were cleaned and disinfected with alcohol wipes, the initial milk stream was diverted, and the subsequent stream was captured into the plastic vial and capped. Milk vials were labeled with quarter (when appropriate), cow identification, and date of collection, and then refrigerated or frozen on the farm until transport in a cooler on ice to the Milk Microbiology Laboratory at CoRFiLaC (**CMML**). On arrival at the laboratory, they were assigned a unique acquisition number.

In the laboratory, samples were handled according to Federation Internationale de Laiterie–International Dairy Federation Laboratory Methods for Use in Mastitis Work, Document 132. Briefly, 0.01 mL of milk was streaked onto a portion of 5% blood agar Petri dishes and incubated at 35°C to 37°C for 24 h. Plates were examined for growth at 24 and 48 h. Bacteria were identified by colony morphology and Gram stain. For gram-positive cocci, catalase tests were performed to distinguish catalase-negative *Streptococcus* spp. from catalase-positive *Staphylococcus* spp. The Christie, Atkins, and Munch-Peterson (CAMP) test on bile esculin agar was used to distinguish *Strep. agalactiae* from other *Streptococcus* spp. Catalase-positive, gram-positive cocci (*Staphylococcus* spp.) were further identified by using a coagulase test, hemolysis patterns, and mannitol salt agar. Gram-negative bacilli were identified by an oxidase test, motility, indole and ornithine decarboxylase, and Simmons citrate.

Organisms were classified as follows: *Strep. agalactiae*, environmental streptococci (*Streptococcus* spp.),

Staph. aureus, CNS, coliform bacteria (*E. coli*, *Klebsiella* spp.), and other organisms. A milk sample from a cow was classified as positive for contagious organisms if at least one colony-forming unit of *Strep. agalactiae* or *Staph. aureus* was isolated. Three or more colony-forming units were necessary to classify a milk sample as positive for CNS, environmental streptococci, or coliform bacteria. Samples were classified as contaminated if growth was not identified as a major mastitis pathogen or if 3 or more bacterial types were isolated from one milk sample.

Classification of Samples

Results of the bacteriological culture for each sample were stored in a database, which was merged with data on the cow, date of collection, and herd of origin. Each sample was assigned a class variable associated with sample type (quarter or composite), bedding type [none, sand, organic (straw or sawdust)], and housing type (bedded pack, free stall, tie stall, paddock) for the lactating group in which the cow was physically present at time of sample collection.

The raw prevalence of bacterial isolate was examined by milk sample and also by cow sample event. A cow was defined as having an IMI based on isolation of bacteria within the date a sample was collected. A cow could have more than one IMI classification because of isolation of different mastitis bacteria from different quarter samples or up to 2 bacteria isolated from an individual milk sample (quarter or composite). Each date of sample collection from a cow was treated as an independent event. If no bacteria were isolated, the cow was defined as not having an IMI. A general category of mastitis (**MAST**) was calculated as the presence of any mastitis pathogen within a milk sample or within a cow for a sample collection date. Prevalence of bacteria and MAST were examined at the level of sample and cow sample event.

Herd Classification and Milk Production Records

Milk records from all Ragusa dairy herds on DHIA test in 2006 were used to create a ranking system based on herd MLS. Herds with an MLS <3.3 (**MLS1**) ranked in the 90th percentile and were considered excellent in control of milk quality. Herds with an MLS between 3.31 and 3.99 (**MLS2**) ranked between the 60th to 90th percentile of herds and were considered good in control of milk quality; herds with a MLS between 4.00 and 4.50 (**MLS3**) ranked between the 40th and 60th percentile of herds and were considered average in control of milk quality, and herds with a MLS greater than 4.50 (**MLS4**) ranked below the 40th percentile and were considered poor in control of milk quality.

Milk records were available for 68 herds from the Ragusa region that also had submitted samples for bacteriological analysis. Herd monthly mean values for milk volume, protein and fat content, linear score, DIM, and lactation number were computed for each herd in Ragusa from January 2001 through June 2006. The production records were merged with monthly cow sample bacteriological IMI prevalence by herd, month, and year. Herd MLS groups were as follows: MLS1 (excellent, n = 11), MLS2 (good, n = 20), MLS3 (average, n = 20), MLS4 (poor, n = 17). Herds with no milk records were assigned a separate MLS category (**MLS5**, no records, n = 33). Prevalence of bacteria in milk samples and cow sample event was examined for each MLS group by using logistic regression.

A program classification (**PROG*n***) was assigned to a herd based on the number of unique years samples were collected: PROG1, 1 yr; PROG2, 2 yr; PROG3, 3 yr; PROG4, 4 yr; PROG5, 5 yr; and PROG6, 6 yr. The prevalence of contagious (*Staph. aureus* and *Strep. agalactiae* combined), environmental (*Streptococcus* spp. and coliform bacteria combined), CNS, and other IMI by year of submission for PROG*n* class was examined by using logistic regression.

Statistical Methods

Prevalence of bacteria was modeled by using logistic regression with the descending option in PROC GENMOD (SAS Institute, 1999). Separate logistic regression models were examined for prevalence of bacteria in a milk sample and prevalence of bacteria for a cow within a sampling event. Independent variables in both models included sample type, housing, bedding, season, and year. Sample type, bedding, and housing were nested within herd, and herd was coded as a repeated measure. The statistical model was as follows:

$$Y_{hijklmn} = \beta_o + \beta_2 \times House_j + \beta_3 \times Bed_k + \beta_4 \times Qsamp_l + \beta_5 \times Seas_m + \beta_6 \times Year_n + \varepsilon_{ijklmn},$$

where $Y_{hijklmn}$ is the prevalence of the hth bacteria or IMI; $house_j$ is the jth housing type (stall, bedded pack); Bed_k is the kth bedding type (none, sand, straw); $Qsamp_l$ is the quarter sample (yes, no); $Seas_m$ is the mth season (winter, spring, summer, fall); $Year_n$ is the nth year; and ε_{ijklmn} is the residual error. Prevalence of IMI was calculated from the least squares mean coefficient for each explanatory variable by using the following formula:

$$\text{Prevalence} = 1/[1 + \exp(-1 \times \text{least square mean coefficient})].$$

The 95% confidence limit (**CL**) was computed by subtracting or adding 1.95 times the SEM to the least squares mean coefficient. To compare IMI prevalence between groups, the odds ratio (**OR**) was calculated by exponentiation of the difference between the least squares mean coefficients of the groups of interest.

Prevalence of IMI was examined for MLS group by using logistic regression as described above (PROC GENMOD, SAS Institute, 1999). Independent variables were MLS group, season, and year of sample submission. Herd was treated as a repeated observation and MLS group was nested within herd. Prevalence of IMI by major contagious organisms (*Staph. aureus* and *Strep. agalactiae*), CNS, and environmental organisms (*Streptococcus* spp. and coliform bacteria) was examined for PROG class by using logistic regression (PROC GENMOD, SAS Institute, 1999). Independent variables included PROG and year, with herd as a repeated observation.

Mean herd monthly milk production was merged with mean herd monthly prevalence of IMI. In addition to each individual bacterial class, overall mastitis prevalence was computed by combing all IMI within a month. Months in which milk samples were not collected from a herd for bacterial culture were deleted. The PROC MIXED in SAS statistical software (1999) was used to examine the association between prevalence of IMI and mean milk production, mean milk protein and fat content, and MLS. Herd, year of sample collection, and month of sample collection were class variables and prevalence was treated as a continuous variable for regression analysis to estimate the association between prevalence and mean herd monthly production variables. Mean DIM and lactation number were treated as continuous variables and were included for covariate adjustment. Herd was treated as a repeated observation.

RESULTS

In total, 18,771 milk samples were collected from October 2000 through June 2006 and submitted to CMML. Samples with missing herd identification, sample date, or cow identifier were deleted, resulting in 18,711 samples for descriptive statistics and raw prevalence data. The number of milk samples collected from an individual herd ranged from 1 to 1,709. The frequency of the number of milk samples collected from a herd and number of herds within each frequency category are presented in Table 1. The modal frequency of milk samples collected was 101 to 200 samples from 23 herds (Table 1). The number of years that samples were collected from a herd was as follows: 49 herds in only 1 yr, 26 herds across 2 yr, 7 herds across 3 yr, 11 herds across 4 yr, 3 herds across 5 yr, 4 herds across 6 yr, and 1 herd across 7 yr (Table 1).

Table 1. Frequency of milk sample submission for bacteriological culture to the milk microbiological laboratory, CoRFiLaC,[1] for the period from October 2000 through June 2006

Frequency of samples	Herds, n	
1 to 10	7	
11 to 25	15	
26 to 50	17	
51 to 100	16	
101 to 200	23	
201 to 400	13	
401 to 600	5	
601 to 1,000	2	
>1,000	3	
Total 18,711	101	
Herd submission by year	**First-time sample submission, n**	**Total herds, n**
2000	2	2
2001	42	44
2002	22	54
2003	10	31
2004	5	25
2005	16	37
2006	4	19

[1]CoRFiLaC = Consorzio Ricerca Filiera Lattiero Casearia (Ragusa, Sicily).

Sample collection by year, month, type (composite vs. quarter sample), and raw prevalence of bacterial growth within a milk sample are presented in Table 2. Individual quarter samples were the majority of samples collected (92.8%, 17,223 of 18,711 samples; Table 2). Yearly sample collection was lowest in 2000 (n = 26) and greatest in 2005 (n = 4,490; Table 2). Monthly sample collection ranged from 248 in August to 2,906 in February (Table 2).

Bacteria were isolated in 35.4% of milk samples (6,632 samples). The number of bacteria isolated within a milk sample was as follows: 1 bacteria, 5,881 samples (31.4% of all samples), 2 bacteria, 654 samples (3.5% of all samples), 3 bacteria, 91 samples (0.5% of all samples), and 4 bacteria, 6 samples (0.03% of all milk samples). Prevalence of bacterial isolation in milk samples by year was greatest in 2000 (80.8%) and next greatest in 2001 (59.0% of samples; Table 2). After 2001, yearly raw prevalence of bacterial isolation was reduced and ranged from 29.8 to 37.1% of samples from 2002 through 2006 (Table 2). Monthly raw prevalence of bacterial growth in milk samples was variable and ranged from 29.9% in June to 41.7% in samples submitted in December (Table 2).

Raw prevalence of bacteria isolated in milk samples was as follows (% of total samples): CNS, 14.6%; *Staph. aureus*, 12.5%; environmental *Streptococcus* spp., 6.6%; *Strep. agalactiae*, 1.4%; coliform bacteria, 1.7%; and

Table 2. Frequency of milk sample submission to the bacteriological laboratory, CoRFiLaC,[1] by year and month, and raw prevalence of bacterial growth and contamination for the period from October 2000 through June 2006

Item	Sample frequency	Sample type[2]		Bacterial prevalence[3]	
		Quarter, n	Composite, n	All, %	Contamination, %
Year					
2000	26	26	0	80.8	23.1
2001	2,568	2,466	102	59.0	10.9
2002	4,045	3,763	282	37.1	3.0
2003	2,884	2,826	58	25.0	1.3
2004	3,289	3,250	39	29.8	1.0
2005	4,490	3,712	778	30.7	1.9
2006	1,409	1,180	229	35.2	5.3
Total	18,711	17,223	1,488	35.4	3.8
Month					
January	945	757	188	39.3	3.0
February	2,906	2,825	81	35.1	4.1
March	2,222	1,801	421	31.8	3.1
April	1,868	1,733	135	35.9	1.1
May	1,295	1,129	166	32.1	4.2
June	1,105	991	114	29.9	3.6
July	1,732	1,680	52	33.9	4.7
August	248	245	3	33.1	2.8
September	2,009	1,861	148	32.9	2.8
October	2,348	2,309	39	41.4	3.5
November	1,112	1,027	85	37.0	4.4
December	921	865	56	41.7	3.6
Total	18,711	17,233	1,488	35.4	3.8

Bacterial isolate	Raw prevalence, % of all milk samples (18,711)
CNS	14.6
Staphylococcus aureus	12.5
Environmental *Streptococcus* spp.	6.6
Streptococcus agalactiae	1.4
Coliform spp.	1.7
Other bacteria	3.3
Multiple bacterial isolates (3 or more)	0.5

[1]CoRFiLaC = Consorzio Ricerca Filiera Lattiero Casearia (Ragusa, Sicily).

[2]Type: Quarter is a milk sample from a functional individual mammary quarter for a cow; composite is a milk sample from a cow in which all functional mammary gland quarters are combined into one sample.

[3]Bacterial prevalence: All represents isolation of any bacteria from a milk sample. Contamination is defined as isolation of more than 2 bacterial species in one milk sample or growth of an unidentified bacterial species within a milk sample.

other bacteria, 3.3% (Table 2). Samples with other growth or with 3 or more bacteria were classified as contaminated (3.3% other growth and 0.5% multiple growth, 3.8% contaminated samples; Table 2). The prevalence of contamination was substantially greater in 2000 (23.1%) and in 2001 (10.9%; Table 2). Growth of bacteria was greater in composite samples than in individual quarter samples (proportion of samples with bacterial growth, 47.4 vs. 34.3% for composite vs. quarter samples, respectively). This was true for every individual class of bacteria. Because only 26 samples were submitted in 2000 and the prevalence of bacterial growth and contamination was extreme, this year was deleted from the logistic regression analysis. The final number of milk samples used in the logistic analysis was 18,685, collected at 9,721 unique cow sample dates from 5,261 individual cows.

The most frequent housing system was free stalls (57 herds, 11,456 milk samples, 5,783 cow samples) followed by a bedded pack (37 herds, 4,943 samples, 3,008 cow samples), an open paddock (17 herds, 1,712 milk samples, 747 cow samples), and tie stalls (4 herds, 574 samples, 183 cow samples). Bedding at the time of sample collection was as follows: straw, 50 herds, 11,165 milk samples, 5,279 cow samples; sawdust, 1 herd, 253 milk samples, 67 cow samples; no bedding, 61 herds, 6,178 milk samples, 3,933 cow samples; sand, 3 herds, 1,089 milk samples, 442 cow samples. For anal-

ysis, straw and sawdust were combined as organic bedding, because only 1 herd used sawdust bedding.

Bedding in free-stall herds was as follows: no bedding, 43 herds; straw, 12 herds; sand, 1 herd; sawdust, 1 herd. Bedded-pack herds most frequently used straw bedding (35 herds); 2 herds used sand bedding. Paddocks were usually not bedded (16 herds) and had a dirt base; however, 1 herd used straw spread through the paddock area. Tie stalls were bedded with straw (2 herds) or nothing (2 herds). Herds that did not use bedding in resting areas housed cows in outside paddocks or in free stalls and tie stalls with mattress surfaces. Within some herds, the housing and bedding system varied with season or lactation group; therefore, the number of herds for housing and bedding systems summed to a total (115) greater than the number of herds (101).

The cow-level logistic models for bacterial prevalence are reported in this paper, because these reflect IMI for a cow and the associations for bacterial prevalence within a milk sample were similar to the cow-level models. Milk samples were collected from 5,261 individual cows at 9,721 cow sampling events. Sixty-one percent of cows had 1 sampling event, 18.9% had 2, 8.7% had 3, and 11.4% of cows had 4 or more sampling events. At a sampling event, a cow could have been classified as having more than one IMI, because at least 2 organisms could be found within one milk sample and different bacteria could be isolated from individual quarter samples. A cow was classified as having an IMI at a sample collection if at least one milk sample was positive for a mastitis pathogen. Each sample collection date was treated as an independent event for a cow.

Prevalence of IMI by cow for a sample collection is presented in Table 3. Bacteria were isolated in 49.4% of cows at a sampling (Table 3). The most common IMI in a cow was CNS (22.6% of cow samples). The next most frequent IMI was *Staph. aureus* (20.6% of cow samples), followed by environmental streptococci (11.1% of cow samples), coliform bacteria (2.9% of cow samples), and *Strep. agalactiae* (2.3% of cow samples; Table 3). Unidentified bacteria and contaminated samples (other) bacteria were isolated in 5.8% of cow samples (Table 3).

Of cows classified with an IMI, 1,391 of the 4,800 positive cows had 2 or more bacterial isolates (29.0% of positive cows; Table 3). Coagulase-negative staphylococci and *Staph. aureus* were the sole IMI in 70.5 and 69.9% of cow samples, respectively, which represented a higher proportion of "pure" IMI than observed for other bacteria. Only 50.7% of *Strep. agalactiae*, 49.8% of *Streptococcus* spp., and 41.8% of coliform bacteria IMI were the sole bacterial isolates for a cow at a sample collection (Table 3).

In cows with more than one bacterial isolate, the isolation of multiple IMI was influenced by bacterial species. A cow with a *Strep. agalactiae* IMI was more likely to have an *Staph. aureus* IMI than a cow without a *Strep. agalactiae* IMI [OR, 1.54 (CL 1.04, 2.27)]. Cows with a CNS IMI were unlikely also to have a *Staph. aureus* IMI compared with cows that did not have a CNS isolate IMI [OR, 0.44 (CL 0.30, 0.66)]. A cow without a coliform bacterial isolate IMI was more likely to have a *Staph. aureus* IMI than a cow with a coliform bacterial isolate IMI [OR 1.85 (CL 1.24, 2.75)]. Cows with a coliform bacteria isolate IMI, if they had a second IMI, were more likely to have an environmental streptococcus isolate ($P < 0.001$) than cows without a coliform bacteria isolate IMI [OR 2.39 (CL 1.69, 3.38)]. Cows with an environmental streptococcus IMI were not different in prevalence of *Staph. aureus* compared with cows that did not have an environmental streptococcus isolate.

Table 4 presents the logistic regression least squares mean coefficients for the prevalence of IMI for a cow at a sample collection adjusted for sample type, herd, housing, bedding, season, and year. The prevalence of *Staph. aureus* IMI in a cow differed by bedding ($P < 0.01$) and tended to differ by season ($P < 0.06$) and year ($P < 0.07$; Table 4). Cows housed with no bedding had a greater least squares mean prevalence of *Staph. aureus* IMI compared with cows housed with organic or sand bedding [prevalence: no bedding, 24.5% (CL 17.4%, 33.3%), organic bedding, 12.7% (CL 8.7%, 18.3%), sand bedding, 12.3% (CL 6.8%, 21.4%); OR: none vs. organic, 2.22 (CL 1.32, 3.73), none vs. sand, 2.31 (CL 1.23, 4.34)]. The least squares mean prevalence of *Staph. aureus* IMI for a cow was greater in winter and spring compared with fall [prevalence: winter, 20.0% (CL 13.7%, 28.3%), spring, 19.0% (CL 13.0%, 26.9%), fall, 11.5% (CL 7.4%, 17.3%); OR: winter vs. fall, 1.93 (CL 1.22, 3.06), spring vs. fall, 1.81 (CL 1.13, 2.90)]. Prevalence of a *Staph. aureus* IMI for a cow in summer (14.1%, CL 8.8%, 21.9%) was not different from prevalence in fall, winter, or spring (Table 4). There was a trend for the prevalence of *Staph. aureus* IMI to decrease across years. The prevalence of *Staph. aureus* IMI was 32.3% (CL, 19.2%, 49.0%) in 2001 and 6.9% (CL 3.4%, 13.4%) in 2006, with the prevalence ranging from 12.4 to 19.3% in the intermediate years.

Prevalence of isolation of CNS IMI for a cow sample collection was influenced by bedding type ($P < 0.04$), and year ($P < 0.01$) and tended to differ by season ($P < 0.07$; Table 4). The least squares mean prevalence of CNS was least for a cow sampled from herd groups with no bedding compared with organic bedding and sand [prevalence: none, 13.1% (CL 9.6%, 17.7%), organic bedding, 27.4% (CL 21.2%, 34.6%), sand, 26.9% (CL 21.8%,

Table 3. Classification of mastitis status based on isolation of bacteria from milk samples collected from a cow on a specific date from January 2001 through June 2006[1]

Item	None[2]	Mastitis organism[3]					
		Staphylococcus spp.	*Staphylococcus aureus*	*Streptococcus* spp.	*Streptococcus agalactiae*	Coliform spp.	Other
Total, n	4,921	2,006	2,201	1,083	227	282	564
Raw prevalence, % of cow records	50.6	20.6	22.6	11.1	2.3	2.9	5.8
Solo growth, n	—	1,403	1,551	539	115	118	311
% of total	—	69.9	70.5	49.8	50.7	41.8	55.1
One additional organism, n							
Total	—	502	546	423	91	116	162
Staph. aureus	—	—	203	169	51	18	61
Staphylococcus spp.	—	203	—	194	23	57	69
Streptococcus spp.	—	169	194	—	10	30	20
Strep. agalactiae	—	51	23	10	—	3	4
Coliform spp.	—	18	57	30	3	—	8
Other	—	61	69	20	4	8	—
Two additional organisms, n							
Total	—	99	102	116	16	40	82
Staph. aureus	—	—	54	72	8	15	48
Staphylococcus spp.	—	54	—	75	6	27	42
Streptococcus spp.	—	72	75	—	6	32	54
Strep. agalactiae	—	8	6	7	—	—	6
Coliform spp.	—	15	27	26	—	—	14
Other	—	46	42	54	1	17	—
Three additional organisms, n							
Total	—	7	7	8	5	9	9
Staph. aureus	—	—	3	5	2	5	6
Staphylococcus spp.	—	3	—	3	5	4	5
Streptococcus spp.	—	5	3	—	2	6	5
Strep. agalactiae	—	2	5	2	—	1	3
Coliform spp.	—	5	4	6	1	—	8
Other	—	6	5	5	3	8	—

[1]Data from 101 herds; 9,721 unique cow sample collections.

[2]None = no growth.

[3]*Staphylococcus* spp. = staphylococcal species, nonhemolytic staphylococcus; *Streptococcus* spp. = streptococcal species, environmental streptococcal species; coliform spp. = *Escherichia coli* and *Klebsiella* spp.; other = growth of unidentified bacterial species.

32.8%); OR: none vs. organic bedding, 0.40 (CL 0.22, 0.72), none vs. sand, OR 0.41 (CL 0.29, 0.57)]. Cows sampled in spring and summer had the least prevalence of CNS IMI compared with cow sampled in winter and fall [prevalence: spring, 19.2% (CL 15.5%, 23.5%), summer, 19.4% (CL 15.0%, 24.7%), winter, 23.1% (CL 19.5%, 27.1%), fall, 25.2% (CL 21.3%, 29.6%); OR: winter vs. spring, 1.26 (CL 0.98, 1.63), winter vs. summer, 1.25 (CL 0.91, 1.71), spring vs. fall, 0.70 (CL 0.54, 0.92), summer vs. fall, 0.71 (CL 0.53, 0.96)]. The highest yearly prevalence of CNS IMI was in cows sampled in 2006, 32.5% (CL 26.5%, 39.2%) and the least prevalence of CNS IMI was in cows sampled in 2002, 12.4% (CL 9.2%, 16.6%). The prevalence in year 2006 was greater than in 2001, 2002, 2003, and 2004 [OR: 2006 vs. 2001, 2.16 (CL 1.36, 3.45), 2006 vs. 2002, 3.39 (CL 2.35, 4.89), 2006 vs. 2003, 1.98 (CL 1.37, 2.86), 2006 vs. 2004, 1.71 (CL 1.18, 2.46)] but was not different from 2005 [OR: 2006 vs. 2005, 1.14 (CL 0.81, 1.62); Table 4].

Prevalence of *Streptococcus* spp. IMI for cows sampled differed by housing ($P < 0.02$) and tended to differ by season ($P < 0.06$; Table 4). The least squares mean prevalence of environmental streptococci IMI for cows sampled by housing type was as follows: tie stalls, 22.1% (CL 13.9%, 33.3%), bedded packs, 12.8% (CL 9.6%, 16.9%), free stalls, 8.4% (CL 6.7%, 10.6%), and paddocks, 7.0% (CL 3.5%, 13.5%; Table 4). Prevalence of *Streptococcus* spp. was significantly less for sampled cows housed in bedded packs, free stalls, or paddocks compared with cows sampled from tie stalls [OR: bedded packs vs. tie stalls, 0.52 (CL 0.32, 0.84), free stalls vs. tie stalls, 0.32 (CL 0.20, 0.54), paddocks vs. tie stalls, 0.26 (CL 0.11, 0.62); Table 4]. The prevalence of *Streptococcus* spp. IMI for cows sampled from bedded packs tended to be greater than the prevalence of *Streptococcus* spp. in cows sampled from free stalls and paddocks [OR: bedded packs vs. free stalls, 1.60 (CL 1.25, 2.03), bedded packs vs. paddocks, 1.95 (CL 0.94, 4.08)]. Prevalence of *Streptococcus* spp. in cows from free-stall housing was not different from prevalence of *Streptococcus* spp. in cows sampled from paddocks. Seasonal prevalence of *Streptococcus* spp. in sampled cows was great-

Table 4. Least squares mean prevalence (± SEM) for cow mastitis based on isolation of *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus* spp., CNS, coliform bacteria, and other bacteria from milk samples submitted to the microbiological laboratory, CoRFiLaC,[1] for the period from January 2001 through June 2006, from logistic regression[2]

		Mastitis organism[3]						
Item	Cow samples, n	*Staph. aureus*	*Strep. agalactiae*	*Streptococcus* spp.	CNS	Coliform bacteria	Other	All
Intercept		–3.39 ± 0.61	–4.48 ± 0.62	–1.74 ± 0.54	–0.19 ± 0.35	–7.55 ± 0.87	–3.12 ± 1.05	–0.20 ± 0.33
House[4]								
Bedded pack	3,008	–1.46 ± 0.34	—	–1.92 ± 0.16	–1.21 ± 0.19	–4.18 ± 0.3	–2.95 ± 0.37	0.14 ± 0.15
Free stall	5,783	–1.78 ± 0.20	—	–2.38 ± 0.13	–0.99 ± 0.12	–3.89 ± 0.25	–3.10 ± 0.28	–0.04 ± 0.10
Paddock	747	–1.66 ± 0.46	—	–2.59 ± 0.37	–1.72 ± 0.24	–4.55 ± 0.52	–3.91 ± 0.65	–0.55 ± 0.22
Tie stall	183	–1.79 ± 0.42	—	–1.26 ± 0.29	–1.23 ± 0.29	–6.32 ± 0.36	–3.01 ± 0.59	–0.07 ± 0.23
Bedding[5]								
None	3,933	–1.13 ± 0.22	—	–1.85 ± 0.14	–1.89 ± 0.18	–4.61 ± 0.24	–2.73 ± 0.23	–0.05 ± 0.13
Organic	5,346	–1.93 ± 0.22	—	–1.92 ± 0.12	–0.97 ± 0.17	–3.85 ± 0.20	–3.38 ± 0.24	–0.12 ± 0.12
Sand	442	–1.96 ± 0.34	—	–2.34 ± 0.40	–1.00 ± 0.14	–5.75 ± 0.69	–3.62 ± 0.82	–0.22 ± 0.13
Season[6]								
Winter	2,415	–1.39 ± 0.23	–3.78 ± 0.56	–1.89 ± 0.19	–1.20 ± 0.11	–4.70 ± 0.30	–3.06 ± 0.36	0.06 ± 0.12
Spring	2,510	–1.45 ± 0.23	–3.52 ± 0.38	–1.80 ± 0.19	–1.44 ± 0.13	–4.57 ± 0.32	–3.08 ± 0.39	–0.04 ± 0.11
Summer	1,677	–1.80 ± 0.27	–4.26 ± 0.57	–2.32 ± 0.20	–1.42 ± 0.16	–4.69 ± 0.31	–3.10 ± 0.35	–0.32 ± 0.12
Fall	3,119	–2.05 ± 0.24	–4.02 ± 0.56	–2.14 ± 0.21	–1.09 ± 0.11	–5.0 ± 0.32	–3.73 ± 0.44	–0.21 ± 0.10
Year[7]								
2001	2,276	–0.74 ± 0.36	–3.27 ± 0.42	–2.11 ± 0.21	–1.50 ± 0.19	–4.99 ± 0.39	–2.17 ± 0.41	0.14 ± 0.16
2002	3,154	–1.59 ± 0.28	–4.55 ± 0.39	–2.21 ± 0.19	–1.95 ± 0.17	–5.37 ± 0.34	–3.70 ± 0.43	–0.56 ± 0.13
2003	1,000	–1.70 ± 0.35	–3.79 ± 0.54	–1.81 ± 0.22	–1.41 ± 0.17	–4.89 ± 0.33	–3.84 ± 0.40	–0.28 ± 0.17
2004	1,156	–1.43 ± 0.32	–4.14 ± 0.79	–2.11 ± 0.25	–1.26 ± 0.15	–4.37 ± 0.30	–4.32 ± 0.56	–0.09 ± 0.15
2005	1,601	–1.96 ± 0.29	–3.27 ± 0.68	–1.86 ± 0.19	–0.87 ± 0.12	–4.11 ± 0.30	–2.94 ± 0.36	0.11 ± 0.14
2006	534	–2.61 ± 0.38	–4.36 ± 0.56	–2.11 ± 0.25	–0.73 ± 0.15	–4.69 ± 0.43	–2.49 ± 0.51	–0.09 ± 0.18
P<								
House		0.8464	—	0.0219	0.2468	0.1758	0.5252	0.2264
Bedding		0.0113	—	0.6435	0.0368	0.1840	0.0431	0.2941
Season		0.0638	0.1374	0.0583	0.0724	0.5254	0.1572	0.0252
Year		0.0724	0.2367	0.2087	0.0056	0.0025	0.0060	0.0018

[1]CoRFiLaC, Consorzio Ricerca Filiera Lattiero Casearia (Ragusa, Sicily).

[2]Data from 101 herds; 9,721 unique cow sample collections.

[3]Mastitis is the prevalence of bacteria isolated from a cow at a milk sample collection and is calculated as Prevalence = 1/{1 + [exp(–1 × least squares mean coefficient)]}; all mastitis = growth of *Staph. aureus* + *Strep. agalactiae* + *Streptococcus* spp. + CNS + coliform bacteria (*Escherichia coli* or *Klebsiella* spp.).

[4]House = housing facility of the cow at the time of sample collection.

[5]Bedding = bedding in housing of the cow at the time of sample collection; organic = straw or sawdust.

[6]Season = winter (December, January, February); spring (March, April, May); summer (June, July, August); fall (September, October, November).

[7]Year = year of sample submission.

est in spring and winter compared with summer [prevalence: spring, 14.2% (CL 10.3%, 19.4%), winter, 13.1% (CL 9.4%, 18.0%), summer 9.0% (CL 6.3%, 12.7%); OR: spring vs. summer, 1.68 (CL 1.18, 2.40), winter vs. summer, 1.53 (CL 1.10, 2.14); Table 4]. Prevalence of *Streptococcus* spp. in cows sampled in summer was not different from fall [prevalence: fall, 10.5% (CL 7.2%, 15.1%); Table 4].

The least squares mean prevalence of coliform bacteria in cows sampled varied across year of sample submission ($P < 0.0026$; Table 4). The highest coliform bacteria prevalence in sampled cows was in 2005, and the least prevalence was in cows sampled in 2002 [prevalence: 2005, 1.6% (CL 0.9%, 2.9%), 2002, 0.5% (CL 0.2%, 0.9%); Table 4]. The prevalence of coliform bacteria in cows sampled in 2002 was lower than the prevalence in cows sampled in 2004 and 2005 [OR: 2002 vs. 2004, 0.37 (CL 0.21, 0.63), 2002 vs. 2005, 0.28 (CL 0.14, 0.58); Table 4]. The prevalence of coliform bacteria in cows sampled in 2001 was less than the prevalence in cows sampled in 2005 [OR: 2001 vs. 2005, 0.41 (CL 0.19, 0.91)], and the prevalence of coliform bacteria in cows sampled in 2003 was less than that in cows sampled in 2005 [OR: 2003 vs. 2005, 0.46 (CL 0.25, 0.83); Table 4].

The model for prevalence of *Strep. agalactiae*, which included housing and bedding, could not be solved; therefore, these terms were not included in the final model in Table 4. The least squares mean prevalence of *Strep. agalactiae* for sampled cows was 1.1% (CL 0.4%, 3.0%) and was not different across season and

Table 5. Least squares mean prevalence of mastitis pathogens by herds grouped by mean linear score (MLS) for milk samples submitted for bacteriological culture to the milk microbiological laboratory, CoRFiLac,[1] for the period from January 2001 through June 2006

	MLS					Linear score class effect, $P<$
Linear score class[2]	MLS1	MLS2	MLS3	MLS4	MLS5	
Mean milk (± SEM), kg	32.03[a] ± 1.23	25.43[b] ± 1.13	24.61[b] ± 1.23	23.71[b] ± 1.27	—	0.0001
MLS (± SEM)	2.91[a] ± 0.07	3.66[b] ± 0.06	4.17[c] ± 0.07	4.90[d] ± 0.07	—	0.0001
Prevalence, %						
Mastitis[2]	35.77[a]	48.22[ab]	48.78[b]	64.50[b]	53.49[b]	0.0017
95% confidence range	29.02, 43.14	40.89, 55.61	41.36, 56.25	54.90, 73.07	46.56, 60.28	
Staphylococcus aureus	5.85[a]	14.03[b]	16.85[bc]	39.92[c]	25.47[bc]	0.0015
95% confidence range	2.92, 11.39	7.41, 24.96	9.10, 29.09	23.16, 59.44	15.36, 39.16	
CNS	19.80	27.22	21.23	17.39	23.40	0.3119
95% confidence range	2.92, 11.39	7.41, 24.96	9.10, 29.09	23.16, 59.44	15.36, 39.16	
Streptococcus spp.	10.56	13.83	13.92	12.69	10.54	0.1203
95% confidence range	7.62, 14.47	10.16, 18.56	10.00, 19.06	8.02, 19.51	7.48, 14.64	
Coliform bacteria	0.98	0.62	0.81	0.82	0.92	0.5469
95% confidence range	0.54, 1.77	0.30, 1.29	0.44, 1.48	0.29, 2.28	0.47, 1.79	
Other bacteria	2.75	3.57	4.09	2.41	5.63	0.205
95% confidence range	1.34, 5.57	1.69, 7.40	1.87, 8.74	0.88, 6.42	2.73, 11.26	
Streptococcus agalactiae	0.11	1.10	6.42	0.80	1.07	0.3431
95% confidence range	0.03, 0.46	0.55, 2.20	3.22, 12.38	0.25, 2.57	0.49, 2.29	

[1]CoRFiLaC, Consorzio Ricerca Filiera Lattiero Casearia (Ragusa, Sicily).

[2]Linear score class; MLS: MLS1 = herds with MLS of <3.3, n = 11; MLS2 = herds with MLS of 3.4 to 3.99, n = 20; MLS3 = herds with MLS of 4.0 to 4.59, n = 20; MLS4 = herds with MLS of ≥4.59, n = 17; MLS5 = herds with milk records not available, n = 18.

[2]Mastitis = growth of *Staph. aureus*, *Strep. agalactiae*, other streptococci, CNS, or a coliform bacteria (*Escherichia coli* or *Klebsiella* spp.) from milk samples from a cow; coliform bacteria = a cow with isolation of *E. coli* or *Klebsiella* spp.; other bacteria = growth of an unidentified bacteria.

year of sample submission. Bedding was aggregated as organic (straw and sawdust) and inorganic (sand and none) in a subsequent model. Prevalence of *Strep. agalactiae* differed by cows housed on inorganic bedding, 3.8% (CL 1.8%, 8.0%), compared with cows housed on organic bedding, 0.6% [CL 0.3%, 1.1%; OR: inorganic vs. organic, 6.39 (CL 2.39, 17.07)].

Prevalence of other bacteria in cows sampled was different across bedding ($P < 0.04$) and year of sampling ($P < 0.01$). Prevalence of other bacteria in cow samples was least in sand and organic bedding and greatest in no bedding [prevalence: sand, 2.6% (CL 0.5%, 11.9%), organic bedding, 3.3% (CL 2.1%, 5.3%), none, 6.2% (CL 4.0%, 9.4%); Table 4]. Prevalence of other bacteria in sampled cows varied by year and was greatest in 2001, 2006, and 2005 compared with 2004, the year with the least prevalence of other IMI [prevalence: 2001, 10.4% (CL 4.9%, 20.7%), 2006, 7.6% (CL 2.9%, 18.6%), 2005, 5.1% (CL 2.6%, 9.9%), 2004, 1.3%, (CL 0.5%, 3.8%)]. Prevalence of other IMI was intermediate in 2002 and 2003 [prevalence: 2002, 2.4% (CL 1.1%, 5.5%), 2003, 2.1% (CL 1.0%, 4.6%); Table 4].

Table 5 presents the least squares mean prevalence of bacterial isolates by herds grouped by MLS. Herds in MLS1 had the greatest mean monthly milk production and least mean monthly linear score compared with the other MLS herd groups (Table 5). Mean linear score class was significantly associated with the prevalence of MAST and *Staph. aureus* IMI. Herds in group MLS1 had the lowest prevalence of all MAST (35.8%) and *Staph. aureus* IMI (5.8%) compared with all other MLS herd groups (Table 5). Herds in categories MLS2 and MLS3 had lower *Staph. aureus* prevalence compared with herds in the MLS4 group (14.0 and 16.8% vs. 64.5%, respectively; Table 5). Prevalence of *Staph. aureus* was the greatest in cows from herds grouped in MLS4 (Table 5). No other bacterial isolates varied by MLS herd group.

Prevalence of contagious (*Staph. aureus* or *Strep. agalactiae*) and environmental IMI (coliform bacteria or *Streptococcus* spp.) by cow samples for the PROG*n* class are presented in Figures 1 and 2. In the initial year of sample submission, prevalence of contagious bacteria in cow samples ranged from 15.0% to just below 30.0%, and were not different across PROG*n* classes (Figure 1). Contagious IMI prevalence in cow samples declined after 1 yr or more of sample submission in the PROG3 through PROG6 herds, suggesting that these herds were following through on a mastitis control program (Figure 1). Herds that were on the program the longest had further significant reduction in chronic infections from the initial year of submission (Figure 1). Prevalence of contagious bacteria in samples from cow samples after the second year of submission was 10.0% or less (Figure 1). Prevalence of CNS did not vary across year of sample submission by PROG*n* class.

Prevalence of environmental IMI in cow samples did not change across year of sample collection except in

Table 6. Association of monthly prevalence of IMI with herd mean monthly test milk production, milk fat and protein content, and mean linear score[1]

	Dependent variable,[2] monthly mean (± SEM) for herd			
Independent variable	Milk kg/d	Milk protein, %	Milk fat, %	Linear score
Model 1				
IMI				
Staphylococcus aureus	−1.42 ± 0.51	NS	NS	1.01 ± 0.16
Streptococcus agalactiae	NS	NS	NS	1.43 ± 0.44
CNS	NS	NS	NS	NS
Streptococcus spp.	−1.31 ± 0.64	NS	NS	0.59 ± 0.22
Coliform bacteria	NS	NS	NS	NS
Model 2				
Mean monthly linear score				
LNSC	0.16 ± 0.23	NS	NS	
LNSC	−0.12 ± 0.03	NS	NS	

[1]Monthly mean production records by herd merged with monthly mean prevalence of mastitis IMI based on cow lactation prevalence. Herds = 62.

[2]Model 1: $Y = u_i + Herd_j + Year_k + Month_l + IMI_m + \varepsilon_{ijklm}$, where Y = mean milk (kg), mean milk content of protein or fat (%), or mean linear score; u_i = overall mean; $Herd_j$ = jth herd; $Year_k$ = kth year (2000 to 2006); $Month_l$ = lth test month (1 to 12); IMI_m = prevalence of mth IMI in cow lactations [*Staph. aureus*, *Strep. agalactiae*, CNS, *Streptococcus* spp., coliform (*Klebsiella* spp. or *Escherichia coli*)]; and ε_{ijklmn} = residual error. In all models, herd, year, and month were significant. NS = indicates the coefficient was not different from zero. Model 2: mean monthly linear score (LNSC) replaced IMI.

PROG6 herds, which had a lower prevalence in cows sampled in yr 3, 4, and 5 (Figure 2). In yr 6 in PROG6 herds, prevalence of environmental IMI in cows sampled was similar to the prevalence in yr 1 and 2 (Figure 2).

Table 6 presents coefficients of association for prevalence of IMI with mean monthly production of milk, milk protein and fat content, and monthly linear score. An increase in the monthly prevalence of *Staph. aureus* was associated with a reduction in the herd monthly mean daily production per cow of −1.42 kg/d (SEM 0.51; Table 6). An increase in prevalence of *Staph. aureus* increased the monthly MLS by 1.00 unit/d (SEM 0.16) per cow (Table 6). An increase in prevalence of *Streptococcus* spp. was associated with a reduction in monthly mean daily milk production of −1.31 kg/d (SEM 0.64) per cow and an increase in the MLS of 0.59 units/d (SEM 0.22) per cow (Table 6). Mean monthly herd linear score per cow increased by 1.43 units/d (SEM 0.44) per cow with an increase in the prevalence of *Strep. agalac-*

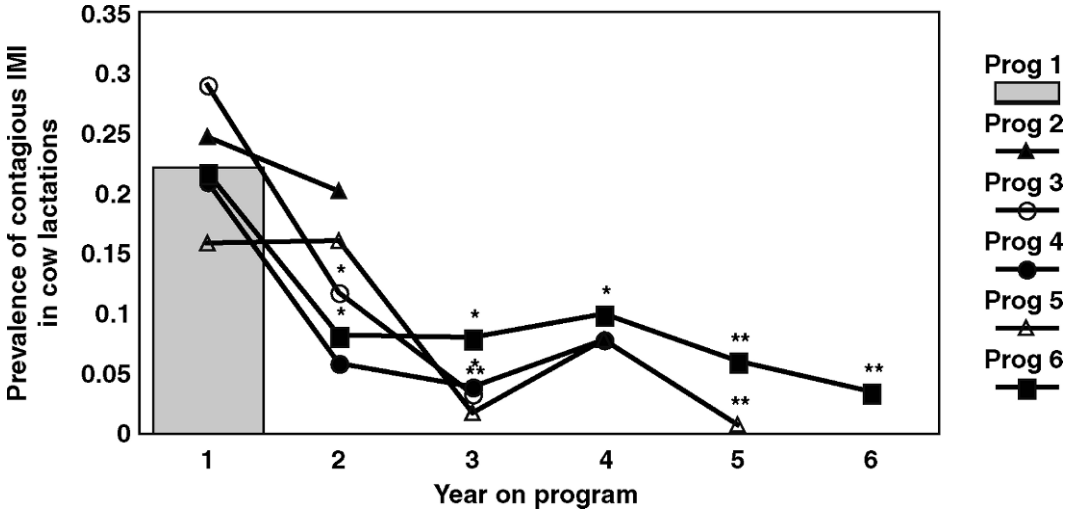

Figure 1. Prevalence of IMI of contagious bacteria (*Staphylococcus aureus* and *Streptococcus agalactiae*) in cows in herds grouped by year of participation in a milk quality program. Prevalence of IMI is plotted by year of participation in the milk quality program for herds that participated 1 to 6 yr. Data points labeled with an asterisk (*) indicate the difference from yr 1: *$P < 0.05$; **$P < 0.01$.

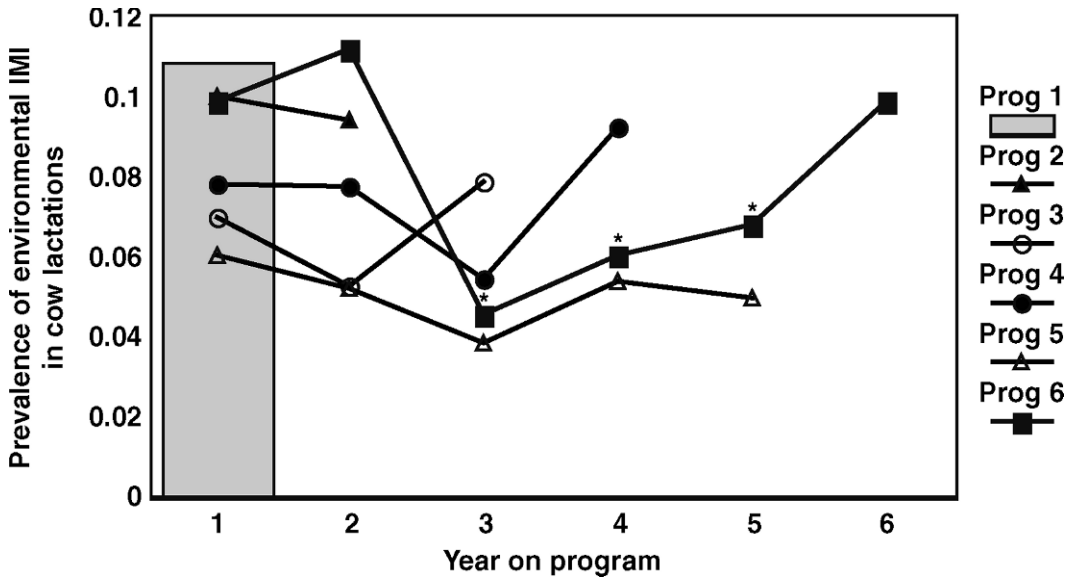

Figure 2. Prevalence of IMI with environmental bacteria (*Streptococcus* spp., excluding *Streptococcus agalactiae*, and coliform spp.) in cows in herds grouped by year of participation in a milk quality program. Prevalence of IMI is plotted by year of participation in the milk quality program for herds that participated 1 to 6 yr. Data points labeled with an asterisk (*) indicate the difference from yr 1: $*P < 0.05$; $**P < 0.01$.

tiae (Table 6), but milk production was not altered significantly by the increase in *Strep. agalactiae*. An increase in the cow sample prevalence of other IMI was associated with a reduction in milk protein content of –0.13%/d (SEM 0.05) per cow (Table 6). Changes in the monthly prevalence of CNS or coliform bacteria were not associated with a change in mean herd monthly test-day milk production, mean milk fat or protein content, or monthly MLS. An increase in monthly MLS was quadratically associated with a reduction in mean monthly milk production, –0.12 kg/d MLS^2 (SEM 0.03) per cow (Table 6).

DISCUSSION

This paper explored 4 questions: 1) What was the prevalence of IMI in Ragusa dairy herds and what management factors were associated with specific bacteria? 2) What was the prevalence of IMI by MLS class? 3) What was the prevalence of IMI across time in herds that submitted samples over a period of years? 4) What was the association of herd prevalence of IMI with herd mean monthly milk production and linear score? We recognize that the interpretation of associations described in this paper should be done with caution because of the limited selection of herds and cows. However, these observations provide a quantitative framework to encourage producers to adopt milk quality protocols and to develop future research projects to address these questions more critically.

The prevalence of mastitis pathogens reported in this paper is based on milk sample submissions to CMML and the laboratory protocols used within the CMML. The CMML identified only major bacterial mastitis pathogens and did not identify other potential pathogens, such as *Mycoplasma* spp. and *Prototheca* spp., or subclassify *Streptococcus* spp. or CNS species. Prevalence estimates of mastitis organisms may differ absolutely from other studies because of the bacteria identified as mastitis pathogens, the proportion of quarter vs. composite milk samples in the study, and the number of colony-forming units used to define a positive milk sample.

Although the raw prevalence of bacterial isolates in milk samples was numerically different compared with other reports (Makovec and Ruegg, 2003; Pitkälä et al., 2004; Østerås et al., 2006; Tenhagen et al., 2006), patterns were similar in that gram-positive bacteria were the predominate isolate, and the prevalence of sterile milk samples and specific bacterial organisms were within ranges reported by others (González et al., 1988; Makovec and Ruegg, 2003; Pitkälä et al., 2004; Østerås et al., 2006; Tenhagen et al., 2006). In Ragusa, 4.0% of milk samples had more than one bacterial species isolate compared with the 17.20% reported by Makovec and Ruegg (2003), 3.7% by Pitkälä et al. (2004), and 6.87% by Østerås et al. (2006). The prevalence of contaminated milk samples (3.8% of all milk samples) in Ragusa was lower than reported by Makovec and Ruegg (2003; 16.58% of samples), by Pitkälä et al. (2004;

4.15% of milk samples), and by Østerås et al. (2006; 6.9% of samples). A total of 50.6% of cow samples in this study had sterile milk samples, which is similar to that observed by Wilson et al. (1997; 51.5% of composite milk samples), but less than those observed by González et al. (1988; 78% of cows) and Pitkälä et al. (2004; 69% of cows).

In cows with clinical mastitis, the National Mastitis Council (1989) reported that 25 to 40% of milk samples do not have a bacterial isolate. For a high probability of bacterial isolation from a sample of milk, there needs to be at least 100 cfu/mL of milk (National Mastitis Council, 1989). A truly infected quarter may have a concentration of bacteria below 100 cfu/mL of milk if the infection is modest and bacteria are shed intermittently; white blood cells in milk have engulfed and sequestered bacteria, preventing isolation; or poor sample handling and collection have reduced the bacterial concentration (National Mastitis Council, 1989). In addition, some less common mastitis pathogens require other culture procedures for isolation, which were not performed by the CMML. The proportion of samples positive for bacterial growth is also influenced by the type of milk sample, whether an individual quarter milk sample or a composite milk sample (Lam et al., 1996).

Prevalence of bacterial isolation from a milk sample was greatest in 2000, next greatest in 2001, reduced in 2002 relative to 2001, but greater in 2002 compared with 2003 through 2006. Makovec and Ruegg (2003) reported a decrease in the prevalence of positive milk samples over a period of time (1994 to 2001), but they also indicated that management factors possibly contributing to a decrease in the prevalence of positive samples across time were not examined. In this study, housing, bedding, and number of years of sample submission from an individual herd were examined in relation to prevalence of IMI. Several factors in this study may have contributed to a reduction in positive samples. First, the proportion of samples collected from cows with clinical mastitis in 2000 and 2001 compared with later years was reduced (M. Gambina, personal observation). After 2001, samples were collected as a component of a milk quality program to identify cows infected with contagious mastitis organisms and ascertain the prevalence of IMI within a herd. The decrease in the proportion of positive samples after 2002 may be related to a change in sampling practices. A high prevalence of samples can be expected to be sterile in a surveillance program compared with samples submitted from cows with a clinical mastitis infection. In addition, it is possible that the emphasis on milk quality improved mastitis control measures on farms and resulted in a reduction in the prevalence of positive samples in years after 2002, but this is unlikely across all farms that submitted samples. Supporting this observation was the lower prevalence of positive milk samples from herds that had been participating in the program for more than 1 yr compared with herds submitting samples for the first time (37.7 vs. 46.5%, respectively, $P < 0.03$). Samples from repeat herd collection represented 61.8% of samples after 2002. In herds submitting samples for the first time, prevalence of bacteria growth was not different in samples from 2001 through 2006. Last, positive samples may have decreased because of improved collection techniques or improved laboratory handling procedures. This would be suggested by the decrease in contaminated milk samples over sequential years. The prevalence of contaminated samples decreased from 10.9% in 2002 to 3.0% or less in 2002, 2003, 2004, and 2005, but increased slightly to 5.3% in 2006. Therefore, the decrease in positive samples over time appears to be related in part to the participation of herds in the program and a reduced prevalence of infection and in part from improved collection techniques after 2001.

The most frequent IMI was gram-positive cocci, CNS (14.7% of milk samples, 22.6% of cows, 86.1% of herds) and *Staph. aureus* (12.5% of milk samples, 20.6% of cows, 88.1% of herds). The next most frequent IMI was *Streptococcus* spp., present in 6.6% of milk samples, 11.1% of cows, and 85.1% of herds. The least frequent gram-positive bacteria was *Strep. agalactiae*, which had a prevalence of 1.4% in milk samples, 2.3% of cows, and 35.6% of herds.

Prevalence of *Staphylococcus aureus* in milk samples (12.5%) and cows at a sample collection (20.6%) was greater than that reported by Hogan et al. (1987; 2.79%), Makovec and Ruegg (2003; 9.70%), Pitkälä et al. (2004; 3.40%), and Tenhagen et al. (2006; 5.7%) but similar to the prevalence of *Staph. aureus* IMI reported by Østerås et al. (2006; 8.2% of quarter samples, 22.2% of cows). Prevalence of *Staph. aureus* in Ragusa for a sampled cow (20.6%) was similar to that reported by Erskine et al. (1987) for high-SCC herds (22.20%) but greater than the cow prevalence reported by González et al. (1988; 9.09%) and Wilson et al. (1997; 9.1%). Prevalence of *Staph. aureus* within a herd was 88.1% based on submitted samples. González et al. (1988) found *Staph. aureus* in 100% of the 50 herds they sampled. The low prevalence of *Staph. aureus* in quarter milk samples reported by Pitkälä et al. (2004; 3.40%) represents a 50-yr effort to eradicate contagious mastitis from dairy herds. Prevalence of *Staph. aureus* in Ragusa suggests that a concerted effort is needed to eradicate this organism. This is especially apparent because increases in the monthly prevalence of *Staph. aureus*

had the greatest association with a reduction in mean monthly milk production.

Herds that subscribed to the milk quality program for more than 3 yr reduced the prevalence of contagious bacteria in cow samples (Figure 1). The reduction in prevalence of *Staph. aureus* may be a dilution effect associated with an increase in samples taken for surveillance, but this is unlikely because these herds had also experienced a reduction in mean monthly herd linear score (data not shown) over a similar time period. Zecconi et al. (2003) observed that after 9 mo on a mastitis control program targeted for control of *Staph. aureus* IMI, the incidence of new IMI with *Staph. aureus* was reduced in 7 of 9 herds. Zecconi et al. (2003) identified routine culture and segregation of infected cows as important components of a control program. Herd managers in Ragusa who were interested in controlling contagious organisms, particularly *Staph. aureus*, were encouraged to create separate groups based on contagious IMI, where possible. However, in this study, specific herd control measures were not examined by prevalence of IMI.

Cow prevalence of IMI was more consistently associated with bedding type (*Staph. aureus*, CNS, *Strep. agalactiae*, and other) than with housing (*Streptococcus* spp.). In general, cows housed with no bedding had a greater prevalence of IMI with *Staph. aureus*, *Strep. agalactiae*, and other bacteria than cows housed with organic or sand bedding. In contrast, cows housed with no bedding had the least prevalence of CNS IMI compared with cows housed with organic or sand bedding. Bedding had no association with *Streptococcus* spp. or coliform bacteria.

Prevalence of *Staph. aureus* and CNS IMI were exactly converse of each other for bedding, and a cow with a CNS IMI had a lower risk of a *Staph. aureus* IMI. This pattern would suggest that an infection with CNS was protective against an IMI with *Staph. aureus*, which was observed by Nickerson and Boddie (1994) in challenge studies. Green et al. (2005) observed that when CNS was isolated from a quarter, the probability of isolating *Staph. aureus* as a secondary IMI was reduced in multiple-quarter infections for cows sampled across the dry period in England. As *Staph. aureus* IMI have decreased in mastitis control programs in Finland, prevalence of CNS has increased (Pitkälä et al., 2004), but this is not necessarily an associative effect. In fact, Nickerson and Boddie (1994) also reported that the protective effect of CNS on *Staph. aureus* IMI has not been observed consistently across studies. Generally, CNS are part of the normal skin flora and may colonize the streak canal. Therefore, habitation of skin surfaces by CNS may competitively inhibit *Staph. aureus* habitation and reduce the risk of *Staph. aureus* IMI yet increase the risk of CNS IMI. Herds using bedding may have encouraged a local environment that supported CNS flora on skin surfaces compared with herds not using bedding.

Typically, organic bedding is considered a risk for environmental organisms (coliform bacteria and *Streptococcus* spp.) when it is wet and contaminated with feces and urine (Hogan and Smith, 1997). However, Elbers et al. (1998) observed that risk factors for clinical mastitis with *Staph. aureus* included irregular disinfection of stalls and replacement of bedding, particularly if bedding was thick, making removal difficult. In the Ragusa data, *Staph. aureus* prevalence was greater in cows (and individual milk samples) from herd groups with no bedding, which represented paddocks or free stalls and tie stalls with mattress surfaces. Management factors that have been associated with *Staph. aureus* IMI, such as udder preparation and postmilking teat disinfection, frequency of disinfection of stall surfaces, and cows leaking milk or with damaged teats, were not examined in this study but should be in future work. It is possible that herds using no bedding routinely follow some other practices that increase the risk of *Staph. aureus* IMI.

Only the prevalence of *Streptococcus* spp. IMI was associated with housing system. Cows housed in tie stalls had the greatest prevalence of *Streptococcus* spp. IMI, cows housed on bedded packs had an intermediate prevalence of IMI, and cows housed in free stalls and paddocks had the least prevalence of *Streptococcus* spp. IMI (Table 4). A buildup of urine and feces in bedded packs and tie stalls may have contributed to the higher prevalence of *Streptococcus* spp. IMI in cows housed in these systems. Cows in tie stalls and on bedded packs may have been exposed to feces and urine for longer periods of time than cows housed in free stalls and paddocks. Paddocks were dirt lots, and cows typically had access to paddocks in drier seasons of the year. Thus, cows in paddocks would not have been exposed to as much wet organic material as cows in tie stalls on bedded packs, which may have reduced the risk of *Streptococcus* spp. IMI. It was surprising that *Streptococcus* spp. and coliform bacteria IMI were not associated with any bedding type, although bedded-pack herds were primarily bedded with straw. Free stalls were bedded with nothing, sand, straw, or sawdust. The interaction between bedding and housing was not examined in this study. A larger data set would be needed to identify interactions between bedding type and housing.

Peeler et al. (2000) observed that straw yard housing, which may be comparable to a bedded pack, increased the risk of contagious mastitis compared with cubicle housing. However, in Ragusa, bedded-pack and tie-stall

housing were associated with an increased prevalence of *Streptococcus* spp. IMI and not contagious mastitis organisms. Herds that used no bedding had a higher prevalence of contagious mastitis organisms. Possibly, the higher prevalence of CNS IMI in herds with organic (primarily straw) and sand bedding was protective against contagious IMI. Peeler et al. (2000) reported on the pattern of mastitis in low-SCC herds, which appear to have a different risk of IMI than high-SCC herds (Barkema et al., 1998, 1999). In this study, the majority of herds would classify as high-SCC herds; thus, risk factors associated with housing and bedding may be different from those observed for low-SCC herds.

The prevalence of CNS IMI was 22.6% in cow samples in this study compared with a prevalence of 0.76% reported by González et al. (1988), 28.20% reported in cows in low-SCC herds and 24.90% reported in cows in high-SCC herds by Erskine et al. (1987), and 11.3% reported in Wilson et al. (1997). Hogan et al. (1987) reported that CNS prevalence was higher in milk samples collected from herds in which teats were not dipped in a germicidal solution following milking (11.0%) compared with herds in which teats were dipped in a germicidal solution following milking (7.2%). In this study, even herds that reduced the prevalence of contagious IMI over time (PROG3, PROG4, PROG5, and PROG6 herds) did not experience a significant change in CNS IMI. Therefore, it is unlikely that the prevalence of CNS IMI was influenced by postmilking practices. The prevalence of CNS IMI has been reported to be 10 to 20% of quarter samples (National Mastitis Council, 2000). The prevalence of CNS was highest in composite milk samples (17.36%) compared with quarter milk samples (10.16%), but the high prevalence of CNS cannot be explained by sample type alone.

There was not a significant association between herd prevalence of CNS IMI and mean monthly milk production or linear score (Table 6). Hogan et al. (1987) observed that CNS did not increase $\log_{10}$ SCC to as great a concentration as *Staph. aureus* or *Strep. agalactiae*, although cell counts were greater than for milk samples with negative bacterial isolation. Wilson et al. (1997) did not find a significant reduction in milk yield associated with CNS infection. Coagulase-negative staphylococci are considered minor milk pathogens (National Mastitis Council, 2000). The significance of CNS as a pathogen may vary from herd to herd (Wilson et al. 1997). In this study, CNS had minor associations with herd-level production variables.

The prevalence of *Strep. agalactiae* in Ragusa was 1.4% in milk samples and 2.3% at the cow level, and prevalence was highest in herds with inorganic bedding, particularly in herd groups with no bedding. Although the overall prevalence of *Strep. agalactiae* in milk samples and cows was low, the herd prevalence was greater than 30%. Therefore, many herds may have a few cows with *Strep. agalactiae*. The association with inorganic bedding may reflect poorer management practices in these herds, particularly because herds with no bedding had a higher prevalence of *Staph. aureus* IMI. Monthly prevalence of *Strep. agalactiae* was not significantly associated with mean monthly milk production, but was significantly associated with mean herd linear score (Table 6). Generally, IMI with *Strep. agalactiae* do not dramatically reduce milk yield unless the infection is chronic, although the SCC may rise quickly. In this study, the small prevalence of *Strep. agalactiae* IMI at the cow level may reflect a moderate infection rate in a herd with insignificant long-term chronic infection; thus, no effect of the prevalence of *Strep. agalactiae* was observed on milk production. The increase in linear score with *Strep. agalactiae* would, however, reduce milk quality and cheese production (Barbano et al. 1991).

Coliform organisms were isolated from 1.6% of milk samples and 2.9% of cow samples, which was higher than reported by Wilson et al. (1997; 0.6%) and Pitkälä et al. (2004; 0.14%), but lower than reported by Makovec and Ruegg (2003; 5.24%). Udder infection with coliform organisms tends to be of short duration compared with gram-positive organisms, because the innate immune system often rapidly kills invading coliform organisms, resulting in negative culture results (Ruegg, 2003). Organic bedding is reported to be a risk factor for coliform IMI, particularly during wet, humid conditions (Smith and Hogan, 1993). Season and bedding type were not associated with prevalence of coliform IMI in this study. Prevalence of coliform bacteria IMI was not associated with a change in monthly mean milk production or mean herd linear score (Table 6). Often a coliform bacteria IMI does not have a dramatic influence on SCC in milk, and production changes may be of short duration.

Season was associated with prevalence of *Staph. aureus*, CNS, and *Streptococcus* spp. Prevalence of *Staph. aureus* was greatest in winter and spring, CNS in fall and winter, and *Streptococcus* spp. in winter and spring. There are 2 seasons in Ragusa, a hot, dry season from April through October and a wet, cool season from November through March. Winter is cool and wet, and cows are confined (Licitra et al., 1998). In warm weather, cows have more access to pasture, particularly in bedded-pack and paddock herds. Surprisingly, coliform bacteria did not increase with season, possibly because the wet season is a cooler season and the hot season is a dry season. Coliform IMI often increase with hot, humid weather and with the use of sawdust bedding (National Mastitis Council, 1989). Summer in Ragusa is hot and dry, whereas the wet season is during

the winter months when weather is cooler. Thus, seasonal effects may be somewhat different in Ragusa compared with more temperate regions.

Herd prevalence of IMI (mastitis) and *Staph. aureus* was significantly associated with MLS classification. The excellent herds (MLS1) had a significantly lower prevalence of all IMI and *Staph. aureus* in cow samples among all other MLS categories. Good and average herds (MLS2, MLS3) had a lower prevalence of mastitis and *Staph. aureus* than poor herds (MLS4). Herds with no MLS data (MLS5) had a high prevalence of all organisms, but were not significantly different from average herds. The mean milk production was higher in the excellent herds (MLS1), although many factors other than the lower prevalence of mastitis may contribute to this observation.

Increasing herd prevalence of *Staph. aureus*, *Streptococcus* spp., and *Strep. agalactiae* increased the monthly MLS (Table 6). Erskine et al. (1987), González et al. (1988), and Elbers et al. (1998) observed that herds with greater bulk tank linear scores had a higher prevalence of contagious IMI and a higher prevalence of infection across all cows. Increasing the mean herd linear score resulted in a quadratic decrease in milk production (Table 6). The magnitude of milk decrease was consistent with that summarized by Ott and Novak (2001) from 8 studies. Green et al. (2006) reported that milk production losses with increasing linear score were not linear and were greater as the SCC increased from less than 200,000 to greater than 400,000 cells/mL of milk. Losses of milk reported by Ott and Novak (2001) and Green et al. (2006) were based on individual cow production. Losses estimated in this paper are based on changes in mean monthly herd milk production and herd monthly MLS.

Increasing prevalence of *Staph. aureus* and *Streptococcus* spp. IMI were associated with decreases in mean herd milk production (Table 6). Wilson et al. (1997) reported mean 305-d production for cows classified by IMI. Cows infected with CNS produced more milk than cows infected with *Staph. aureus* and cows infected with *Strep. agalactiae* produced less milk than cows positive for *Staph. aureus* (Wilson et al., 1997). It is generally recognized that chronic udder infections, which create long-term inflammation, may damage alveoli, resulting in scarring and loss of productive tissue. Infections of short duration have less of an impact on milk production because less permanent damage is caused in the udder. The milk loss associated with *Staph. aureus* and *Streptococcus* spp. suggest more udder damage. An increasing prevalence of *Strep. agalactiae* did not reduce milk yield, which is not consistent with the behavior of this organism in dairy herds. However, the prevalence of IMI with *Strep. agalactiae* was low, and this may have contributed to the lack of a significant herd-level association.

CONCLUSIONS

Gram-positive organisms were the primary mastitis pathogens isolated in milk samples submitted for culture in Ragusa, Sicily. Prevalence was similar to that reported in other studies. *Staphylococcus aureus*, *Staphylococcus* spp. and *Streptococcus* spp. were the major bacterial isolates. Associations with housing and bedding were observed for these 3 organisms. Herds with lower MLS had a lower prevalence of mastitis pathogens in milk samples. *Streptococcus agalactiae* was isolated in fewer than 2% of milk samples but was present in 30% of herds. Reducing the prevalence of contagious udder pathogens would improve milk production and milk quality.

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