An Observational Study of *Corynebacterium bovis* in Selected Ontario Dairy Herds

B.W. Brooks, D.A. Barnum and A.H. Meek*

ABSTRACT

An observational study of *Corynebacterium bovis* was conducted in 74 Ontario dairy herds. The levels of infection with *C. bovis* were 19.9, 36.2 and 85.6% at the quarter, cow and herd level, respectively. Teat disinfection was found to be the variable best able to distinguish between herds with a high or low *C. bovis* quarter infection rate. Mean total milk somatic cell counts for 1103 quarters and 107 cows infected with only *C. bovis* ranged between 150,000 and 200,000/mL and were significantly higher than for uninfected quarters or cows. The rate of infection with mastitis pathogens was not significantly different in quarters previously colonized with only *C. bovis* compared to previously uninfected quarters.

RÉSUMÉ

Cette étude consistait à rechercher la présence de *Corynebacterium bovis*, dans le pis des vaches de 74 troupeaux laitiers ontariens. Le taux d'infection des quartiers, des vaches et des troupeaux atteignit respectivement 19.9%, 32,2% et 85,6%. La désinfection des trayons s'avéra la variante la plus susceptible de permettre d'établir une distinction entre les troupeaux aux prises avec un taux d'infection des quartiers, plus ou moins élevé. La numération moyenne des cellules somatiques présentes dans le lait, relative à 1 103 quartiers et à 107 vaches, infectés seulement par *C. bovis*, varia de 150 000 à 200 000/mL et se révéla sensiblement plus élevée que celle des quartiers et des vaches exempts d'une telle infection. Le taux d'infection par des agents pathogènes, capables de causer la mammite, n'afficha pas de différence appréciable dans les quartiers préalablement infectés par *C. bovis* seulement, comparativement aux quartiers antérieurement sains.

INTRODUCTION

Bovine udder infection is a major problem of the dairy industry and results in economically significant losses due to reduced milk production and quality. Routine use of teat disinfection and antibiotic dry cow therapy have been shown to be effective in reducing the level of infection (17), but widespread application has led to concerns regarding residues of antibiotics (15) and chemicals (11) in milk and the development of resistant strains of pathogenic bacteria (21). Alternative methods of control need to be investigated.

Corynebacterium bovis may play an important role as a biological control mechanism in the bovine udder but has received little attention. Microbial interactions resulting in protection of the host against pathogenic bacteria have been shown to be important in the gut and oral cavity (24) and there is limited evidence that *C. bovis* may protect the udder against infection with pathogenic bacteria (1, 3).

Increased interest in the role of *C. bovis* as a natural protective mechanism is associated with the widespread use of teat disinfection and antibiotic dry cow therapy. Although currently available teat dips reduce new infections due to Gram-positive bacteria they do not appear to be as effective against Gram-negatives (9) and thus there is some concern that the frequency of infection with the latter organisms may increase (8). In addition, routine use of teat disinfection and dry cow therapy reduces the prevalence of *C. bovis* (4) and may leave the udder more susceptible to infection with mastitis pathogens (7).

The purpose of the present study was to determine the frequency and distribution of *C. bovis* under natural field conditions and to investigate the association of the organism with bovine udder infection involving pathogenic bacteria.

MATERIALS AND METHODS

COLLECTION OF MILK SAMPLES

Individual quarter and composite (cow) milk samples were collected using standard procedures (6) from all lactating cows in each of 74 Ontario dairy herds on one occasion during the period February 1979 to July 1979. A bulk tank milk sample was also obtained

*Department of Veterinary Microbiology and Immunology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1. Present address of senior author: Animal Diseases Research Institute, Agriculture Canada, P.O. Box 11300, Station H, Nepean, Ontario K2H 8P9.

Submitted May 14, 1982.

from each herd. Herds were randomly selected from among the producers enrolled in the Dairy Herd Improvement (DHI) program in the counties of Hastings, Lennox-Addington, Prince Edward and Northumberland. Quarter samples were obtained after discarding the first 10-15 mL of milk. Composite samples were collected immediately after quarter samples and were made up of approximately equal volumes of milk from each lactating quarter. The latter milk samples will subsequently be referred to as the first set of samples.

Quarter, composite and bulk tank milk samples were collected from 70 of the above 74 herds on a second occasion during the period November 1979 to May 1980 and will subsequently be referred to as the second set of samples.

In addition, bulk tank milk samples were collected monthly during the period February 1979 to Apirl 1979 from all producers (approximately 800) in the above counties.

All milk samples were shipped to the Ontario Veterinary College in refrigerated coolers.

COLLECTION OF MANAGEMENT AND PRODUCTION DATA

Data regarding the use of mastitis control procedures and management practices were collected at the same time as the above quarter samples were obtained. The birthdate, last calving date and daily milk production, as recorded at the closest DHI test prior to sampling, were recorded for each cow.

EXAMINATION OF MILK SAMPLES

Bacteriological examinations and somatic cell count (SCC) determinations were carried out using methods described by Brooks *et al* (5). A California mastitis test (CMT) was performed on each quarter and composite sample. California mastitis test scores of negative, trace, 1, 2 and 3 were assigned values of zero, one, two, three and four respectively for the purpose of statistical analysis.

Corynebacterium bovis infection status of a quarter or cow (composite sample) was determined solely on the basis of bacteriological results using a critical level of 120 colony forming units (CFU)/mL. A quarter or cow was classified as infected with only *C. bovis* if at least 120 CFU/mL of *C. bovis* and less than 120 CFU/mL of any other bacterial species were isolated from a single quarter or composite milk sample respectively. A quarter or cow was classified as uninfected if less than 120 CFU/mL of any bacterial species were isolated from a single quarter or composite milk sample. Cows were also classified with respect to *C. bovis* infection status on the basis of quarter sample bacteriological results.

Infection of a quarter or cow (composite sample) with a bacterial pathogen was determined by two different methods. The first was based solely on the isolation of at least 120 CFU of a particular pathogen per mL of milk sample. The second method involved consideration of both bacteriological and CMT results and quarters were classified as being positive, negative, group 3 (isolation of a pathogen but low CMT) or group 4 (elevated CMT but no pathogen).

ANALYTIC METHODS

Four data files were created. The first contained data on management practices and use of various mastitis control procedures. The second and third files contained results for the first and second set of samples respectively. The fourth file contained data from the first and second set of samples for those cows which had been sampled on both occasions.

Analysis was carried out using the Statistical Package for the Social Sciences (19).

The quarter infection rate (QIR) with *C. bovis* was determined for each herd. On the basis of the mean QIR with *C. bovis* for all herds, the herds were divided into two groups. Discriminant analysis, a statistical technique used to distinguish between two or more groups of cases, was used to determine which of the two variables TEATDIP (no = 0; yes = 1) or DRYCOW (treat all quarters: no = 0; yes = 1) was better able to differentiate between herds with a *C. bovis* QIR below (QIRCB = 1) or above (QIRCB = 2) the mean. Regression analysis was used to investigate the relationship between the presence (= 1) or absence (= 0) of *C. bovis* and each of actual cow age (years) and actual days in milk.

Total and differential SCC were logarithmically transformed prior to statistical analysis.

The QIR with any bacterial pathogen was determined for each herd. On the basis of the mean QIR with any bacterial pathogen (QIRP) for all herds, the herds were divided into two groups. Discriminant analysis was used to determine which of the three variables TEATDIP (no = 0; yes = 1), DRYCOW (treat all quarters: no = 0; yes = 1) or QIRCB (below mean = 1; above mean = 2) was best able to differentiate between herds with a QIR with any pathogen below the mean for all herds (QIRP = 1) and above (QIRP = 2). Stepwise discriminant analysis was used to look at the effect of TEATDIP AND DRYCOW having controlled for the effect of QIRBC.

RESULTS

The percentage isolation of *C. bovis* (≥ 120 CFU/mL) from the first set of quarter milk samples collected from herds in which various combinations of teat disinfection and antibiotic dry cow therapy were routinely used is shown in Table I. The highest QIR with *C. bovis* was found in herds in which no teat disinfection and no or selected (treat selected quarters of selected cows) dry cow therapy were used (42.2% and 42.4% respectively). The lowest QIR with *C. bovis* was found in herds in which both teat disinfection and complete (treat all quarters of all cows) dry cow therapy were used (11.5%).

Similar results were obtained for the second set of samples. *Corynebacterium bovis* was isolated (≥ 120 CFU/mL) from 19.5%

TABLE I. The Percentage Isolation of *C. bovis* from Quarter Milk Samples[a] Collected from Selected Dairy Herds in Which Various Combinations of Teat Disinfection and Antibiotic Dry Cow Therapy were Used

Use of Teat Disinfection (TD) and Dry Cow Therapy (DCT)	Percentage Isolation of *C. bovis*		
	Herd Level	Cow Level	Quarter Level
All herds	85.6 (74)[b]	36.2 (2381)[c]	19.9 (9620)[d]
No DCT and no TD	100.00 (6)	69.3 (163)	42.2 (649)
DCT (selected)[e] and no TD	100.0 (5)	73.2 (164)	42.4 (672)
DCT (complete)[f] and no TD	80.0 (5)	60.7 (135)	35.9 (543)
TD and no DCT	100.0 (10)	31.7 (303)	16.2 (1226)
TD and DCT (selected)	96.0 (25)	33.2 (843)	17.7 (3409)
TD and DCT (complete)	69.6 (23)	22.0 (773)	11.5 (3121)

[a]Results from first set of samples only are shown
[b]% of herds in which at least one quarter of one cow was infected with *C. bovis* (≥ 120 CFU/mL); number of herds in parentheses
[c]% of cows with at least one quarter infected with *C. bovis* (≥ 120 CFU/mL); number of cows in parentheses
[d]% of quarters infected with *C. bovis* (≥ 120 CFU/mL); number of quarters in parentheses
[e]Treat only selected quarters of selected cows
[f]Treat all quarters of all cows

of 8994 quarters. The highest QIR with *C. bovis* was in herds in which no teat disinfection and selective, complete or no dry cow therapy were used (63.5%, 51.9% and 48.1% respectively). The lowest QIR with *C. bovis* was in herds in which both teat disinfection and no or complete dry cow therapy were used (9.4% and 11.1% respectively).

Discriminant analysis revealed that the variable TEATDIP was better able to distinguish between herds with a *C. bovis* QIR above and below the mean for all herds (21.3 ± 19.0 and 21.1 ± 20.5 for the first and second set of samples respectively) than was the variable DRYCOW. Standardized discriminant coefficients for TEATDIP were 0.91 and 0.94 (first and second set of samples respectively) and for DRYCOW and 0.40 and 0.33.

The percentage isolation of *C. bovis* (≥ 120 CFU/mL) from composite samples was also determined. *Corynebacterium bovis* was isolated from 21.3% and 22.8% of 2386 and 2240 composite samples respectively (first and second set of samples).

The influence of cow age and stage of lactation on the percentage isolation of *C. bovis* (≥ 120 CFU/mL) were investigated using results from cow (composite) samples. The percentage isolation of *C. bovis* was found to increase ($p < 0.05$) as cow age increased for both the first and second set of samples when all herds were considered together and for the first set of samples only for herds in which both teat disinfection and complete dry cow therapy were used. For the first and second set of samples, no change ($p > 0.05$) in the percentage isolation of *C. bovis* was observed with an increase in cow age for samples from herds in which no teat disinfection and no or only selected dry cow therapy were used. An increase in stage of lactation was not found to be associated ($p > 0.05$) with an increase or decrease in the percentage isolation of *C. bovis* for any of the circumstances investigated for the first or second set of samples.

Corynebacterium bovis was isolated from less than 20% of bulk tank milk samples collected from herds known to have a *C. bovis* QIR greater than 20% and from less than 10% of bulk tank milk samples from the 800 herds. In most cases less than 120 CFU of the organism were isolated per mL of sample.

Somatic cell counts for milk samples from quarters or cows classified as uninfected or infected with only *C. bovis* are shown in Table II. Mean total and differential SCC for quarters infected with only *C. bovis* were significantly higher ($p < 0.05$) than those for uninfected quarters in the first and second set of samples. Similar results were obtained from analyses carried out to minimize the effect of cow level variables. Separate analyses were also carried out to investigate the effect of the critical level used to establish the quarter infection status with *C. bovis* on mean total SCC. Using a critical level of ≥ 40 or ≥ 400 CFU/mL, a statistically significant ($p < 0.05$) elevation in mean total SCC was observed for quarters classified as infected with *C. bovis* compared to uninfected quarters.

Mean total and differential SCC for composite samples from which only *C. bovis* was isolated (≥ 120 CFU/mL) were significantly higher ($p < 0.05$) than those for composite samples from which no

TABLE II. Geometric Mean Total and Differential Somatic Cell Counts (SCC) for Milk Samples from Uninfected Quarters and Cows and from Quarters and Cows Infected with Only *C. bovis*

Total or Differential SCC	Set of Samples	Somatic Cell Count					
		Uninfected Quarters		Quarters Infected with only *C. bovis*	Uninfected Cows[a]		Cows[a] Infected with only *C. bovis*
Total	1	125.4 ± 2.7 (2672)[b]	*	171.3 ± 2.7 (702)	122.2 ± 2.4 (408)	*	165.9 ± 2.4 (107)
	2	120.6 ± 2.5 (2099)	*	170.6 ± 2.5 (401)	118.1 ± 2.5 (301)		156.1 ± 2.6 (70)
Differential	1	5.7 ± 2.3 (2672)[c]	*	8.7 ± 2.1 (702)	5.6 ± 2.3 (408)	*	8.6 ± 2.2 (107)
	2	6.0 ± 2.1 (2099)	*	9.0 ± 2.1 (401)	5.9 ± 2.3 (301)		7.6 ± 2.2 (70)

[a]Composite sample results
[b]Geometric mean of total SCC ± SD × 10^3/mL; number of observations in parentheses
[c]Geometric mean of differential (% of total cell volume in channel 8) SCC ± SD; number of observations in parentheses
*($P < 0.05$) Student's t-test

bacteria were isolated in the first but not the second set of samples. Composite sample results for cows from which only *C. bovis* was isolated from one or more quarters (quarter sample results) or for cows from which no organism was isolated from any quarter did not suggest ($p > 0.05$) that total or differential SCC increased as the number of quarters per cow infected with *C. bovis* increased.

California mastitis test reactions for quarters (0.58 ± 0.84 and 0.65 ± 0.83 — first and second set of samples respectively) or cows (0.56 ± 0.78 and 0.66 ± 0.90) infected with only *C. bovis* were significantly higher ($p < 0.05$) than those for uninfected quarters (0.25 ± 0.67 and 0.27 ± 0.65) or cows (0.33 ± 0.69 and 0.38 ± 0.68) respectively.

Mean daily milk production for cows (composite sample results) infected with only *C. bovis* (21.2 ± 8.1 and 19.6 ± 7.5 kg — first and second set of samples respectively) was not significantly different (p 0.05) from that of uninfected cows (21.5 ± 7.0 and 19.2 ± 7.2). No increase or decrease ($p > 0.05$) in milk production was observed with an increase in cow age for cows infected with only *C. bovis* in the first or second set of samples and for uninfected cows in the second but not the first set of samples. A decrease in milk production was associated ($p < 0.05$) with an increase in stage of lactation for cows infected with only *C. bovis* and for uninfected cows in the first and second set of samples.

The frequency of infection with any bacterial pathogen (based on bacteriological results alone) in quarters classified as infected or uninfected with *C. bovis* is shown in Table III. The results for the first and second set of samples suggested that a quarter was more likely (relative risk = 1.29) to be classified as infected with a bacterial pathogen if it was classified as infected with *C. bovis*. Similar results were obtained when only quarters classified as positive or negative for any bacterial pathogen (based on bacteriological and CMT results) were included in the analysis.

TABLE III. Frequency of Infection with any Bacterial Pathogen as Determined on Two Separate Occasions in Quarters Classified as Infected or Uninfected with *C. bovis*

Set of Samples	Infected[a] or Uninfected[b] with *C. bovis*	Infected or uninfected with any bacterial pathogen[c]	
		Infected[a]	Uninfected[b]
1	Infected	215[d]	535
	Uninfected	679	2376
			($p < 0.05$) chi-squared test
2	Infected	180	286
	Uninfected	490	1851
			($p < 0.05$) chi-squared test

[a] $\geq$ 120 CFU/mL of *C. bovis* or any bacterial pathogen and < 120 CFU/mL of coagulase negative staphylococci
[b] < 120 CFU/mL of *C. bovis* or any bacterial pathogen and < 120 CFU/mL of coagulase negative staphylococci
[c] Includes *Staph. aureus*, *Strep. agalactiae*, other streptococcal species, coliform, *Pseudomonas* species, *Corynebacterium pyogenes*
[d] Number of quarters

Separate analyses were also carried out to determine the significance of association between *C. bovis* and each of *Staphylococcus aureus*, *Streptococcus agalactiae* or other streptococcal species. For each of the latter pathogens it was observed that if a quarter was classified as infected with the pathogen it was more likely ($p < 0.05$) to be classified as infected rather than uninfected with *C. bovis*. The frequency of infection with coliform bacteria was too low (< 1.0% of all quarters) to allow any conclusions to be drawn.

The mean quarter infection rates with any bacterial pathogen for all herds were 19.2 ± 15.4 and 15.6 ± 13.0 for the first and second set of samples respectively. The standardized discriminant function coefficients obtained using stepwise discriminant analysis and forcing the variable QIRCB to enter the analysis first were: QIRCB 0.81 and 0.94 (first and second set of samples respectively), TEATDIP -0.40 and -0.22 and DRYCOW 0.02 and 0.25.

The rate of infection with bacterial pathogens over a period of nine months in quarters previously classified as uninfected or infected with only *C. bovis* is shown in Table IV. The results were obtained from examination of quarter samples from cows sampled on both occasions and in the same lactation on both occasions. No significant ($p > 0.05$) association was found between the rate of infection with a pathogen in previously uninfected quarters compared to that for quarters previously infected with only *C. bovis*.

TABLE IV. Rate of Infection with Any Bacterial Pathogen over a Period of Nine Months in Quarters Previously Classified as Uninfected or Infected with Only *C. bovis*

Quarters Previously Uninfected or Infected[a] with only *C. bovis*	Percentage of Quarters Becoming or not Becoming Infected with any Bacterial Pathogen[c]	
	Infected	Not Infected
Previously infected with only *C. bovis*	10.8 (4)[d]	89.2 (33)
Previously uninfected	9.6 (38)	90.4 (359)
		($p > 0.05$) chi-squared test

[a] As determined from bacteriological results for the first set of quarter samples
[b] As determined from bacteriological results for the second set of quarter samples
[c] Includes *Staph. aureus*, *Strep. agalactiae*, other streptococcal species, coliform, *Pseudomonas* species, *Corynebacterium pyogenes*
[d] Percentage of quarters; number of observations in parentheses

TABLE V. Rate of Infection with *C. bovis* over a Period of Nine Months in Quarters Previously Classified as Uninfected or Infected with Only a Bacterial Pathogen

Quarters Previously Uninfected or Infected[a] with any Bacterial Pathogen	Percentage of Quarters Becoming or Not Becoming[b] Infected with *C. bovis*	
	Infected	Not Infected
Previously infected with only a bacterial pathogen	24.6 (14)[d]	75.4 (43)
Previously uninfected	6.8 (26)	93.2 (359)
		($p < 0.05$) chi-squared test

[a]As determined from bacteriological results for the first set of quarter samples
[b]As determined from bacteriological results for the second set of quarter samples
[c]Includes *Staph. aureus*, *Strep. agalactiae*, other streptococcal species, coliform, *Pseudomonas* species, *Corynebacterium pyogenes*
[d]Percentage of quarters, number of observations in parentheses

The rate of infection with *C. bovis* in quarters previously uninfected or infected with only a bacterial pathogen is shown in Table V. The results suggested that a quarter previously infected with a pathogen was more likely ($p < 0.05$) to become infected with *C. bovis* than was a previously uninfected quarter.

DISCUSSION

Except for the general statement that in the absence of teat disinfection and antibiotic dry cow therapy *C. bovis* is usually the most common organism isolated from bovine milk samples (2, 3), reports in the literature which provide data regarding the prevalence of *C. bovis* are rare. Many surveys have been conducted to determine the prevalence of bacteria in bovine milk samples (10, 23) but these have been concerned with pathogenic organisms associated with significant economic loss. *Corynebacterium bovis* is generally considered to be a harmless commensal (18) and thus has been ignored. In the present study the QIR with *C. bovis* at the herd level was found to be as high as 88% and as low as 0%.

The influence of mastitis control procedures on the prevalence of *C. bovis* in 30 herds was observed by Bramley *et al* (4). Over a three year period during which teat disinfection and antibiotic dry cow therapy were routinely applied, the mean percentage of quarters infected with *C. bovis* declined from 47 to five. Similar results were obtained in the present study which involved a larger number of herds. The lowest QIR with *C. bovis* was found in herds in which teat disinfection and no or complete dry cow therapy were used.

Results of previous studies have suggested that infection with *C. bovis* is associated with a mild increase in milk SCC. Black *et al* (1) observed that CMT scores for samples from quarters infected with only *C. bovis* were mildly elevated compared to those for uninfected quarters (1.1 and 0.5 respectively). Bramley (3) reported arithmetic mean SCC of 460,000 and 193,000 per mL for quarters infected with *C. bovis* or coagulase negative micrococci and for uninfected quarters respectively but no data were reported for mean SCC for quarters infected with only *C. bovis*.

A mild but statistically significant elevation in total SCC was observed in the present study for quarters infected with only *C. bovis* compared to uninfected quarters. The elevation was found to be significant using CMT or automated cell counting results. The elevation was considered to be mild as the geometric mean SCC was less than 200,000/mL, a level not generally considered to be associated with a decrease in milk production (22) or a change in milk composition (13). These observations and the results obtained for mean daily milk production suggested that colonization with *C. bovis* does not significantly lower milk production and were not similar to the findings of Natzke *et al* (16).

The mean differential SCC was also found to be significantly higher in milk from quarters infected with only *C. bovis* compared to that for uninfected quarters. This is of particular importance as a neutrophil leukocytosis has been shown to protect the mammary gland against establishment of infection with mastitis pathogens (12, 20).

Black *et al* (1) and Bramley (3) reported that the new infection rate with bacterial pathogens was lower in quarters previously infected with *C. bovis* than in previously uninfected quarters.

In the present study the proportion of quarters infected with *C. bovis* that were also infected with a pathogen was found to be significantly higher than the proportion of quarters not infected with *C. bovis* that were infected with a pathogen. However, prevalence data do not provide an indication of the order of infection and data from the present study also suggested that *C. bovis* may become established in quarters infected with a mastitis pathogen. In addition teat disinfection and dry cow therapy were being used in many herds and these practices have been associated with lower *C. bovis* (4) and pathogen quarter infection rates (14).

Results obtained in the studies of Black *et al* (1) and Bramley (3) suggest that colonization of the udder with *C. bovis* is associated with protection against subsequent infection with pathogenic bacteria. In the present study, although the rate of infection with bacteria pathogens in quarters previously infected with only *C. bovis* was not different from that in previously uninfected quarters, a protective effect associated with *C. bovis* may not have been observed because of the long time period between collection of milk samples (nine months compared to one week and three months in the previous studies). Further studies are required to investigate the role of *C. bovis* in the control of bovine udder infection.

ACKNOWLEDGMENTS

This work was supported in part by the Ontario Milk Marketing Board, the Ontario Ministry of Agriculture and Food and Agriculture Canada. The field assistance of E. Johnston and the excellent technical work of R. Johnston, R. Johnson and D. Moore is greatly appreciated. The senior author was supported on a sholarship from the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

1. **BLACK, R.T., C.T. BOURLAND** and **R.T. MARSHALL.** California mastitis test reactivity and bacterial invasions in quarters infected with *Corynebacterium bovis*. J. Dairy Sci. 55: 1016-1017. 1972.
2. **BLACK, R.T., R.T. MARSHALL** and **C.T. BOURLAND.** Locus of mammary gland infections of *Corynebacterium bovis*. J. Dairy Sci. 55: 413-416. 1972.
3. **BRAMLEY, A.J.** Infection of the udder with coagulase negative micrococci and *Corynebacterium bovis*. Proc. of Seminar on Mastitis Contro. Doc. 85: 377-381. Int. Dairy Fed., Brussels, Belgium. 1975.
4. **BRAMLEY, A.J., R.G. KINGWILL, T.K. GRIFFIN** and **D.L. SIMPKIN.** Prevalence of *Corynebacterium bovis* in bovine milk samples. Vet. Rec. 99: 275. 1976.
5. **BROOKS, B.W., D.A. BARNUM** and **A.H. MEEK.** A survey of mastitis in selected Ontario dairy herds. Can. vet. J. 23: 156-159. 1982.
6. **BROWN, R.W., D.A. BARNUM, D.E. JASPER, J.S. MCDONALD** and **W.D. SCHULTZE.** Microbiological Procedures for Use in the Diagnosis of Bovine Mastitis. 2nd Edition. National Mastitis Council, Washington, D.C. 1981.
7. **DODD, F.H.** and **T.K. GRIFFIN.** The role of antibiotics treatment at drying off in control of mastitis. Proc. of Seminar on Mastitis Control. Doc. 85: 282-302. Int. Diary Fed., Brussels, Belgium. 1975.
8. **EBERHART, R.J.** and **J.M. BUCKALEW.** Evaluation of a hygiene and dry period therapy program for mastitis control. J. Dairy Sci. 55: 1683-1691. 1972.
9. **EBERHART, R.J., H.C. GILLMORE, L.J. HUTCHINSON** and **S.B. SPENCER.** Somatic cell counts in DHI samples. Proc. Natn. Mastitis Council. pp. 32-40. 1979.
10. **ELLIOTT, R.E.W., J.G. TATTERSFIELD** and **E.D. BROOKBANKS.** New Zealand national mastitis survey: 1965-6. 3. The microflora of bovine composite milk samples. N. Z. vet. J. 24: 80-84. 1976.
11. **HEMKEN, R.W.** Milk and meat iodine content: relation to human health. J. Am. vet. med. Ass. 176: 1119-1121. 1980.
12. **JAIN, N.C., O.W. SCHALM, E.J. CARROLL** and **J. LASMANIS.** Experimental mastitis in leukopenic cows: Immunologically induced neutropenia and response to intramammary inoculation of *Aerobacter aerogenes*. Am. J. vet. Res. 29: 2089-2097. 1968.
13. **KING, J.O.L.** Cell counts and composition of bovine milk. Vet. Rec. 103: 397-398. 1978.
14. **KINGWILL, R.G., F.K. NEAVE, F.H. DODD, T.K. GRIFFIN** and **D.R. WESTGARTH.** The effect of a mastitis control system on levels of subclinical and clinical mastitis in two years. Vet. Rec. 87: 94-100. 1970.
15. **MOL, H.** Antibiotics and Milk. Rotterdam, Netherlands: A.A. Balkema. 1975.
16. **NATZKE, R.P., R.W. EVERETT, R.S. GUTHRIE, J.F. KEOWN, A.M. MEEK, W.G. MERRILL, S.J. ROBERTS** and **G.H. SCHMIDT.** Mastitis control programs: effect on milk production. J. Dairy Sci. 55: 1256-1260. 1972.
17. **NEAVE, F.K., F.H. DODD** and **R.G. KINGWILL.** A method of controlling bovine udder disease. Vet. Rec. 78: 521-523. 1966.
18. **NEWBOULD F.H.S.** Microbial diseases of the mammary gland. *In* Lactation, a Comprehensive Treatise. Vol. II. Edited by B.L. Larson and V.R. Smith. New York: Academic Press, Inc. 1974.
19. **NIE, N.H., C.H. HULL, J.G. JENKINS, K. STEINBRENNER** and **D.H. BENT.** Statistical Package for the Social Sciences. Second Edition. New York: McGraw-Hill Inc. 1975.
20. **SCHALM, O.W., E.J. CARROLL** and **J. LASMANIS.** The leukocyte barrier and serologic investigations of experimental coliform (*Aerobacter aerogenes*) mastitis in cattle. Am. J. vet. Res. 25: 90-96. 1964.
21. **STOVLBAEK-PEDERSEN, P.** Penicillin-resistant *Staphylococcus aureus* in the bovine udder — definition and assay methods. *In* Proc. of Seminar on Mastitis control Doc. 85: 352-357. Int. Dairy Fed., Brussells Belgium. 1975.
22. **WARD, G.E.** and **L.H. SCHULTZ.** Relationship of somatic cells in quarter milk to type of bacteria and production. J. Dairy Sci. 55: 1428-1431. 1972.
23. **WILSON, C.D.** and **M.S. RICHARDS.** A survey of mastitis in the British dairy herd. Vet. Rec. 106: 431-435. 1980.
24. **WOOLCOCK, J.B.** Bacterial Infection and Immunity in Domestic Animals. Amsterdam: Elsevier Scientific Publishing Co. 1979.