EFFECT OF ULTRASONIC TREATMENT ON RECOVERY OF BACTERIA FROM MILK

by

ISABEL CRISTINA LARRIERA

B.S., Universidad de la Republica,
Facultad de Agronomia,
Montevideo, Uruguay, 1976

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

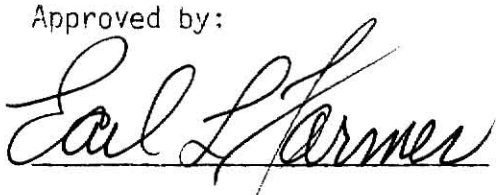
MASTER OF SCIENCE

Department of Animal Sciences and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1982

Approved by:



Major Professor

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Bacteriological analysis of quarter milk samples is an important step in the diagnosis of infectious mastitis. Recommended procedures for bacteriological assay involve aseptic sampling, proper handling and storage followed by plating on one or more kinds of agar (1, 19). Identification of microorganisms involves further steps (19). However, failure to recover pathogenic microorganisms from clinical cases is not uncommon (10, 14, 17, 20). Reasons for this could be: a) no infection present at time of sampling (14), b) post-sampling destruction of microorganisms by leucocytic action (37), c) error due to plating small volumes from a milk sample with a low bacteria count (19, 20), d) presence of potentially viable bacteria entrapped inside the leucocytes and not recovered by usual procedures. Although the polymorphonuclear leucocyte (PMN) is an important mammary defense mechanism against infection (32, 33), its action is known to be impaired in milk with respect to both ingestion and killing of bacteria, as reviewed by Paape (25, 32, 33). This has been attributed to one or more of the following factors: ingestion of fat (26, 27, 30, 31), ingestion of casein (31, 34, 35), casein coating (35), lack of energy (18, 21, 22), presence of cream (9), lack of opsonins (21, 22), inability to lower the pH of phagocytic vacuole (25, 33) and stress (28). However, some authors do not consider lack of opsonins (35) or stress (11) to be factors depressing PMN phagocytic activity.

Our hypothesis was that some bacteria not recovered by standard plating techniques might have been ingested but not destroyed by the PMNs. Therefore the use of a PMN disruptive procedure not affecting bacteria viability could give more reliable plating results.

Cellular disruptive techniques have been used extensively in studying

PMN activity. They involve the use of lysis, either by hypertonic (23,24) or hypotonic solutions (9, 15, 34, 39), freeze-thaw cycle (44) and sonication (4, 11, 12, 26, 30, 42, 43). In all the foregoing cases release of phagocytosed bacteria was achieved. However, no reports were found that indicated the use of disruptive procedures in routine bacteriological assays. Blending by an instrument called the stomacher is an easy to use method to extract bacteria from food and other products (36); although no reference has been found on the effect of the stomacher on somatic cell disruption, its potential was considered worthy of evaluation.

In the agar plate method bacteria are counted as colony forming units (1, 8). Since each colony could have originated from a single cell or a group of cells (clump, chain), counts are only an estimate (8) and often are lower than actual counts. In view of this, the possibility that one or more PMN disruptive procedures might disperse bacterial aggregates could lead to more accurate plate counts. Sonication has been used to disperse staphylococci clumps (9, 26, 42) with a resultant almost twofold increase in viable cell counts (42).

The purposes of our study were: 1) to find a suitable minimum time-expense method to disrupt mammary PMNs without decreasing bacteria viability; and 2) to test the quantitative effect of that procedure on recovery of mammary bacteria.

MATERIALS AND METHODS

A) Preliminary observations on somatic cell disruptive procedures.

Stomacher blending, sonication and lysis were the cellular disruptive procedures selected for preliminary testing. Somatic cell disruption was

measured by comparing pre and post treatment Electronic Somatic Cell Counts (ESCC)[1] and confirmed by microscopic observation of smears stained with Wright's stain[2]. Pre and post treatment viable cell counts (VCC) (1) were compared for stomacher blending and sonication procedures to determine effect on bacteria viability.

Clinically mastitic quarters were considered to be a suitable source of high somatic cell count milk for observing effects of disruptive procedures. Therefore, mastitic cows in the Kansas State University herd that had not received antibiotic treatment four days prior to sampling were used. A California Mastitis Test (CMT) was performed on all samples upon arrival in the laboratory. Those samples showing a CMT score of 3 and not excessively flaky were selected for procedural observations. Samples used for sonication and stomacher blending were inoculated with Staphylococcus aureus strain S-6 (courtesy of Dr. Daniel Y. C. Fung, Animal Sci. Department, Kansas State University), to assure presence of bacteria for viability observations.

For stomacher blending observations, each milk sample was divided into 80 ml sub-samples, placed in 400 ml sterile polyethylene bags and subsequently blended in a Stomacher Lab-Blender 400[3] for 0, 1, 2, 4, or 8 minutes. Effects of stomaching on ESCC and VCC are presented in Table 1. This method was not effective in disrupting somatic cells, post treatment ESCC values

---

1.- Performed at DHIA laboratory on a Fossomatic cell counter (A/S N. Foss Electric, DK-3400 Hillerød, Denmark).

2.- Diff-Quik. (Manufactured by Dade Diagnostic, Inc., Aguada, Puerto Rico 00602).

3.- Seward Laboratory. (A member of the Medical Division of UAC International Limited, London, England).

being only slightly negative or positive. Moreover, these small differences in ESCC could have been the result of sampling error rather than treatment. Microscopic observations confirmed failure of disruption. To extend stomacher treatment time beyond 8 minutes was thought impractical, thus no further attempts were made.

For sonication observations, each milk sample was divided into 75 ml sub-samples and sonicated for one minute at 0 (no treatment), 10, 20, 40, 60, 80, 90, or 100 setting with a Bronwill Biosonik II sonicator[1], all settings represent percent of maximum instrument output. Ultrasound proved practical and effective in somatic cell disruption as shown in Table 1 and corroborated by microscopic observation. Somatic cell disruption increased with increasing power settings and more than 80% of the cells were disrupted by the 80 setting. Microscopic observations verified these findings. At settings above 80 few recognizable cells were found in the stained smears. Viable cell counts consistently increased with increasing power indicating bacterial viability was not destroyed. Time required for sonication was estimated to be 3 minutes per sample.

Two different approaches were attempted for disruption with lysis, a hypotonic (lysis-1) and a hypertonic (lysis-2) approach. Lysis-1 was performed according to Russell et al. (34), except that larger volumes were used. Two ml of milk sample and 98 ml of distilled water were vigorously shaken for one minute. In lysis-2 a 70 ml sample consisting of equal parts milk sample and 30 % NaCl solution were shaken with glass beads for one minute (24). The lysis procedures were quickly recognized as an undesirable method. It was tedious, unsuitable for ESCC and complicated VCC procedures.

1.- Frequency of ultrasonic output: 20 Kcps $\pm$ 400 cps

Thus it was promptly discarded.

Based on the results obtained in this preliminary work, sonication at 90 % power setting was adopted as standard method for further study.

B) Effect of sonication on somatic cell and bacteria counts in milk.

a) Sampling.

Representative milk samples with ESCCs ranging from normal, for healthy mammary quarters, to those expected from subclinically mastitic quarters, were used for testing sonication effects. CMT scores of 1 and 2 were considered appropriate for sample selection.

The Kansas State University dairy herd was used. One hundred and fifty ml milk samples were aseptically (19) collected from quarters with CMT scores of 1 or 2 from cows that had not received antibiotic treatment in the last four days. Samples were refrigerated within two hours after collection.

b) Sonication.

Samples were thoroughly mixed in sterile Erlenmeyer flasks prior to division into 75 ml sub-samples, one for control observations, stored at $5^{o}C$ until plating. The second sub-sample was poured into a sterile 150 ml plastic beaker and sonicated with a Bronwill Biosonik II sonicator[1] at a power setting of 90 for one minute. Between samples the probe was rinsed with distilled water, dried with paper tissue and sterilized with a sterile cotton ball soaked with absolute ethanol.

c) Plating.

Two types of media were used, Standard Plate Count (SPC) agar for general recovery of bacteria (1), and Mannitol Salt (MS) agar for recovery

---

1.- Standard ½" titanium tip probe.

of mannitol fermentative staphylococci (6, 29, 40, 41). Counts were made and recorded according to Standard Methods for the Examination of Dairy Products (1). Representative mannitol fermentative colonies were Gram stained and subjected to coagulase test to determine presence of Staphylococcus aureus and/or mannitol fermentative-coagulase negative staphylococci.

d) Somatic cell count.

Duplicate aliquots were taken for electronic somatic cell count (ESCC) using the Fossomatic cell counter (13). Data were recorded and grouped in three categories as follows: A) $< 3 \times 10^5$ cells/ml, presumed healthy; B) $> 3 \times 10^5$ and $< 8 \times 10^5$ cells/ml, subclinical; C) $> 8 \times 10^5$ cells/ml, subclinical with high SCC.

e) Analysis of data (38).

Data were analyzed according to the following model

$$Y_{ijk} = \mu + \alpha_i + \beta_{ij} + \varepsilon_{ijk}$$

where:

μ: Population mean.

α_i: Somatic cell count group effect. Three levels of somatic cells were used: A) less than 3×10^5 cells per ml,

B) between 3×10^5 and 8×10^5 cells per ml,

C) more than 8×10^5 cells per ml.

β_{ij}: Cow within somatic cell count group effect.

ε_{ijk}: Random error.

$Y_{ijk} = \{x_{ijk(1)} - x_{ijk(2)}\}$

$x_{ijk(1)}$ = The k^{th} sample from the j^{th} cow of the i^{th} somatic cell group that was sonicated.

$x_{ijk(2)}$ = The k^{th} sample from the j^{th} cow of the i^{th} somatic cell group that was not sonicated.

Note:

Y_{ijk}: a) Viable cell count, where $Y = \{x_{(1)} - x_{(2)}\}$, and x is expressed as log {actual count + 1}.

b) Mannitol fermentative staphylococci count, where $Y = \{x_{(1)} - x_{(2)}\}$, and x is expressed as log {actual count + 1}.

c) Somatic cell count and $Y = \dfrac{\{x_{(2)} - x_{(1)}\}}{x_{(2)}} \times 100$

The analysis of variance and results for the above model are shown in the following table,

Source of variance	d.f.	Mean squares	Expected means
Somatic cell count groups	2	MS_{SCC}	$\sigma^2 + 1.471\sigma^2_{C(SCC)} + Q_{SCC}$
Cow within SCC group	92	$MS_{C(SCC)}$	$\sigma^2 + 1.317\sigma^2_{C(SCC)}$
Residual	31	MS_E	σ^2

LSD multiple range test followed an F test for the SCC means.

TABLE 1.- Effect of stomaching, sonication and lysis on electronic somatic cell and viable cell counts.

Experimental method	ESCC[a] (% change)	VCC[b] (difference from control) log[c] means	VCC[b] (difference from control) geometric means
Stomaching			
2[d]	+ 4.56	+ 0.06	1.15
4	+ 2.33	+ 0.14	1.38
8	- 1.36	+ 0.30	2.00
Sonication			
10[e]	- 0.24	+ 0.08	1.20
20	- 0.16	+ 0.05	1.12
40	- 12.50	+ 0.17	1.48
60	- 53.99	+ 0.42	2.63
80	- 85.76	+ 0.55	3.55
90	- 93.60	+ 0.69	4.90
100	- 96.36	+ 0.77	5.89
Lysis			
lysis-1	+ 100.00	nd	nd
lysis-2	+ 15.64	nd	nd

a.- Electronic somatic cell count.

b.- Viable cell count.

c.- Expressed as difference of logarithms of actual counts.

d.- Minutes.

e.- Percent of maximum power for a Biosonik II sonicator.

RESULTS AND DISCUSSION

Preliminary work using stomacher blending, lysis and ultrasound procedures to disrupt somatic cells in milk without decreasing bacteria viability showed ultrasound to be the most suitable method. Therefore, sonication treatment was selected for investigation of effect on viable cell count and somatic cell disruption when applied to milk samples containing a wide range of somatic cells.

The effect of ultrasound on somatic cell disruption is shown in Table 2. Somatic cell disruption was effective for all somatic cell count (SCC) groups averaging 74.7 % cell disruption. Percent of somatic cell disruption tended to increase as the somatic cell content of milk increased. Percent disruption (66.83 %) for group A ($< 3 \times 10^5$ cells/ml) was significantly different from groups B (79.02 %) and C (85.21 %). However, groups B and C did not differ. The increase in SCC disruption with increasing somatic cell count could be due to a higher probability of cellular material moving into the ultrasound effect-zone when cell concentrations increase (5). This may also explain in part why average values in somatic cell disruption for any of the three SCC groups were lower than the values attained in the preliminary work (93.60 %), since the preliminary work involved only milk samples with high somatic cell content ($> 2 \times 10^6$ cells/ml). Additional variability in cellular disruption was explained by significant differences due to cow effect ($p < 0.01$) as seen in Table 3.

Effects of sonication on viable cell count (VCC) and mannitol fermentative staphylococci counts (MFC) are shown in Table 2. Sonication of milk samples caused increased bacteria counts with Standard Plate Count (SPC) agar (avg. 0.42 log) and Mannitol Salt (MS) agar (avg. 0.17 log), when compared to

untreated samples. The increase was highly significant with SPC ($p < 0.001$) and significant for recovery of mannitol fermentative staphylococci[1] on MS ($p < 0.05$), for all SCC groups. MFS colonies were observed in 37 % of the control samples and represented 22 % of the colonies in each sample (Table 4). The increase obtained in MFC due to sonication was compared to the increase in VCC but no correlation was found between these variables.

Increases in VCC due to sonication were correlated with initial VCC counts ($r = 0.545$; $p < 0.001$) as shown in Figure 1. When viable cell control counts were larger than 100 per ml, response to sonication was consistent and positive. Samples with counts lower than 100 per ml tended to vary directly as the control VCC, however, many negative values were recorded. This variable response for low initial VCC milk samples could be due to sampling error (19). When there are only one or two colonies per plate, interpretation is in doubt (19, 44), and a minimum of 5 colonies per plate has been recommended as evidence of intramammary infection (19, 41). When dealing with low bacteria count samples, effectiveness of detection of infection depends on aliquot size for plating. Plating with small aliquots (0.05 ml or less) is common practice as cited by Neave (20) and others (2, 3, 23). Larger aliquots should be considered because about 5 % of the milk samples from staphylococcal and streptococcal infected quarters are expected to have less than 100 bacteria per ml (19). Standard Methods procedures (1) were considered to be adequate for milk samples with low bacteria counts because larger aliquots (1.0 ml) increase effectiveness of bacteria recovery.

1.- Mannitol fermentative bacteria growing in MS agar were classified as mannitol fermentative-coagulase negative staphylococci, after appropriate tests were made.

Increases in bacteria counts were to be expected either because of disruption of somatic cells with subsequent liberation of engulfed but not killed bacteria, and/or because of dispersion of staphylococci clumps or other bacterial aggregates. A tendency for bacterial counts to vary directly as the percent of somatic cell disruption was observed, suggesting that release of phagocytosed bacteria may be contributing to the VCC and MFC increases. Although no correlation was found between increased VCC and/or MFC and increase in somatic cell disruption,findings by other workers (23) suggests that the release of PMN phagocytosed bacteria could lead to improved diagnostic accuracy. Dispersion of bacterial aggregates could also cause increases in bacteria counts. Ultrasonic dispersion of staphylococci clumps has been previously reported (9, 26, 42). In addition to staphylococci dispersion, the application of ultrasonic treatment to other bacterial groups may result in a similar dispersion. Since it was not the purpose of this study to determine effects of ultrasound on frequency of appearance and disappearance of different bacteria species, further work on this could be important and relevant. The fact that the ultrasonic treatment response for bacterial count increases was unusually high for some milk samples (1.5 log, Figure 1) indicates the need for further study on the effect of ultrasound on each of the primary mastitis causing bacteria.Studies including pure cultures as well as bacteria recovered from the mammary gland could aid in distinguishing between the relative importance of clump dispersion and bacterial release from leucocytes with regard to accuracy of mammary bacterial assays.

Of interest in our experiment was that no correlation was found between viable cell control counts and somatic cell control counts for any SCC group. This observation tends to confirm findings by other workers wherein SCC

alone is not conclusive evidence of infection (7, 16). Bacteriological analysis of suspect samples is necessary for dependable diagnosis.

CONCLUSIONS

Application of ultrasound to milk samples resulted in effective disruption of somatic cells and increased quantitative recovery of bacteria on SPC and MS agar. Increase in bacteria counts seemed to be due to release of phagocytosed bacteria from disrupted somatic cells and dispersion of bacterial aggregates. Whenever low viable cell counts are suspected, the use of ultrasound prior to plating should give more reliable plate counts and decrease the incidence of false negatives in bacteriological assay.

TABLE 2.- Least square means and standard deviations for sonication effects.

SCC group	Number of observations	Change in viable cell count (log)		Change in mannitol fermentative count (log)		Percent somatic cell disruption	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
A[1]	61	0.304**	0.069	0.140*	0.059	66.835[a]**	1.880
B	32	0.486**	0.094	0.202*	0.081	79.024[b]**	2.565
C	33	0.552**	0.091	0.209*	0.078	85.211[b]**	2.474

1.- A: $< 3 \times 10^5$ cells/ml; B: $> 3 \times 10^5$ and $< 8 \times 10^5$ cells/ml; C: $> 8 \times 10^5$ cells/ml.

*.- ($p < 0.05$).

**.- ($p < 0.001$).

a, b.- Means with different superscripts differ ($p < 0.01$).

TABLE 3.- Analysis of variance of viable cell count, mannitol fermentative staphylococci count, and percent somatic cell disruption.

Source	d.f.	Mean squares		
		Change in viable cell count (log).	Change in mannitol fermentative count (log).	Percent somatic cell disruption.
SCC[1]	2	0.689	0.059	3516.073**
Cow(SCC)	92	0.248*	0.182	182.459**
Residual	31	0.090	0.134	38.686

1.- Somatic cell count.

*.- ($p < 0.05$).

**.- ($p < 0.01$).

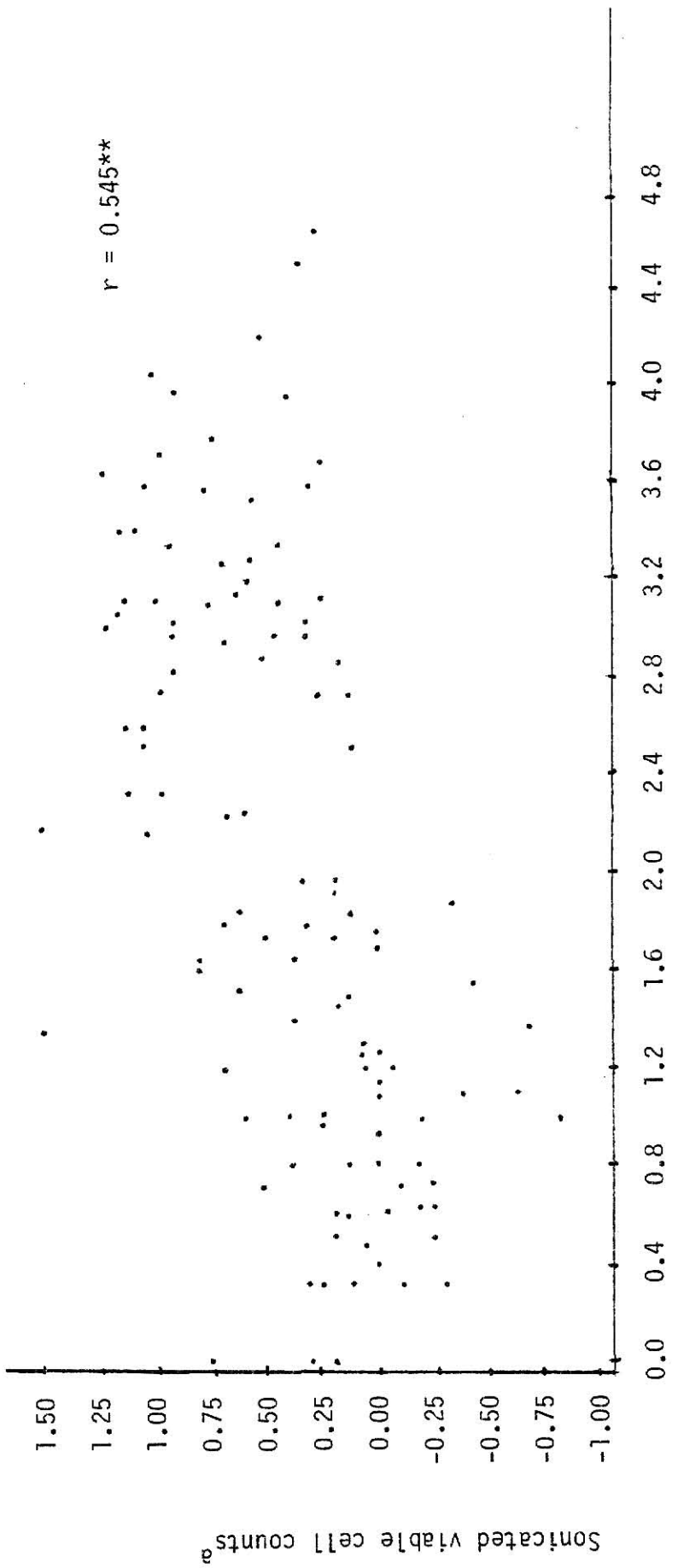


FIGURE 1.- Effect of sonication on viable cell counts. a) log (VCC_S + 1) - log (VCC_C + 1). b) log (VCC_C + 1).

**.- ($p < 0.001$).

TABLE 4.- Percent of samples containing mannitol fermentative staphylococci (MFS) and percent of MFS in each sample.

Group SCC	Number of observations	Percent of samples containing mannitol fermentative staphylococci.		Percent of mannitol fermentative staphylococci in each sample.	
		Control	Sonicated	Control	Sonicated
A[a]	61	34	42	20.426	27.219
B	32	40	50	27.861	28.567
C	33	39	48	19.315	22.898
Weighted average		37	46	22.094	26.094

a.- A: $< 3 \times 10^5$ cells/ml

B: $> 3 \times 10^5$ and $< 8 \times 10^5$ cells/ml

C: $> 8 \times 10^5$ cells/ml.

REFERENCES

1.- American Public Health Association. 1978. Standard methods for the examination of dairy products. 14th ed. APHA. New York.

2.- Blackburn, P.S. 1968. The cell count of cow's milk and the microorganisms cultured from the milk. J. Dairy Res. 35:59.

3.- Cousins, C.L., T.M. Higghs, E.R. Jacksoner, F.K. Neave, and F.H. Dodd. 1980. Susceptibility of the bovine udder to bacterial infection in the dry period. J. Dairy Res. 47:11.

4.- Craven, N., and J.C. Anderson. 1980. The effects of cloxacillin on staphylococci phagocytosed by bovine neutrophils. Res. Vet. Sci. 29:57.

5.- Davidson, E.A., and T. Rosett. 1967. Some biological applications of ultrasound. In: Ultrasonic techniques in biology and medicine. Chapter 11. B. Brown and D. Gordon ed. London Iliffe books.

6.- Difco manual. 1977. In: Difco manual of dehydrated culture media and reagents for microbiological and clinical laboratory procedures. 9th ed. Difco Lab., Detroit, MI. Page 150.

7.- Dohoo, I.R., and A.H. Meek. 1982. Somatic cell counts in bovine milk. Can. Vet. J. 23:119.

8.- Elliker, P.R. 1949. Practical dairy bacteriology. Chapter 6: Methods to determine sanitary quality of milk and milk products. 1st ed. McGraw-Hill Book Company, Inc. New York, Toronto, London.

9.- Eshelman, J.R., R.J. Eberhart, and R.W. Scholz. 1981. Effects of cream on bactericidal and metabolic functions of bovine PMN neutrophils. Am. J. Vet. Res. 42:738.

10.- Farmer, E.L. 1974 . Personal communications. Department of Animal Sci. and Industry, Kansas State University, Manhattan, Kansas.

11.- Fox, L.K., and C.W. Heald. 1981. Effect of cortisol on the bactericidal function of the bovine milk neutrophil in vitro. Am. J. Vet. Res.42:1933.

12.- Guidry, A.J., M.J. Paape, R.E. Pearson, and W.F. Williams. 1980. Effect of local immunization of the mammary gland on phagocytosis and intracellular kill of Staphylococcus aureus by polymorphonuclear neutrophils. Am. J. Vet. Res.41:1427.

13.- Heeschen, W. 1975. Determination of somatic cells in milk (technical aspects of counting). In: Proc. IDF seminar on mastitis control, Reading University, April 7-11. Pages 79-92.

14.- Hill, A.W., A.L. Shears, and K.G. Hibbitt. 1978. The elimination of serum-resistant Escherichia coli from experimentally infected single mammary glands of healthy cows. Res. Vet. Sci. 25:89.

15.- Holmes, B., P.G. Quie, W.B. Windhorst, B. Pollara, and R.A. Good. 1966. Protection of phagocytyzed bacteria from killing action of antibodies. Nature 210:1131.

16.- Lindstrom, V.B., H. Kenttamies, J. Arstila, and R. Touvila. 1981. Usefulness of cell counts in predicting bovine mastitis. Acta Agric. Scand. 31:199.

17.- Logan, E.F., S.D. Neill, and D.F. Mackie. 1982. Mastitis in dairy cows associated with an aerotolerant Campylobacter. Vet. Rec. 110:229.

18.- Naidu, T.G., and F.H.S. Newbould. 1973. Glycogen in leukocytes from bovine blood and milk. Can. J. Comp. Med. 37:47.

19.- National Mastitis Council Inc. 1981. Microbiological procedures for use in the diagnosis of bovine mastitis. 2nd ed. NMC, Inc. Washington, D.C.

20.- Neave, F.K. 1975. Diagnosis of mastitis by bacteriological methods alone. In: Proc. IDF seminar on mastitis control. Reading University, April 7-11. Pages 19-36.

21.- Newbould, F.H.S. 1973. The effect of added serum and glucose, and some inherent factors, on phagocytosis in vitro by milk leukocytes from several cows. Can. J. Comp.Med. 37:189.

22.- Newbould, F.H.S. 1970. Enhancement of phagocytosis in bovine milk leukocytes in vitro. Can. J. Comp. Med. 34:261.

23.- Newbould, F.H.S., and F.K. Neave. 1965. The recovery of small numbers of Staphylococcus aureus infused into the bovine teat cistern. J. Dairy Res. 32:157.

24.- Newbould, F.H.S., H.G. Saurasti, and D.A. Barnum. 1966. Studies on phagocytosis of Staphylococcus aureus by leukocytes from the bovine udder. I.- Stimulation of staphylococci by milk leukocytes. J. Comp. Path. 76:127.

25.- Paape, M.J. 1979. Somatic cells in milk. Nature and action. In: 18th annual meeting National Mastitis Council, Lousville, Kentucky. Page 5.

26.- Paape, M.J., and A.J. Guidry. 1977. Effect of fat and casein on intracellular killing of Staphylococcus aureus by milk leukocytes. Proc. Soc. Exp. Biol. Med. 155:588.

27.- Paape, M.J., A.J. Guidry, S.T. Kirk, and D.J. Bolt. 1975. Measurement of phagocytosis of ^{32}P-labeled Staphylococcus aureus by bovine leukocytes: Lysostaphin digestion and inhibitory effect of cream. Am. J. Vet. Res. 36:1737.

28.- Paape, M.J., F.C. Gwazdauskas, A.J. Guidry, and B.T. Weinland. 1981. Concentrations of corticosteroids, leukocytes, and immunoglobulins in blood and milk after administration of ACTH to lactating dairy cattle: effects on phagocytosis of Staphylococcus aureus by polymorphonuclear leukocytes. Am. J. Vet. Res. 42:2081.

29.- Paape, M.J., W.D. Schultze, A.J. Guidry, and W.M. Kortum. 1981. Effect

of an intramammary polyethylene device on the concentration of leukocytes and Immunoglobulins in milk and on the leukocyte response to Escherichia coli endotoxin and challenge exposure with Staphylococcus aureus. Am. J. Vet. Res. 42:774.

30.- Paape, M.J., and W.P. Wergin. 1977. The leukocyte as a defense mechanism. J. Am. Vet. Med. Assoc. 170:1214.

31.- Paape, M.J., and W.P. Wergin. 1977. Scanning and transmission electron microscopy of PMN leukocytes isolated from milk. Fed. Proc. 36:1202 (Abstract).

32.- Paape, M.J., W.P. Wergin, A.J. Guidry, and R.E. Pearson. 1979. Leukocytes. Second line of defense against invading mastitis pathogens. J. Dairy Sci. 62:135.

33.- Paape, M.J., W.P. Wergin, A.J. Guidry, and W.D. Schultze. 1981. Phagocytic defense of the ruminant mammary gland. In: The ruminant immune system. J.E. Butler ed. Plenum publishing corporation. Page 555.

34.- Russell, M.W., B.E. Brooker, and B. Reiter. 1976. Inhibition of the bactericidal activity of bovine PMN leucocytes and related systems by casein. Res. Vet. Sci. 20:30.

35.- Russell, M.W., and B. Reiter. 1975. Phagocytic deficiency of bovine milk leucocytes: an effect of casein. J. Reticuloend. Soc. 18:1.

36.- Sharpe, A.N., and A.K. Jackson. 1972. Stomaching: a new concept in bacteriological sample preparation. Appl. Microb. 24:175.

37.- Singh, B., and R.T. Marshall. 1966. Effects of temperature and storage time on the phagocytic activity of leucocytes of milk. J. Dairy Sci. 49:1113.

38.- Snedecor, G.W., and W.G. Cochran. 1980. Statistical methods. 7th ed. Iowa State University Press. Ames, Iowa.

39.- Tan, J.S., C. Watanakunakorn, and J.P. Phair. 1971. A modified assay of neutrophil function. Use of lysostaphin to differenciate defective phagocytosis from impaired intracellular killing. J. Lab. Clin. Med. 78:316.

40.- Ward, G.E., and L.H. Schultz. 1972. Relationship of somatic cell in quarter milk to type of bacteria and production. J. Dairy Sci. 55:1428.

41.- Ward, G.E., J.E. Madl, and R.H. Lyon. 1981. Mannitol agar for microbiological diagnosis of bovine mastitis. J. Am. Vet. Med. Assoc. 178:1061.

42.- White, E.A., M.J. Paape, B.T. Weinland, and I.H. Mather. 1978. Virulence of 3 strains of Staphylococcus aureus isolated from clinically infected bovine mammary glands. J. Dairy Sci. 63:1128.

43.- White, E.A., M.J. Paape, B.T. Weinland, and I.H. Mather. 1978. Variation in the interaction among strains of Staphylococcus aureus and polymorphonuclear neutrophils (PMN). Fed. Proc. 37:937. Fed. Am. Soc. Exp. Biol. (Abstract).

44.- White, A.G., and S.M. Walker. 1981. A new assay for the assessment of staphylococcal killing by human leucocytes. J. Immun. Methods. 42:203.

ACKNOWLEDGMENTS

I wish to thank Dr. E. L. Farmer for his advice during the course of this study. I am also grateful to Dr. D. Y. C. Fung for his help and guidance as professor and committee member. Thanks are also given to Dr. J. M. Morrill for serving as the other member of my supervisory committee.

I express my gratitude to my fiance, Villiam, for his help, love and understanding.

EFFECT OF ULTRASONIC TREATMENT ON RECOVERY OF BACTERIA FROM MILK

by

ISABEL CRISTINA LARRIERA

B.S., Universidad de la Republica,
Facultad de Agronomia,
Montevideo, Uruguay, 1976

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1982

ABSTRACT

One hundred and twenty six milk samples from lactating cows in the Kansas State University dairy herd were used to determine the efficacy of ultrasound on somatic cell disruption and quantitative recovery of bacteria from milk samples. Results showed effective disruption of somatic cells ($p < 0.001$) and significantly higher bacterial counts with ultrasound treatment compared to controls. Increases in bacteria counts were highly significant on standard plate count agar ($p < 0.001$) and significant for mannitol salt agar ($p < 0.05$). Conclusions were that the use of ultrasound as a routine practice in the analysis of milk samples would decrease the incidence of false negatives. Possible factors involved are presented and discussed.