

Epidemiologic analysis of *Mycoplasma* spp isolated from bulk-tank milk samples obtained from dairy herds that were members of a milk cooperative

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Objective—To determine the prevalence of *Mycoplasma* spp in herds that were members of a milk cooperative.

Design—Epidemiologic study.

Sample Population—267 dairy herds that were members of a milk cooperative.

Procedure—Bulk-tank milk samples were collected monthly during a 6-year period from all dairies in the cooperative. Samples were submitted to the cooperative's laboratory for bacterial culture for *Mycoplasma* spp, using direct plating. Milk samples positive for *Mycoplasma* organisms were speciated.

Results—Prevalence of positive samples varied from 1.8 to 5.8% for all species of *Mycoplasma* and from 1.2 to 3.1% for *Mycoplasma* spp known to be mastitis pathogens. One mycoplasmal species was isolated initially on 99 of 198 (50.0%) dairies, but 68 of 198 (34.3%) dairies had 2 species isolated. *Mycoplasma bovis*, *M californicum*, and *M bovisgenitalium* were consistently isolated, but *M bovis* (243/499; 48.6%) was the most commonly isolated species. *Acholeplasma laidlawii* was more prevalent in 1989 and 1995 than other years. *Mycoplasma bovisgenitalium* and *M californicum* had a seasonal distribution. Less than 50 colonies per plate were isolated for most (317/500; 63.4%) bulk-tank samples. Of the milk samples with > 100 colonies/plate, *Mycoplasma bovis* was isolated most frequently (73/243; 30.0%).

Clinical Implications—Distribution of *Mycoplasma* spp varied by year, number of colonies isolated per sample, season, and herd. Therefore, it may be necessary to routinely sample bulk-tank milk, and all isolates should be speciated. Culture results from milk cooperatives should be used with other monitoring information to determine the *Mycoplasma* status of herds. (*J Am Vet Med Assoc* 1997;211:1036–1038)

Bacterial culture of bulk-tank milk samples is a useful procedure to detect *Mycoplasma*-infected cows in a herd.¹⁻³ Prevalence of *Mycoplasma* organisms varies between 1 and 4% for bulk-tank milk in which routine bacterial culturing is performed.^{3,4} Because cows with latent infections may not be shedding organisms,⁵ and subclinically infected cows may shed intermittently,⁶ collection of multiple samples of bulk-tank milk on a routine basis is more likely to yield *Mycoplasma* spp. Specifically modified media is required to enhance the

growth of *Mycoplasma* spp and inhibit the growth of other bacteria.⁴ *Mycoplasma bovis* is the most commonly isolated mycoplasmal mastitis pathogen.^{4,6} As the mastitic pathogenicity of mycoplasmal species varies, it is important to determine the genus and species of *Mycoplasma* isolated.⁴ Serologic tests or polymerase chain reaction analyses are necessary for speciation.⁴

The objective of the study reported here was to determine the prevalence of *Mycoplasma* organisms by genus and species isolated from bulk-tank milk samples collected monthly between 1989 and 1995 from dairy herds that were members of a milk cooperative in California.

Material and Methods

During the study period, the cooperative received milk from 260 dairies during any given month. Bulk-tank samples were collected monthly from each patron and submitted for bacterial culture of *Mycoplasma* spp in the cooperative's laboratory. The laboratory used direct plating with a 1:100 loop dilution on a quarter plate. Plates were incubated at 37 C (98.6 F) in candle jars for up to 7 days on media designed for isolation of mycoplasmas.⁸ Samples that were positive for *Mycoplasma* spp at the cooperative's laboratory were sent to the UC-Davis Milk Quality Laboratory for speciation. Speciation was by the agar-block technique, using fluorescent antibody.⁷ The study was conducted by using results obtained from the Milk Quality Laboratory from January 1989 through December 1995. Primary isolations were those that were the initial isolation for a species on a dairy or that were isolated again after ≥ 1 year from a previous isolation of the same species on a given dairy.

Statistical tests for seasonality of *Mycoplasma* isolations were performed separately for each species by means of χ^2 goodness-of-fit tests. The null hypothesis tested was that isolation of *Mycoplasma* organisms would be distributed randomly among months of the year with all months having an equal probability.

Results

During the study, 267 dairies sold milk to the cooperative. There were 3,120 (ie, 260 herds/mo \times 12 months) bulk-tank samples submitted for culture each year. *Mycoplasma* spp were isolated from bulk-tank milk from each of the 267 dairies. The number of bulk-tank milk samples yielding positive results at the cooperative's laboratory and submitted for speciation was 870. Of 232 nonpathogenic isolates, 107 were identified as *Acholeplasma laidlawii*, 107 were nontypeable isolates, and 18 were other infrequent isolates. Speciations were performed on 499 primary pathogenic *Mycoplasma* isolates (Table 1). Using 3,120 as the maximum number of positive

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bulk-tank samples possible per year, the percentage of positive bulk-tank samples per year was estimated to range from 1.8 to 5.8% for all samples submitted to the Milk Quality Laboratory, and from 1.2 to 3.1% for pathogenic *Mycoplasma* isolates. Large numbers of *A laidlawii* isolates were identified in 1989 (54) and 1995 (32).

Of the 638 pathogenic isolates, 498 (78.0%) were primary isolations. *Mycoplasma bovis* was the most common isolate (350/638, 54.9%) and was the most common primary isolate (311/638, 48.7%; Table 2). The number of *Mycoplasma*-positive bulk-tank samples per dairy varied from 1 to 4 for primary isolates and from 1 to 8 for all isolates. The majority of the dairies (296/389; 74.3%) had a single primary isolate, whereas 80 (20.6%) had 2 primary isolates, 10 (2.6%) had 3 primary isolates, and 3 (0.8%) had 4 primary isolates.

Mycoplasma bovigenitalium ($P < 0.0001$) and *M californicum* ($P = 0.0001$) had seasonal patterns of distribution, whereas *M bovis*, *M alkalescens*, and *M canadense* did not have a seasonal distribution (Table 3). Of the dairies from which primary *Mycoplasma* isolates were obtained, a single species was isolated from 99 of 198 (50.0%), 2 species were isolated from 68 of 198 (34.3%), 3 species were isolated from 25 of 198 (12.6%), and 4 species were isolated from 6 of 198 (0.03%). Most of the *Mycoplasma*-positive bulk-tank milk samples had < 50 colonies/plate (Table 4). *Mycoplasma bovis* had the most isolates with > 400 colonies/plate.

Table 1—Distribution of bulk-tank milk isolates positive for *Mycoplasma* spp in samples obtained from a California cooperative (1989 to 1995)

Year	All submissions	Pathogen-only submissions	Primary, pathogen-only submissions
1989	150 (4.8)	66 (2.1)	63 (2.0)
1990	112 (3.6)	82 (2.6)	68 (2.2)
1991	124 (4.0)	106 (3.4)	88 (2.8)
1992	111 (3.6)	94 (3.0)	79 (2.5)
1993	84 (2.7)	73 (2.3)	50 (1.6)
1994	55 (1.8)	50 (1.6)	38 (1.2)
1995	182 (5.8)	139 (4.5)	97 (3.1)
Total	870	638	499

Numbers in parenthesis are the estimated yearly percentage of bulk-tank milk samples with positive results.

Table 2—Distribution of major mycoplasmal isolates from a California cooperative (1989 to 1995). Total number of isolates is indicated first, followed by the number of primary isolates. Primary isolates are the first isolate or the first isolate after a 1-year interval from the original identification of the organism

Year	<i>M bovis</i>	<i>M bovigenitalium</i>	<i>M californicum</i>	<i>M alkalescens</i>	<i>M canadense</i>
1989	33/31	12/12	3/3	10/10	8/7
1990	30/22	16/16	3/3	17/16	14/13
1991	48/39	17/12	8/8	18/17	15/13
1992	43/31	30/29	3/3	8/8	10/10
1993	52/31	18/16	2/2	1/1	0/0
1994	35/24	13/12	0/0	0/0	2/2
1995	90/56	38/24	8/6	3/3	10/8
Total	350/243	140/126	30/27	59/50	59/53

Table 3—Distribution of primary isolations of *Mycoplasma* spp from bulk-tank milk samples obtained from patrons of a California cooperative (1989 to 1995)

Month	<i>M bovis</i>	<i>M bovigenitalium</i>	<i>M californicum</i>	<i>M alkalescens</i>	<i>M canadense</i>
Jan	22	32	14	2	3
Feb	19	17	8	4	5
Mar	25	19	6	4	3
Apr	20	9	5	6	2
May	20	8	5	3	3
Jun	30	11	1	7	0
Jul	13	3	1	4	4
Aug	20	5	1	6	2
Sep	19	2	5	3	0
Oct	18	4	4	7	1
Nov	15	6	1	2	2
Dec	22	10	2	2	2

Table 4—Distribution of number of colonies per plate for primary isolates detected in *Mycoplasma*-positive bulk-tank milk samples obtained from a California cooperative (1989 to 1995)

No. of colonies/plate	<i>M bovis</i>	<i>M bovigenitalium</i>	<i>M californicum</i>	<i>M alkalescens</i>	<i>M canadense</i>
<50	142	94	19	32	30
51-100	28	16	2	6	12
101-200	23	9	1	4	5
201-300	9	3	1	4	1
301-400	5	1	2	1	3
>400	36	3	2	3	2

Discussion

Reports of culture results of repeated monthly bulk-tank milk samples are scarce in the literature. During the 6-year period, 267 dairies had *Mycoplasma* spp isolated from their bulk-tank milk samples. Of these, 209 dairies had isolates of potentially pathogenic *Mycoplasma* organisms (*M bovis*, *M bovigenitalium*, *M californicum*, *M canadense*, and *M alkalescens*). The remaining 58 dairies had nonpathogenic *Mycoplasma* isolates in the bulk-tank milk samples. This illustrated the importance of speciating *Mycoplasma* isolates to determine whether some of the isolates were species considered to be nonpathogenic.^{1,4,8-10} In Tulare County, California, where many of the cooperative herds were located, mean herd size was approximately 1,000 cows. The cost to determine *Mycoplasma* spp of isolates from bulk-tank milk samples on these farms would be minimal, compared with the cost of culturing samples from all milking cows in a dairy belonging to this cooperative. Speciation of *Mycoplasma* organisms cultured from bulk-tank milk should always be done prior to beginning an aggressive control program.

On the basis of a yearly sample size of 3,120 bulk-tank samples, the estimated yearly prevalence of *Mycoplasma*-positive bulk tanks (Table 1) was similar to the 1 to 4% reported elsewhere^{3,4} when routine testing or surveys of bulk tanks was done on a statewide basis. Of the farms that had potentially pathogenic *Mycoplasma* spp in their bulk-tank milk samples, 74.3% had a positive result on only a single bulk-tank sample. At the opposite extreme, 1 dairy had 6 *Mycoplasma* spp isolated, and 4 other dairies had 5 species isolated during the study. Only 3.4% of the dairies had positive

isolates on > 3 tests. The most common species isolated from the *Mycoplasma*-positive bulk-tank samples was *M bovis*. It was found in 243 of 499 (48.6%) of the positive bulk-tank samples, which was similar to values reported in a survey conducted on dairies in California in 1980 to 1981.^{8,9} The second most common isolate was *M bovis genitalium*.

During the 6-year study, *M bovis*, *M californicum*, and *M bovis genitalium* were routinely identified each year (Table 2). Although other species were identified each year, the number identified varied considerably by year. For example, there was a greater number of *A laidlawii* isolated in 1989 and 1995, compared with other years. We could not determine reasons for this extreme variation; however, rainfall was above average in those years, which may have been a contributing factor.

Mycoplasma bovis, *M alkalescens*, and *M canadense* did not have a seasonal distribution pattern, but *M bovis genitalium* and *M californicum* were seasonal (Table 3). The lack of apparent seasonal distribution is contrary to findings previously reported for California dairies.¹¹

On 500 bulk-tank milk samples, the number of colonies isolated per plate was recorded by the cooperative's laboratory. More than 63.4% of the bulk tank samples confirmed in our laboratory had < 50 colonies/plate, whereas 12.8% had 51 to 100 colonies/plate. Although these bulk-tank milk samples with a few colonies may have resulted from the dilution of a few *Mycoplasma*-infected cows, they may also have been an indication of chronically infected cows, recovering clinically infected cows, or intermittent shedders. Few bulk-tank samples had > 400 colonies/plate (Table 4).

Acholeplasma laidlawii had the most (94.7%) bulk-tank milk samples with < 10 colonies/plate, whereas other *Mycoplasma* spp varied from 28.0 to 49.6%. Finding *A laidlawii* with few colonies per plate illustrated the need to use caution when deciding whether to take aggressive action after isolating mycoplasmas without obtaining information on genus and species involved. This may be even more important when enrichment techniques are used, because they may disclose more positive results for bulk-tank milk samples than direct plating. For example, finding a few colonies of *A laidlawii*, as was the case many times in the study reported here, has much less importance than finding a few colonies of *M bovis* or finding 101 to 200 colonies of *M bovis*/plate.

Analysis of our data indicated that *Mycoplasma* species seem to differ in distribution by year, number of colonies isolated per plate, seasonal patterns, and dairy. We suggest that it may be necessary to routinely sample bulk-tank milk for *Mycoplasma* organisms and

to identify the genus and species of any *Mycoplasma* isolates before beginning a total herd bacterial culturing program or an aggressive culling program. Identification of genus and species will preclude errors when decisions are made on the basis of genus alone, which may lead to costly attempts to control *Mycoplasma* spp when cows are infected with only nonpathogenic strains. Similarly, the number of colonies isolated may be important, depending on the desired outcome of the plan for sample collection. Monthly screening of bulk-tank samples by the cooperative is a useful procedure to assist their patrons in early identification of mastitis caused by *Mycoplasma* organisms. Results of these *Mycoplasma* screening tests should be used along with other monitoring information (eg, bacterial culture results of milk samples from postparturient cows, culture results of samples obtained from all cows with clinical mastitis, bulk-tank somatic cell counts, and incidence of cows with chronic nonresponsive mastitis) to determine the extent and nature of any problem within a dairy herd that could be attributed to *Mycoplasma* spp. Because our data was generated only from dairies in California, caution should be exercised when extrapolating our results to dairies in other geographic areas.

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