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The relationship of the bacterial flora of the bovine udder to the California mastitis test with emphasis on the staphylococci

Joel A. Collins

To the Graduate Council:

I am submitting herewith a thesis written by Joel A. Collins entitled "The relationship of the bacterial flora of the bovine udder to the California mastitis test with emphasis on the staphylococci." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

W.W. Overcast, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

August 7, 1964

To the Graduate Council:

I am submitting herewith a thesis written by Joel A. Collins entitled "The Relationship of the Bacterial Flora of the Bovine Udder to the California Mastitis Test with Emphasis on the Staphylococci." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairying.

Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:

Dean of the Graduate School

THE RELATIONSHIP OF THE BACTERIAL FLORA OF THE BOVINE UDDER TO THE CALIFORNIA MASTITIS TEST WITH EMPHASIS ON THE STAPHYLOCOCCI

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Joel A. Collins

August 1964

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J. A. C.

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CHAPTER I

INTRODUCTION

Since the advent of organized dairying mastitis has been a constant problem to the dairy industry. Modern developments in production such as confinement of cattle, increased production, and management practices, have aggravated this problem, especially since farm labor has been hard to obtain and train. In the United States mastitis reduces the milk yield and shortens the productive life of affected cows, causing an estimated loss of 225,504,000 dollars annually (1,15). This amounts to an average annual loss of 10 dollars per cow for each dairy cow in the United States, with the loss for some individual cows as high as 100 dollars, and total loss in case of death. Mastitis is, without question, one of the dairyman's largest economic losses.

Research conducted on many phases of mastitis has produced volumes of literature on the predisposing factors, etiology, diagnosis, prevention, control, and treatment. Study of these research data have resulted in the initiation of many educational programs, only to have more complexing problems evolve. This has been the case with the causative organisms of mastitis. In former years streptococci were almost the sole species responsible for mastitis. However, in recent years staphylococci have emerged as a more dangerous threat to the dairyman since they are one of the most difficult organisms to control.

McCoy (54), presenting figures to the Mastitis Conference at Chicago in 1960, indicated that: one cow in four was shedding staphylococci from at least one quarter; this was a twenty times higher incidence of mastitis than the herd owner was aware of or than the veterinarian could detect on routine examination, and there was no correlation between the shedding of staphylococci and total plate count or methylene blue reduction time. Therefore, there was no way for the owner to detect the presence of staphylococci at an early stage. The rise in staphylococci and fall in streptococci as the etiological agent of mastitis has been explained on the basis that antibiotics are highly effective against streptococci and the way has been opened for the staphylococci to invade the udder without considerable competition. Jezeski (36) observed that an increased number of isolated organisms showed characteristics similar to those associated with human disease. The possibility exists that cows have become infected with the so-called "human strains" of staphylococci. The udder thus may become a reservoir for staphylococci capable of causing disease in humans.

Many times the bovine udder harbors various species of bacteria without noticeable symptoms of infection. These mild or chronic cases may be quite dangerous as they may be carriers and spreaders of the organisms to other cows in the herd. Likewise, these same organisms may be responsible for acute infections when favorable conditions develop in the udder. Therefore, a good management program should include some method for diagnosis at regular intervals. Some barn tests that have been used to determine the presence and extent of mastitis in a herd are:

physical examinations of milk and the milked out udder for abnormalities, strip-cup test for clots, bromthymol blue test for altered pH, Whiteside Mastitis Test, Negretti Mastitis Test, and the California Mastitis Test, referred to hereafter as CMT. Of these tests the CMT has been found to be the most effective diagnostic test at the time of milking.

The CMT, developed by Schalm (77), estimates the degree of udder irritation. This test has been broadly related to the number of leucocytes present in the milk. However, the CMT does not necessarily detect infection, nor can it distinguish between infectious and non-infectious mastitis; it is merely an estimate of udder irritation. The CMT has been found to be a good screening test for dairymen to use. However, the CMT should not be the sole criteria for establishing a mastitis treatment program.

Because of the increased use of the CMT as a field test for detecting udder irritation, and the observed increase in the incidence of staphylococcal mastitis, and because few studies have been made correlating the incidence of staphylococci with the CMT in raw milk, the present investigation was undertaken.

CHAPTER II

REVIEW OF LITERATURE

Public Health Aspects of Staphylococci and Streptococci

An alarming increase of mastitis has occurred over the last twenty years. Drury et al. (15) indicated that in 1939, 86 per cent of the herds and 26 per cent of the cows were infected. However, in 1960, their survey showed 100 per cent of the herds and 59 per cent of the cows were infected in one or more quarters. Also in 1939, 98 per cent of the infections were streptococci compared to 58.8 per cent in 1960; while staphylococci increased from practically zero to 39.4 per cent over the same period. The problem of mastitis caused by staphylococci has become increasingly important to human health. Slanetz, Howe, and MacLeod (82) stated that the staphylococci responsible for bovine mastitis may be similar to those of human infection, and that staphylococcal mastitis was not caused by specific staphylococci of bovine origin. Therefore, the udder could be a reservoir for potentially pathogenic staphylococci. McCoy (54) pointed out this reservoir as a potential danger to man because under certain conditions enterotoxin can develop and cause food poisoning as occurred in dry milk in England in 1955, Puerto Rico in 1956, and in cheese in Canada in 1955, and in this country in 1959. Other investigators (2,5,20,29,36,54,56,82,88) have established the causative agent of food poisioning to be staphylococcal enterotoxin in dairy products.

Jezeski (36) found Staphylococcus aureus to be a principle cause for concern by the dairy processor and the health department because of the possible production of enterotoxin which was not destroyed by pasteurization. Foltz et al. (20) examined 207 samples of pasteurized dairy products and found coagulase-positive staphylococci in 3.4 per cent of the products. Allen and Stovall (2) found seventy-eight staphylococci, seventy-two of which were coagulase-positive, from eighty-seven samples of Colby cheese. Michelsen et al. (56) analyzed 125 samples of cheese, representing twenty varieties, and found 76 per cent contained staphylococci; 70.4 per cent of which were Staphylococcus aureus and 7.2 per cent were potentially pathogenic coagulase-positive. Thatcher and Simon (90), Williams (96), and Wallace et al. (94) have found Staphylococcus aureus of bacteriophage type 80/81, a common causative agent of human enteritis and food poisoning, in dairy products. This type has been commonly referred to as the "hospital staph," and of particular importance because of its resistance to penicillin, dihydrostreptomycin, chlortetracycline, and other common antibiotics (94).

Jezeski (36) indicated that the presence of Staphylococcus aureus in dairy products kept under proper conditions so that growth was prevented did not constitute a health hazard. Other workers (15,48,54,69) agreed that when sufficient growth has occurred to produce enterotoxin, then a potential health hazard exists.

Incidence of Staphylococci and Streptococci in Mastitis

Numerous organisms may be associated with mastitis. While it is not uncommon for other organisms to be primarily responsible for mastitis in a given locality, species of streptococci and staphylococci are by far the most important causative agents in the world's cattle population. This has been shown by many surveys made in various countries of the world (3,6,10,18,27,28,32,37,47,57,60,65,67,68,71,72,75,97).

Hughes (32) made a survey over a five year period from 1949 to 1953 from a population of 4000 heifers and cows in which clinical cases of mastitis were detected. The results were analyzed on the basis of overall incidence of mastitis and the different bacterial species contributing to the total number of infections. The different bacterial infections showed certain trends over the five years. Streptococcus agalactiae infections were the most frequent in each year, but there was a definite decrease in their numbers over the period. Staphylococcus aureus showed an increase toward the end of the survey while Streptococcus dysgalactiae, Corynebacterium pyogenes, and Steptococcus uberis fluctuated irregularly or remained steady over the five year period. The overall incidence of mastitis infections was 13.9 per cent; Streptococcus agalactiae averaged 33.0 per cent, and Staphylococcus aureus 12.5 per cent.

Rivera and Berrocal (75) examined 8120 udder quarters of cows in Puerto Rico and found 38.03 per cent of the cows free of mastitis, 22.35 per cent free in three quarters, 17.05 per cent free in two quarters, 11.63 per cent free in one quarter, and all quarters were infected in 9.95 per cent. An incidence of mastitis in India of 10.23 per cent was reported by Kalra and Dhanda (37). In Italy (68) 19.4 per cent of 2102 cows were found to be infected in one or more quarters. In another

Italian survey, Guallini and Vocino (27) found the presence of chronic mastitis in 33 per cent of the animals and 10.3 per cent of the quarters. Barnum (6) reported a significant number of mastitis pathogens in over 15 per cent of the milk samples tested in Ontario each year from 1958 until 1961. An incidence of 44.2 per cent of 776 quarter-milk samples from 200 cows in Sweden was found to be infected with mastitic organisms (39). Numerous other surveys have been made in Australia (24), England (85), Norway (40), India (38), Sweden (73), Germany (89), Bulgaria (86, 92), Japan (80), and in the Netherlands (98).

The incidence of mastitis in the United States has been reported for Minnesota (3), Connecticut (69), Iowa (67), New York (69), New Jersey (60), and others. Pounden and Frank (71) reported an incidence of 50 per cent in four high yielding thirty to fifty cow herds. Oliver *et al.* (65) reported 979 infections in 530 complete lactations over an eight year period in a dairy herd.

Barnum (6) and Rendel and Sundberg (73) reported over 80 per cent of the infected quarters were caused by *hemolytic staphylococci* and *Streptococcus agalactiae*. Results of a survey of forty-four herds conducted by Frost (24) showed that *Streptococcus agalactiae* and *Staphylococcus aureus* were the major causes of subclinical mastitis, and that 29.7 per cent and 46.8 per cent of the cows were infected with these organisms, respectively. Rako *et al.* (72) reported 14.3 per cent infections; 60 per cent due to *Streptococcus agalactiae* and 28 per cent due to pathogenic staphylococci. An examination of 200 cows (39) showed 63.3 per cent of all infections due to staphylococci, 29.3 per cent by streptococci, and 6.8 per cent by mixed streptococci and staphylococci. Chernevski (12) isolated four strains of pathogenic streptococci and forty-three strains of pathogenic staphylococci from 609 cows over a two year period (1960-1961). Frank and Pounder (23) reported a 91.6 per cent incidence of streptococci and 78.7 per cent staphylococci in 1000 bulk samples and 40.9 and 39.8 per cent infection, respectively, from 1000 quarter samples.

Previous to 1940 mastitis was primarily caused by streptococci. Ziegler (97) found streptococci in 61.0 per cent of 2142 milk samples collected in 1959-1960. Murphy (61), in 1947, reported 90 per cent of mastitis infections with Streptococcus agalactiae as the causative agent. Other investigators observed varying percentages: Zwanenburg (98) found 47 to 61 per cent, Signorini (81) found 44 per cent, Shimizer (80) found 11.9 per cent, and Marshall (53) found 10 per cent. Leidl and Schalm (45) found infections in eleven of twelve herds were due to Streptococcus agalactiae. An eight year survey (40) showed that Streptococcus agalactiae mastitis fell from 34.0 per cent to 10.5 per cent between 1949 and 1957. However, in recent years, the incidence of staphylococci has attracted wide-spread attention. McCoy (54) indicated that staphylococcal mastitis invaded 70 per cent of all herds in America by 1960. Kalra and Dhanda (37) and Krejakovic-Miljkovic and Milojevic (41) observed staphylococci as the chief cause of clinical mastitis. Marshall (53) reported 70 per cent of the infecting organisms causing mastitis as staphylococci. In 7009 cases of clinical mastitis, Kjos-Hanssen (40) showed that the incidence of Staphylococcus aureus increased from 20.5

per cent in 1949 to 48 per cent in 1957. Forty-nine out of 604 mastitic milk samples collected by Todorov (92) contained pathogenic staphylococci. Steede and Iredale (87) investigating a food poisoning outbreak found fifteen of fifty-five and eighteen of thirty-nine farmers' milk samples received at two local dairies to contain *Staphylococcus aureus*. Guallini and Vocino (27) found 13.4 per cent of infections to be caused by *Staphylococcus aureus*; Loken (49) observed 21.9 per cent; but Ziegler (97) found only 3.0 per cent.

Composite morning milk samples obtained once a week during a nine week period contained 61 per cent coagulase-positive staphylococci (95). The recovery of staphylococci from individual farms varied from 11 to 88 per cent. Milk from 110 apparently healthy cows tested by Kaushal and Saxena (38) contained coagulase-positive staphylococci. Murray (62), on examining 175 samples of Grade A raw milk, found coagulase-positive staphylococci to exceed 50 per milliliter in 57.7 per cent of the samples and 5000 per milliliter in 1.7 per cent. Steelemann *et al*. (89) tested thirty-four herds for the presence of staphylococci. Of 514 samples, 38.2 per cent were free of staphylococci, 28.9 per cent contained less than 500 per milliliter, 95.0 per cent contained less than 1000 per milliliter, 10.8 per cent contained less than 2500 per milliliter, but 12.6 per cent contained more than 2500 per milliliter.

Only four of forty-four cows from which pathogenic staphylococci were isolated showed clinical signs of mastitis (86) while Thorne *et al*. (91) observed that nine of eleven cows having staphylococci showed clinical signs of mastitis.

California Mastitis Test

The California Mastitis Test, hereafter referred to as CMT, developed by Schalm and Noorlander (78) in 1956, employs a specially designed paddle with four cups and a test reagent containing 1.5 per cent sodium hydroxide and a bromcresol purple indicator (dibromo-o-cresol-sulfonaphthalein) at a concentration of 1 to 10,000. Milk is drawn directly from each quarter into the respective cup of the paddle. The test reagent is added in an estimated equal quantity to the milk from a polyethylene wash bottle. The reactions that occur are scored while the milk and the reagent are mixed by a gentle circular motion of the paddle. The CMT uses a grading system depicting five gel reactions as described by Schalm (77). Symbols for these grades or scores are negative (-), trace (T), 1, 2, and 3. The degree of gelation tends to be related to the leucocyte count (77,69). The CMT does not detect infection, nor does it distinguish between noninfectious and infectious mastitis; it is merely an estimate of udder irritation. Even though the amount of gel is closely related to the number of leucocytes present, other factors may cause gel formation (69). Also, leucocytes may or may not indicate infectious organisms; however, the shedding of these white blood cells is indicative of an irritation in the udder.

Abnormal milk, such as that which contains small flakes, may result from the action of enzymes on the milk in the teat cistern. Harmless bacteria may also cause flaking. Even though flaking of the milk in the teat cistern occurs, the milk in the upper portion of the gland may be a wholesome product. Gray and Schalm (25) concluded that when foremost milk was used, a CMT score of 1 may be regarded as a suspect and a score of 2 and 3 as positive for mastitis. Also, they concluded that after the first streams are discarded, the next 15 to 20 milliliters are representative of the total milk in the quarter as far as the CMT was concerned.

Many observers (7,8,9,13,16,17,19,22,25,33,34,35,41,42,48,50,53, 70,79) have agreed that the CMT was a fast and fairly good test for the farmers to use to detect mastitis. Drury and Reed (16) comparing over 9000 CMT scores with the results of direct microscopic examination, and in many cases with Blood Tryptose Agar plates, found that treatment of quarters with drugs should not be based on a single CMT score. These findings were confirmed by other investigators (7,9,17,19,41,48,64,74,76, 84,93). Other environmental, physiological, and compositional factors such as pH, chloride content, acidity, lactose, stage of lactation, herd size, annual milk yield, preparation for milking, hygienic precautions, vacuum fluctuations, age of milk, temperature of milk, previous bacterial growth, type of organisms present, age of cow, and management may affect or alter the CMT score (9,11,31,43,48,53,64,66).

Varying estimates have been made comparing bacterial counts with CMT scores. Jackson (33) in a herd survey of five hundred lactating cows found that 31 per cent of the quarters were positive to CMT while 56 per cent were infected according to bacterial count. Under Yugoslov conditions (41) 1022 individual milk samples were examined. Bacteriological tests agreed with CMT 81.3 per cent of the time, 39.3 per cent being positive and 42.0 per cent negative. Marshall (53) found that 90 to 95 per cent of those samples strongly positive to CMT were culturally positive. The CMT was carried out on 567 quarter milk samples from cows in twenty-one mastitic herds. For samples giving CMT scores of 3, 2, 1, trace, and negative, the number of quarters bacteriologically positive were 61.7, 39.1, 25.5, and 9.5 per cent, respectively.

The number of leucocytes found in the udder varies. Anderson (4) reported that the average leucocyte count of milk from quarters infected with *Micrococcus pyogenes*, streptococci other than *Streptococcus agalactiae*, and *Streptococcus agalactiae* was 1,400,000, 1,900,000 and 2,100,000 per milliliter, respectively. In comparison, the average leucocyte count of 3789 samples from uninfected quarters was 180,000 per milliliter. Most investigators agreed that milk from normal quarters rarely contains more than 500,000 leucocytes per milliliter (4,13,45,48, 55,59,63).

Luedecke (50) analyzed 718 quarter milk samples and found an increase in leucocyte count with increase in CMT scores. The arithmetic average leucocyte count per milliliter of samples corresponding to the various CMT scores were: negative 51,000; trace 96,000; one 340,000; two 1,600,000; and three, 7,300,000. Milojevid and Milenkovic (58) reported the average leucocyte counts per milliliter of 150,000; 269,000; 514,000; and 1,276,000 for negative, doubtful, weakly positive, and positive samples. An increase in number of leucocytes present in milk with increasing CMT readings was confirmed by Leidl *et al.* (46), Blomberg and Gastrin (7), Marshall (53), Ewbank (17), Bortree *et al.* (8), Figueiredo (19), Leali and Vallis (42), Jarrtsveld (35), Richter *et al.* (74) and others (13,41,79).

A trace reaction appeared to contain about 500,000 leucocytes per milliliter (13). Ewbank (17) suggested that a positive CMT reading contained more than 100,000 leucocytes per milliliter. However, a doubtful or negative CMT reading could contain as many as 250,000 per milliliter.

The CMT has also been used as a quality test for bulk milk samples (25,41,45,48,51,52,70,74,78). In tests made by MacLeod et al. (51) on milk from 31 herds, counts of 1,000,000 or more per milliliter were usually observed when the percentage of udder infection was 40 per cent or more. Cows were classed as infected when the milk from one or more quarters contained either Streptococcus agalactiae and 500,000 or more leucocytes per milliliter, or other organisms and 1,000,000 or more leucocytes per milliliter. Factors other than infection apparently affected the leucocyte count sufficiently to prevent a close correlation between the leucocyte count of herd milk and the degree of infection, when the degree of infection was under 40 per cent.

Leidl and Schalm (45) found that 20 liter containers of milk with a CMT score of 1 or more generally contained over 500,000 leucocytes per milliliter, and an average of 22 per cent of the individual quarter samples from cows in the herd which produced this milk had a CMT score of 1, 2, or 3. Gray and Schalm (25) suggest that milk samples from individual cows with a CMT score of 1, 2, or 3 are indicative of abnormally high leucocyte counts in one or more quarters, and a herd score of 2 or 3 indicate a serious mastitis problem. CMT test on bulk milk from 983 farms with 8357 milk samples from individual cows making up the bulk milk, was compared with bacteriological results (74). The CMT detected 86.4 per cent (503 out ot a total of 582) of the farms with milk giving positive cultural and microscopic results. Marquardt and Forster (52) averaged the CMT score for herds and referred to this average as the CMT index. Herds with a low CMT index tended to have a high proportion of quarters with CMT negative scores while herds with a high CMT index tended to have a larger proportion of quarters with high CMT scores. Due to the dilution factor in bulk milk a negative CMT score tolerates up to 25 per cent CMT positive cows (25). Post (70) observed results on 277 cows on fifteen farms and concluded that mastitis be considered a serious problem in herds of ten or more cows when milk from half or more of the cows give a positive CMT reaction.

Jarrtsveld (35), examining 1121 samples, found that the CMT tended to give a number of false positive results. Other workers (41) reported 11.1 per cent false positive and 7.6 per cent false negative CMT results. Richter _et al_. (74) found 29.8 per cent false positive results.

The CMT has been compared with other field tests which determine udder irritation (44,30). The Whiteside Mastitis Test, from which the CMT was developed, was compared to the CMT by Frank and Pounden (22). Agreement between the two tests was obtained in 63.3 per cent of 2000 quarter milk samples. The disagreements were for the most part in normal milk. Schipper (79) found the proportion of positive results in the CMT and Whiteside Test were, respectively, 5.7 and 9.8 per cent of samples containing less than 200,000 leucocytes, 21.6 and 21.9 per cent of those between 500,000 and 1,000,000 and 54.9 and 48.2 per cent of those containing more than 1,000,000 leucocytes. A number of false negative Whiteside Test results were observed by Jarrtsveld (35). Some observers found comparable results between the two tests (13,19,30), but the majority agreed that the CMT was a better detector of milks with mild or severe udder irritation.

The CMT was considered a superior farm test of quarter samples over the catalase test for detecting infection (84). However, the catalase test was considered superior for bulk milk. Contradictory results were obtained by Leali and Vallis (42) comparing CMT and the Antiformin Test. A modified CMT, the California Mastitis Tube Test, agreed well with CMT and Whiteside Test results (34,35). Ewbank (17) and Vallis (93) compared the CMT with the Negretti Mastitis Test and found the Negretti Test to be slightly more sensitive to infection, but the CMT was easier to perform.

Carrolland Schalm (11) found that 1.65 micrograms per milliliter of desoxyribonuclease, from nuclei of cells of inflammatory exudate, added to milk with a CMT score of 3 completely inhibited the reaction. As a result of this evidence that desoxyribonucleic acid was the active agent responsible for the CMT reaction, the Feulgen reaction was applied to the detection of udder irritation by Poape *et al*. (66). The correlation between CMT and Feulgen reaction was 0.936. The Feulgen reaction demonstrated its superiority as a laboratory test, but cannot be performed conveniently at the barn.

The association of CMT score with milk yield has been demonstrated (21,26). Gray and Schalm (26) obtained 12,438 samples from 1243

Holstein-Friesian cows over a nineteen month period and the results of the test were correlated with milk yield. The average decreases in milk yield of 6.0, 10.0, 16.0, and 24.5 per cent were observed from CMT scores of trace 1, 2, and 3, respectively, using CMT negative cows as controls. Forster (21) estimated daily decreases of 277 cows, based on the production from opposite quarters which differed in CMT score, at 0.76, 2.26, 4.07, and 5.86 pounds per quarter for CMT scores of trace 1, 2, and 3, respectively. These differences were statistically significant in both cases.

CHAPTER III

EXPERIMENTAL METHODS

Samples for the California Mastitis Test (CMT) were taken at the evening milking from the University of Tennessee, Knoxville, dairy herd. Prior to sampling, the teats, udder, and flanks of each cow were washed with an iodophor solution containing 25 to 50 parts per million available iodine and dried with a clean cloth towel. The first stream of milk from each quarter was discarded. Each quarter sample was then milked into the appropriate cup of the white plastic paddle. To the three to five milliliter sample of milk was added an approximately equal volume of the CMT reagent. The sample was mixed by a circular motion of the paddle and after observing mixing, a reading or score was assessed and recorded. The samples were scored on the basis of the suggestions offered by the manufacturer (77) as follows:

1. Mixture remained liquid with no evidence of a precipitate was scored negative (-).

2. A slight precipitate was observed, but may disappear with continued mixing was scored trace (T).

3. A distinct precipitate was observed and was scored 1 for weak positive.

4. A gel formation was observed which tended to move toward the center of the cup with swirling motion of the paddle was scored 2 for distinct positive.

5. A convex gel formation was observed which tended to adhere to the bottom of the cup was scored 3 for strong positive.

The reagent used was Mastitis Quality Test (MQT) brand manufactured by Dairy Equipment Company, Madison, Wisconsin. One pint of concentrated reagent was mixed with one gallon distilled water and put into polyethylene bottles until used.

Immediately following the scoring of each quarter with CMT, the floor of the udder and teats (taking care to clean the ends of the teat near the opening to the streak canal) were thoroughly washed with a cheese cloth saturated with 70 per cent alcohol. Approximately ten milliliters of milk from each quarter was drawn into a sterile test tube held horizonally so as to prevent falling contamination and to permit aseptic collection of the milk sample. After collection, each quarter milk sample was properly labeled and immediately placed in an ice water bath until it was processed in the laboratory.

The milk samples for bacterial counts were removed from the barn and processed within three hours after collection. A composite sample was prepared by taking one milliliter from each of the cooled quarter milk samples of each cow. Sterile 1.1 milliliter pipettes were used to prepare composite samples by drawing 1.0 milliliter from each quarter sample and transferring to a separate sterile test tube, taking care to flame the test tube before and after each addition to the composite. One-tenth milliliter of the composite was placed on Bacto-Staphylococcus Medium No. 110 (14), prepared by Difco Laboratories, Detroit 1, Michigan, which is a selective medium for isolation of staphylococci. A sterile bent glass rod was used to spread the milk evenly over the plates. After forty-eight hours incubation at 30° C., each of the plates was observed for the total number of colonies growing on Bacto-Staphylococcus Medium No. 110. A Quebec Colony Counter was used to aid the counting of colonies.

One-tenth milliliter of the composite milk sample was placed on blood agar plates. These plates were made of Blood Agar Base Media (heart muscle infusion), prepared by Baltimore Biological Laboratory, Baltimore, Maryland, and 5.0 per cent sterile defibrinated bovine blood. The blood was added just prior to pouring the plates. A blood plate, selected at random, was incubated twenty-four hours as a control for sterility of the plates. Total number of bacterial colonies, hemolytic colonies, and typical pin-point streptococci colonies were observed on blood plates after incubating at 35° C. for twenty-four hours.

The procedure just explained was followed each month during the test period with all equipment and media sterilized in an autoclave under fifteen pounds pressure at 250° F. for fifteen minutes. The work presented covered a period from October, 1962, to June, 1963, and from August, 1963 to June, 1964, with each month requiring two or three separate test days to complete sample collection of the entire herd.

CHAPTER IV

RESULTS AND DISCUSSION

The results of this study are based on 7,468 quarter milk samples and 1,872 individual cow samples obtained over a two year period, 1962-1964, from the University of Tennessee, Knoxville, dairy herd. A California Mastitis Test (CMT) score was obtained for each quarter sample. Total bacteria, staphylococcal, hemolytic, and streptococcal counts were obtained on composite milk samples from individual cows. For the comparisons presented in this study, each composite sample was given a CMT score equal to the highest quarter milk sample contained in that particular composite.

In Table I, which contains data for the 1962-1963 year, the staphylococci, total and hemolytic counts, increased with increases in CMT score. Similar results were obtained for the 1963-1964 year, as shown in Table II. The number of samples for CMT scores of 2 and 3 increased from 29.0 per cent for the first year (Table I) to 47.8 per cent for the second year (Table II). However, with CMT scores of 2 and 3 the median of the total bacteria counts decreased in the second year by as much as 50 per cent. Because of a noticeable increase in number of streptococci colonies on blood agar during the latter part of the first year, streptococci were included in the counts made during the second year (Table II). The number of staphylococci from cows with CMT scores of 2 and 3 decreased markedly from the first to the second year.

TABLE I

COMPARISON OF CMT WITH MEDIAL BACTERIAL COUNTS OF THE COMPOSITE MILK SAMPLES FOR 1962-1963

CMT Score	No. of Samples	% of Samples	Staphylo-cocci[a]	Total Count[b]	Hemolytic Count
3	115	12.6	4,310	10,000	5,600
2	150	16.4	1,420	4.600	2,910
1	181	19.8	480	2,500	1,210
T	248	27.2	410	1,600	1,000
-	219	24.0	90	670	160
Total	913				

[a]Counts on Bacto-Staphylococcus Medium No. 110.

[b]Counts on Blood Agar Base.

TABLE II

COMPARISON OF CMT WITH MEDIAL BACTERIAL COUNTS OF THE COMPOSITE MILK SAMPLES FOR 1963-1964

CMT Score	No. of Samples	% of Samples	Staphylo-cocci[a]	Total Count[b]	Hemolytic Count	Strepto-cocci[c]
3	328	34.2	1,000	5,400	1,850	150
2	130	13.6	610	4,500	1,800	120
1	136	14.2	480	3,730	1,450	130
T	93	9.7	380	2,400	430	190
-	272	28.3	140	1,500	280	110
Total	959					

[a]Counts on Bacto-Staphylococcus Medium No. 110.

[b]Counts on Blood Agar Base.

[c]Streptococci count on Blood Agar Base.

This may be partially due to culling of cows in the summer of 1963, which were known to have high CMT scores and bacterial counts.

The data for 1963-1964 is presented by breed in Table III (Holstein-Friesian) and Table IV (Jersey). Each breed showed an increase in all bacterial counts with an increase in CMT, except the streptococci which varied without a definite pattern. One will note that the staphylococcal and hemolytic counts for the Holstein-Friesians were approximately double that for the Jerseys, except for the staphylococcal counts for a negative CMT score.

An Irritation Index was calculated from the CMT scores obtained from 7,468 quarter milk samples. The numbers 4, 3, 2, 1, and 0 were assigned for CMT scores of 3, 2, 1, Trace and negative, respectively, as suggested by Drury and Reed (16). The numerical value for each cow calculated on this basis could range from 0 to 16. A sum of these numerical values for all quarter samples divided by the number of cows in the herd gave the Irritation Index. In this study for the 1962-1963 year, an index of 4.31 was calculated. This increased to 5.44 for 1963-1964; with Holstein-Friesians having an index of 5.71 and Jerseys 4.92. An index of 5.01 was calculated for the two year testing period. In comparison with the work of Drury and Reed (16), these indexes are considered high. These workers indicated that a herd index of more than 3 should be reduced by an improved mastitis control program.

The data in Table V reveal that 128 of 913 composite samples (14.0 per cent) contained no staphylococci. For the second year, Table VI, only 114 of 959 (10.8 per cent) were free of staphylococci. This means

TABLE III

COMPARISON OF CMT WITH MEDIAL BACTERIAL COUNTS OF THE COMPOSITE MILK SAMPLES FOR 1963-1964 (HOLSTEIN-FRIESIANS)

CMT Score	No. of Samples	% of Samples	Staphylo-cocci[a]	Total Count[b]	Hemolytic Count	Strepto-cocci[c]
3	228	35.8	1,200	5,400	2,150	200
2	79	12.4	850	4,900	2,150	160
1	81	12.7	800	4,400	1,850	90
T	67	10.5	500	2,440	680	190
-	182	28.6	160	1,890	400	300
Total	637					

[a]Counts on Bacto-Staphylococcus Medium No. 110.

[b]Counts on Blood Agar Base.

[c]Streptococci count on Blood Agar Base.

TABLE IV

COMPARISON OF CMT WITH MEDIAL BACTERIAL COUNTS OF THE COMPOSITE MILK SAMPLES FOR 1963-1964 (JERSEYS)

CMT Score	No. of Samples	% of Samples	Staphylo-cocci[a]	Total Count[b]	Hemolytic Count	Strepto-cocci[c]
3	100	31.0	540	4,550	1,250	0
2	51	15.8	420	3,700	960	70
1	55	17.1	300	2,400	750	150
T	126	8.1	280	2,000	380	180
-	90	28.0	130	1,300	200	10
Total	322					

[a]Counts on Bacto-Staphylococcus Medium No. 110.

[b]Counts on Blood Agar Base.

[c]Streptococci count on Blood Agar Base.

TABLE V

COMPARISON OF CMT WITH NUMBER OF COWS SHEDDING NO BACTERIA IN 0.1 ML. OF THE COMPOSITE MILK SAMPLE FOR 1962-1963

CMT Score	No. of Samples	Composite Samples with No Growth on Staph 110 Media		No. of Samples with No Growth on Blood Agar	No. of Samples with No Growth on Staph 110 or Blood Agar
		No.	% Within Each CMT Score		
3	115	9	7.8	0	0
2	150	14	9.3	0	0
1	181	21	11.6	1	1
T	248	36	14.5	1	0
-	219	48	21.9	16	10
Total	913	128		18	11

TABLE VI

COMPARISON OF CMT WITH NUMBER OF COWS SHEDDING NO BACTERIA IN 0.1 ML. OF THE COMPOSITE MILK SAMPLE FOR 1963-1964

CMT Score	No. of Samples	Composite Samples with No Growth on Staph 110 Media: No.	Composite Samples with No Growth on Staph 110 Media: % Within Each CMT Score	No. of Samples with No Growth on Blood Agar	No. of Samples with No Growth on Staph 110 or Blood Agar
3	328	27	8.2	0	0
2	130	18	13.8	1	0
1	136	11	8.1	1	0
T	93	13	14.0	1	1
-	272	45	16.5	2	0
Total	959	114		5	1

that 86.0 and 89.2 per cent of the samples for the first and second years, respectively, contained staphylococci. As the CMP score increased, as shown in Table V, the number of cows free of staphylococci decreased. However, the data for the second year, Table VI, indicate a large number of samples free of staphylococci for the CMT scores of 3 and negative.

Only 18 (2.0 per cent) of 913 observations shown in Table V had no growth on blood agar. This means that 98.0 per cent of the cows tested for 1962-1963 were shedding organisms that would grow on blood agar. The data in Table VI show that only 5 of 959 samples (.5 per cent) had no bacterial growth on blood agar. This means that 99.5 per cent of all cows tested during 1963-1964 were shedding bacteria detectable in 0.1 milliliter quantities.

During the two year period only 12 composite samples (1.3 per cent) were free of bacteria in 0.1 milliliter quantities. This is a much smaller percentage than was observed by Smith and Shultz (83) who found 1,450 samples from 105 quarters of 39 cows to be free of culturable microorganisms. This difference may be due to the use of 0.1 milliliter of milk for the bacteria counts in this study while Smith and Shultz (83) used only 0.01 milliliter.

CHAPTER V

SUMMARY AND CONCLUSIONS

A total of 7,468 quarter milk samples and 1,872 composite milk samples was collected from the University of Tennessee, Knoxville, dairy herd over a two year period (1962-1964). A California Mastitis Test (CMT) was performed on all quarter milk samples from which an Irritation Index of 5.01 was calculated; 4.31 and 5.44 for the first and second year, respectively. For the first year, the percentages were 12.6, 16.4, 19.8, 27.2, and 24.0 for CMT scores of 3, 2, 1, Trace and negative, respectively, and the percentages for the second year were 34.2, 13.6, 14.2, 9.7, and 28.3. For the second year, the number of samples for a CMT score of 3 substantially increased, while a CMT score of trace decreased. An inverse relationship was obtained between the CMT scores and the medial number of staphylococci and the total bacteria counts. A noticeable decrease for the second year in the medial number of staphylococci, total and hemolytic counts, for CMT scores of 2 and 3 was observed.

A total of 86.0 and 89.2 per cent of the samples contained staphylococci for the first and second year, respectively, and 98.0 and 99.5 per cent contained some culturable bacteria. Only 12 of 1,872 samples (1.3 per cent) were free of bacteria in 0.1 milliliter quantities.

REFERENCES

REFERENCES

(1) Agricultural Research Service. Losses in Agriculture. USDA. ARS, 20-1. 1954.

(2) Allen, V. D., and Stovall, W. D. Laboratory Aspects of Staphylococcal Food Poisoning from Colby Cheese. J. Milk and Food Technol. 23:271. 1960.

(3) Andberg, W. G., and Weirether, F. J. The Incidence of Bovine Mastitis in Minnesota. Cornell Vet. 35:289. 1944.

(4) Anderson, E. O. Relation of Bovine Mastitis to Quality of Dairy Products. In: Bovine Mastitis, First Edition. Little and Plastridge. McGraw-Hill Book Co. New York. 1946.

(5) Anderson, P. H. R., and Stone, D. M. Staphylococcal Food Poisoning Associated with Spray-Dried Milk. J. Hygiene. 53:387. 1955.

(6) Barnum, D. A. A Method for the Control of Staphylococcal and Streptococcal Mastitis in Ontario. Dairy Science Abstr. 26:507. 1964.

(7) Blomberg, H., and Gastrin, B. Investigations into the Reliability of Whiteside Mastitis Test and the California Mastitis Test. Dairy Science Abstr. 23:1426. 1961.

(8) Bortree, A. L., Carroll, E. J., and Schalm, O. W. Whey Protein Patterns of Milk from Cows with Experimentally Produced Mastitis. J. Dairy Sci. 45:1465-71. 1962.

(9) Braund, D. G. and Schultz, L. H. Physiological and Environmental Factors Affecting the California Mastitis Test Under Field Conditions. J. Dairy Sci. 46:197-203. 1963.

(10) Bryan, C. S. The Incidence of Streptococcal Mastitis Among Dairy Cattle. Vet. Med. 32:70. 1937.

(11) Carroll, E. J., and Schalm, O. W. Effect of Deoxyribonuclease on the California Test for Mastitis. J. Dairy Sci. 45:1094-97. 1962.

(12) Chernevski, N. Study of the Incidence of Pathogenic Streptococci and Staphylococci in Cows' Milk in the Kolarovgrad Region. Dairy Sci. Abstr. 26:213. 1964.

(13) Comparison of California Mastitis Test and Whiteside Tests. J. Am. Vet. Assoc. 136:226. 1960.

(14) Difco Laboratories. Difco Manual. Ninth Edition. P. 151. 1953.

(15) Drury, A. R., Murray, D. L., and Reed, G. W. Mastitis Control in Michigan Herds. Mich. St. Univ. Ext. Bulletin 344. Rev. 1962.

(16) Drury, A. R., and Reed, G. W. A Herd Irritation Index Using the California Mastitis Test. Vet. Med. 56:147-52. 1961.

(17) Ewbank, R. An Evaluation of the California Mastitis Test and the Negretti Field Test (NFT) as Indicators of Subclinical Bovine Mastitis. Vet. Rec. 74:1017-20. 1962.

(18) Ferguson, Jean. The Distribution of the Mastitis Streptococci in Dairy Herds. Cornell Vet. 28:211. 1938.

(19) Figueiredo, J. B. A Comparison of the California Mastitis Test with Other Commonly Employed Diagnostic Tests. Dairy Sci. Abstr. 22:3202. 1960.

(20) Foltz, V. D., Mickelsen, Ross, Martin, W. H., and Hunter, Charles A. The Incidence of Potentially Pathogenic Staphylococci in Dairy Products at the Consumer Level. I. Fluid Milk and Fluid Milk By-Products. J. Milk and Food Technol. 23:280. 1960.

(21) Forster, T. L. Relationship Between California Mastitis Test Reaction and Production of Milk from Opposite Quarters. J. Dairy Sci. 47:696. 1964.

(22) Frank, N. A., and Pounden, W. D. A Comparison of the California Mastitis Test and Whiteside Mastitis Test. J. Am. Vet. Med. Assoc. 132:98. 1958.

(23) Frank, Norma A., and Pounden, W. E. Mastitis Tests of Bulk Tank Milk and Quarter Samples from the Same Dairy Herds. J. Am. Vet. Assoc. 142:1390-94. 1963.

(24) Frost, A. J. Incidence of Udder Infection and Mastitis in South-East Queensland. Aust. Vet. J. 38:110-13. 1962.

(25) Gray, D. M., and Schalm, O. W. Interpretation of the California Mastitis Test Results on Milk from Individual Mammary Quarters, Bucket Milk and Bulk Herd Milk. J. Am. Vet. Med. Assoc. 136:195. 1960.

(26) Gray, D. M., and Schalm, O. W. The Mastitis Variable in Milk Yield as Estimated by the California Mastitis Test. Am. J. Vet. Res. 23:541-43. 1962.

(27) Guallini, L., and Vocino, G. Chronic Mastitis Induced by Bacteria Other than Streptococcus agalactiae. Dairy Sci. Abstr. 26:211. 1964.

(28) Hastings, E. G., Beach, B. A., and Johnson, Mildred. Observations on Mastitis in an Experimental Herd. J. Dairy Sci. 26:471. 1943.

(29) Hendricks, S. L., Belknap, R. A., and Hausler, W. J., Jr. Staphylococcal Food Intoxication Due to Cheddar Cheese. I. Epidermiology. J. Milk and Food Technol. 23:313. 1959.

(30) Hobbiger, A. Rapid Tests for the Detection of Mastitis Milk in Bulk Milk. Dairy Sci. Abstr. 23:3533. 1961.

(31) Hosoya, H., Tokumura, K., Igarashi, Y., Kosasa, F., Miyamoto, Y., and Kinoshita, Y. Studies on Improvement of the Quality of Raw Milk. II. Relationship Between California Mastitis Test and the Quality of Milk. Dairy Sci. Abstr. 25:1756. 1963.

(32) Hughes, D. L. Some Epidemiological Patterns in Bovine Mastitis. Vet. Rec. 72:485. 1961.

(33) Jackson, R. A. A Practical Mastitis Control Program. Vet. Med. 56:143-46. 1961.

(34) Jarrtsveld, F. H., Jr. Diagnosis of Bovine Mastitis in an Organized Control Scheme. Dairy Sci. Abstr. 25:853. 1963.

(35) Jarrtsveld, F. H., Jr. Contribution to the Diagnosis of Mastitis in Connection with Organized Control Measures. Dairy Sci. Abstr. 23:2327. 1961.

(36) Jezeski, J. J. Is Staphylococcus Our Next Problem? Fifty-fifth Annual Processor's Milk Industry Foundation Convention. 1961. Washington, D. C.

(37) Kalra, D. S., and Dhanda, M. R. Incidence of Mastitis in Cows and Buffaloes in Northwest India. Vet. Rec. 76:219-22. 1964.

(38) Kaushal, R. L., and Saxena, S. P. Normal Bacteria Flora of Udders of Cows and Buffaloes in Malwa Tract. I. Staphylococcus pyogenes as carriers and Their Important Properties. Dairy Sci. Abstr. 23:176. 1961.

(39) Krebs, M. Study of Udder Infections Among Slaughter House Cows. Dairy Sci. Abstr. 22:1076. 1960.

(40) Kjos-Hanssen, B. Clinical Mastitis. Dairy Sci. Abstr. 22:166. 1960.

(41) Krejakovic-Miljkovic, V., and Milojevic, Z. The Application of the Schalm Test for the Diagnosis of Bovine Mastitis. Dairy Sci. Abstr. 25:199. 1963.

(42) Leali, L., and Vallis, P. Comparative Evaluation Between the Negretti and California Test in Rapid Bovine Mastitis Diagnosis. Dairy Sci. Abstr. 22:771. 1960.

(43) Leidl, W. Comparison of the Schalm Mastitis Test with the Chlorine Content of Milk. Dairy Science Abstr. 24:794. 1962.

(44) Leidl, W., and Lagemann, H. Comparison of the Schalm Mastitis Test with the Catalase-Thybromol Test of Roeder. Dairy Sci. Abstr. 24:793. 1962.

(45) Leidl, W., and Schalm, O. W. The Application of Schalm Mastitis Test to Bulk Milk for the Determination of Low Quality Milk Due to High Cell Count. Dairy Sci. Abstr. 23:3532. 1961.

(46) Leidl, W., Schalm, O. W., Krieger, A., and Lagemann, H. Comparison of Results of Schalm's Mastitis Test with Cell Content, the Catalase-Thybromol Test and the Chlorine Content of Milk. I. Comparison of the Schalm Mastitis Test with the Cell Content. Dairy Sci. Abstr. 25:559. 1963.

(47) Little, R. B., and Foley, E. J. Staphylococci Associated with Mastitis. J. Am. Vet. Med. Assoc. 87:637-49. 1935.

(48) Little, R. B., and Plastridge, W. N. Bovine Mastitis. First Edition. McGraw-Hill Book Co. New York. 1946.

(49) Loken, K. I. Studies on Infection of the Bovine Mammary Gland with Staphylococci. Diss. Abstr. 20:1130. 1959.

(50) Luedecke, L. O. Relationship Between California Mastitis Test Reactions and Leucocyte Count, Catalase Activity, and A-esterase Activity of Milk from Opposite Quarters. J. Dairy Sci. 47:696. 1964.

(51) MacLeod, P., Plastridge, W. N., Anderson, E. O., Gullet, V. N., and Hale, H. H. Leucocyte Count of Herd Milk Compared to the Incidence of Mastitis. J. Dairy Sci. 36:1267. 1953.

(52) Marquardt, R. R., and Forster, T. L. The Relationship Between the Mastitis Condition of Individual Quarters and the Level of Abnormal Milk Found in the Bulk Tank. J. Dairy Sci. 47:663. 1964.

(53) Marshall, R. T. The Evaluation of a Mastitis Control Program in Southwest Missouri. Diss. Abstr. 21:2435-36. 1961.

(54) McCoy, Elizabeth. Staphylococcal Problems in the Dairy Industry. Milk Prod. J. 50. 1959.

(55) McEwen, A. D., and Cooper, M. B. Bovine Mastitis. Vet. Rec. 59:655. 1947.

(56) Mickelsen, Ross, Foltz, V. D., Martin, W. H., and Hunter, Charles A. The Incidence of Potentially Pathogenic Staphylococci in Dairy Products at the Commer Level. II. Cheese. J. Milk and Food Technol. 24:342. 1961.

(57) Miller, W. T. The Incidence and Types of Streptococci in First-Calf Heifers at Parturition. Cornell Vet. 26:241. 1936.

(58) Milojevic, Z., and Milenkovic, D. The Incidence of Abnormal Milk in Bulk Supplies. Dairy Sci. Abstr. 25:1155. 1963.

(59) Murphy, J. M. The General Effect of Staphylococcal Infection on the Biochemical Composition of Strict Foremilk. Cornell Vet. 33:52. 1943.

(60) Murphy, J. M. The Genesis of Bovine Udder Infection and Mastitis: I. The Incidence of Streptococcal and Staphylococcal Infection in Relation to Predisposing Factors. Proceedings Forty-ninth Annual Meeting. U. S. Livestock Sanitarian Assoc. 30. 1945.

(61) Murphy, J. M. The Genesis of Bovine Udder Infection and Mastitis: II. The Occurrence of Streptococcal Infection in a Cow Population during a 7-year Period and Its Relationship to Age. Am. J. Vet. Res. 8:29. 1947.

(62) Murray, J. G. The Incidence and Possible Significance of *Staphylococcus aureus* in Milk. Dairy Sci. Abstr. 24:2593. 1962.

(63) Murphy, J. M., and Stuart, O. M. Some Results of the Application of *Streptococcus agalactiae* (Cornell 48 Strain) to the Bovine Teat Canal by Means of the Hadley-Wisconsin Swab Technique. Cornell Vet. 43:465. 1953.

(64) Obiger, G. Why is the California Mastitis Test not Reliable? The Mechanism of the Test. Dairy Sci. Abstr. 24:2043. 1962.

(65) Oliver, J., Dodd, F. H., and Neane, F. K. Infection and Mastitis in a Dairy Herd. J. Dairy Res. 23:169. 1956.

(66) Poape, M. J., Snyder, W. W., and Hafs, H. D. Feulgen DNA in Milk as a Measure of Udder Irritation. J. Animal Sci. 21:1028. 1962.

(67) Packer, R. A. Incidence of Various Mastitis Organisms in Iowa. N. Am. Vet. 28:591. 1947.

(68) Pisanu, S. The Incidence of Bovine Streptococcal Mastitis in Sardinia. Dairy Sci. Abstr. 26:205. 1964.

(69) Plastridge, W. N. Bovine Mastitis: A. Review. J. Dairy Sci. 41:1141. 1958.

(70) Post, R. Some Observations on Overcoming Mastitis. Neth. Milk Dairy J. (English Summary). 14 (1):66-74. 1960.

(71) Pounden, W. E., and Frank, Norma A. Influence of Forage on Mastitis. J. Am. Vet. Med. Assoc. 138:146-150. 1961.

(72) Rako, A., Okljesa, B., and Jakovoc, M. Mastitis--An Economic Problem in Cattle Breeding. Dairy Sci. Abstr. 25:1156. 1963.

(73) Rendel, J., and Sundberg, T. Factors Influencing the Type and Incidence of Mastitis in Swedish Dairy Cattle. Dairy Sci. Abstr. 24:2616. 1962.

(74) Richter, O., Kleinschroth, E., Gruber, R., and Haugg, G. The California Mastitis Test for the Examination of Can Samples in Relation to Control of Udder Health. Dairy Sci. Abstr. 23:3531. 1961.

(75) Rivera, Anaya, J. D., and Berrocal, C. M. Mastitis in Puerto Rican Dairy Cattle. Dairy Sci. Abstr. 25:1465. 1963.

(76) Rude, T. A., Jr. Comparison of California Mastitis Test Readings and Bacteriological Culture Results. Vet. Med. 58:322 and 327. 1963.

(77) Schalm, O. W. Why, When, and How to Use the California Mastitis Test. Masco Bulletin Publication.

(78) Schalm, O. W., and Noorlander, D. O. Experiments and Observations Leading to Development of the California Mastitis Test. J. Am. Vet. Med. Assoc. 130:199. 1957.

(79) Schipper, C. J. The Cell Count of Milk of Individual Cows and Some Methods of Detecting Udder Infection. Dairy Sci. Abstr. 26:209. 1964.

(80) Shimizer, K. Bacteriological Studies on Streptococci from Bovine Udder. I. Serological and Biochemical Observations on Group B Streptococci and a General Description of Streptococci from Bovine Milk in Hokkaido. Dairy Sci. Abstr. 22:2629. 1960.

(81) Signorini, G. The Incidence of Streptococcal Mastitis Caused by Streptococcus agalactiae. Dairy Sci. Abstr. 22:164. 1960.

(82) Slanetz, L. W., Howe, A. F., and MacLeod, H. P. Characteristics of Staphylococci and Staphylococcal Toxins. Agric. Exper. Sta., Univ. New Hampshire. Tech. Bulletin 84. 1945.

(83) Smith, J. W., and Schultze, W. D. Cell Count Variations in Milk from Bacteria-Free and Clinically Normal Individual Quarters. J. Dairy Sci. 47:696. 1964.

(84) Spencer, G. R., and Simon, J. The Catalase, California, and Cell Count Tests for Detecting Abnormalities in Milk. Am. J. Vet. Res. 21:578-84. 1960.

(85) Stableforth, A. W. The Incidence of the Various Serological Types of Streptococcus agalactiae in Herds of Cows in Great Britian.

(86) Stamatov, T. Study of the Incidence of Pathogenic Streptococci and Staphylococci in Cows' Milk in the Tolbukhin District. Dairy Sci. Abstr. 26:212. 1964.

(87) Steede, F. D. F., and Iredale, J. L. G. Staphylococcal Infection of Raw Milk as a Cause of Food Poisoning. Mon. Bull. Minist. Hlth. Lab. Serv. 21:122-27. 1962.

(88) Steele, J. H. Epidemiological Aspects of Mastitis as Related to Public Health. Proceedings Mastitis Conference in Chicago. 1960.

(89) Steelemann, M., Obiger, G., Rackow, H. G., and Welz, W. The Incidence of Staphylococci and Their Importance in the Control of Certified Raw Milk. Dairy Sci. Abstr. 25:3270. 1963.

(90) Thatcher, S. S., and Simon, W. A Comparative Appraisal of the Properties of Staphylococci Isolated from Clinical Sites and from Dairy Products. Canadian J. Microbiol. 2:703-714. 1956.

(91) Thorne, H., and Wallmark, G. Studies on Staphylococcal Mastitis in an Individual Herd with Special Reference to Phoge-types of Isolated Staphylococci. Dairy Sci. Abstr. 22:2016. 1960.

(92) Todorov, I. Study of the Incidence of Pathogenic Staphylococci in Cows' Milk in the Burgas Region. Dairy Sci. Abstr. 26:501. 1964.

(93) Vallis, P. The Negretti Test for the Diagnosis of Bovine Chronic Catarrhal Mastitis in the Field. Dairy Sci. Abstr. 23:198. 1961.

(94) Wallace, G. D., Quisenberry, W. B., and Deharne, M. A. Preliminary Report of Human Staphylococcal Infection Associated with Mastitis in Dairy Cattle. Public Health Report. 75:457-460. 1960.

(95) Walters, A. H. The Isolation of Coagulase Positive Staphylococci from Routine Composite Milk Samples. J. Applied Bact. 22:248-52. 1959.

(96) Williams, Smith H. The Typing of Staphylococci of Animal Origin by the Bacteriophoge Method. J. Comp. Path. Ther. 58:179-188. 1948.

(97) Ziegler, H. G. Incidence of Mastitis Organisms and Mastitis Treatment. Dairy Sci. Abstr. 24:3317. 1964.

(98) Zwanenburg, T. S. Streptococcal Mastitis and Milk Hygiene. (English Summary). Neth. Milk Dairy J. 14:149-59. 1960.