

J. W. CUNKELMAN



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PENICILLIN THERAPY IN FIELD
STUDIES OF BOVINE MASTITIS
CONTROL

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE

J. W. Cunkelman
1945

THESIS

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STUDIES OF BOVINE MASTITIS CONTROL

by

J. W. Cunkelman

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FIELD STUDIES OF
BOVINE MASTITIS CONTROL

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PENICILLIN THERAPY IN FIELD STUDIES OF BOVINE
MASTITIS CONTROL

Bovine mastitis is the greatest single cause of economic loss in the dairy industry today. Bryan (12) found that 26.2% of the cattle in 322 Michigan herds totaling 2715 cows, harbored streptococci, usually associated with mastitis, in the mammary gland. In the current Illinois mastitis control program, a survey of 300 cooperating herds which included over 6000 dairy cows, revealed that 99% of the herds and 40% of the cows harbored the organism (11). In the ambulatory clinic at Michigan State College 100% of the herds comprised of three cows or more, and 52%, or 110 cows, of the 212 lactating cows in 12 representative herds were infected. Alberts (11) estimates the annual loss in the state of Illinois due to bovine mastitis to be \$8,000,000. The loss for the national dairy industry can be estimated accordingly.

It is fitting that a problem of such economic importance should be the subject of much investigation and research. A considerable

amount of knowledge including the anatomy and physiology of the udder, the types and causes of mastitis, and the chemo-therapy of established cases, has been made available. Many articles have appeared in publications available to the laymen which have portrayed only the favorable side of the reports of these investigations and research efforts, particularly in the field of chemo-therapy of bovine mastitis. Failure to have a balanced view of the problem and the proper place of chemo-therapy in mastitis control has been the source of much dissatisfaction and discouragement to the client and often to the practitioner as well. A criticism of the research work has been that most of it has been done under conditions much more favorable to success than those found on the average farm. It is the purpose of this thesis to present the methods used in controlling mastitis in the small farm herd, using penicillin as the chemo-therapeutic agent of choice.

Mastitis Defined

Mastitis is a very general term which refers to any inflammation of the udder irrespective of the cause and severity of the condition.

It may be of non-infectious or infectious origin. Non-infectious udder inflammation may be due to trauma, chilling, irregular milking procedures, or any other mechanical or thermal factor which interferes with the normal physiological function of the udder. Often non-infectious mastitis precedes infectious mastitis, particularly if the cow is repeatedly exposed to the same cause of the non-infectious mastitis. Infectious mastitis is the result of invasion of the udder by one or more of several organisms. The most common of these is Streptococcus agalactiae. Corynebacterium pyogenes, staphylococci, and coliform bacteria also may produce infectious mastitis usually of the acute form.

Both non-infectious and infectious mastitis may be chronic, sub-acute, or acute. As a rule the onset is chronic, although it may be acute. The chronic form often passes unobserved by the milker until careful examination of the udder by the veterinarian reveals abnormal milk and some degree of fibrosis of the glandular tissue. If the milker has been observant, there is usually a record of "garget" or flakes, in the milk.

The sub-acute form usually is the result

of a relatively slight increase in activity of a chronic case, although it may be the symptom of an initial attack in the udder. This form is characterized by some swelling and edema, tenderness, and gargety milk, and is usually recognized by the milker. These cases are usually transient with or without any special treatment, and are "recovered" within the span of two or three milking periods, although they may progress to the acute type.

The acute form is manifested by heat, pain, swelling, and a decided change in the amount and character of the secretion. The condition is generally obvious, and of greater severity and longer duration than the sub-acute form.

All three of these forms of mastitis, chronic, sub-acute, and acute, may be local or systemic in their effect on the host. When the condition is local, the symptoms are limited to the physical condition of the udder and the character of the milk secretion. When the condition is systemic various combinations of the symptoms of fever and septicemia are seen in addition to the local symptoms of the udder. These systemic symptoms may include high body temperature, rapid

pulse, rapid and shallow respirations, anorexia, chilling, tremors, weakness, and even prostration. As a general rule chronic cases are local, sub-acute cases are usually local, and acute cases commonly are systemic.

Penicillin Therapy

Seldom in the history of clinical medicine has a therapeutic agent excited the interest that has penicillin. Although some knowledge of the antibacterial activity of organisms was held by workers for some fifty years previously, it was not until 1929 that Fleming (1) observed that staphylococcal growth on an agar plate was undergoing lysis in a zone immediately around a mold contamination. Some work was done, but little was accomplished in stimulating interest in the clinical value of this discovery until 1941, when a report (13) was made on the unique therapeutic properties of the drug. Several commercial companies in the United States in conjunction with Federal agencies went to work on the problems of production and purification, which, stimulated by the increased demand for antibacterial agents brought about by the war, resulted in the product, penicillin, being

available in every drug store in the land only four years later.

Penicillin, a powerful antibiotic, is obtained from a common mold, Penicillium notatum. It differs from chemical disinfectants in the manner in which it's antibacterial influence is exerted. Chemical disinfectants are protoplasmic poisons, without power to distinguish between invading bacteria and the cell structure of the host. Penicillin accomplishes it's antibiotic influence by affecting the functional activity of the micro-organisms against which it is effective. This affect on the functional activity does not produce immediate death to the organisms, but does inhibit their ability to reproduce. The fact that it is not a protoplasmic poison and it's use is followed by a minimum of irritation to the host makes the drug a very desirable therapeutic agent for intra-mammary infusion.

Since penicillin was made available for experimentation in the field of veterinary clinical medicine, several reports have been published having to do with it's use in mastitis control. Kakavas (2) reported the use of penicillin

in treating several refractory mastitis cases in which a "drug fast" strain of Strep. agalactiae would not respond to sulphanilamide-in-oil therapy. He found that the infusion of sodium penicillin solution into the udder produced no unfavorable local or systemic reactions. The results obtained from the limited cases reported in this study warranted the view that sodium penicillin solution had promising chemotherapeutic value in streptococcic and staphylococcic mastitis.

Bryan and associates (3) demonstrated that the intravenous administration of relatively large doses of penicillin (50,000 Oxford units every 3 hours) was ineffective in the treatment of chronic Strep. agalactiae infection. Individual quarters treated by intra-mammary infusion of penicillin dosages ranging from 1000 to 20,000 units per quarter, uniformly recovered from chronic Strep. agalactiae infection with one treatment in a high percentage of the cases. The daily milk production records of 12 cows for seven days prior to the intra-mammary infusion which effected a recovery from the Strep. agalactiae infection, and for ten succeeding days, show that there was

either very little or no drop in production; actually three cows showed a slight increase in production. No cow with marked induration of the udder, as determined by a physical examination was treated.

The U. S. Bureau of Animal Industry in cooperation with the University of Maryland (5) reported the recovery of 58.8% of 76 quarters infected with Strep. agalactiae, and 60% of five quarters infected with staphylococci, following the use of penicillin.

Weirether and associates (4) found that an aqueous solution of penicillin containing 5000 Oxford units per ml. had but slightly irritating effects on the normal bovine mammary gland as measured by physical examination of the gland, body temperature, and changes in the physical character and quantity of the milk produced. The largest dose was 40 ml. (200,000 Oxford units) per quarter. In those cows whose glands were infused with a larger volume of water (950 ml. containing 990,000 Oxford units of penicillin) an increase in body temperature, tremors, and decided changes in the character

of the milk were observed. When the glands were infused with a small volume of water containing 5000 Oxford units per ml., the rate of decline of penicillin per ml. of milk was greatest during the first 10 hours. The milk contained 0.5 or more units of penicillin per ml. 24 hours after the infusion of 30,000 or more Oxford units. The disappearance rate from pathologic glands was of the same order as for the normal gland except in coliform infections, where the rate of decline was much greater.

Slanetz and Allen (6) concluded that penicillin is highly effective in bovine mastitis, and that streptococcic mastitis may be cured by one injection of 100,000 Oxford units of penicillin administered in 100 ml. of sterile saline or distilled water, via the teat canal. In long standing chronic streptococcic infections, and in staphylococcic mastitis, one or more injections of 200,000 Oxford units of penicillin in 100 ml. of sterile water may be necessary.

Seeley and associates (7) reported that penicillin activity was not observed in any milk samples obtained within a 24 hour period following

the intravenous injection of 80,000 Oxford units, in one trial, and 500,000 units in a second trial. The results showed that the lactating mammary gland does not serve as a major systemic exit for penicillin from the blood.

Murphy and Pfau (8) treated 52 quarters of 19 cows with both varying dosages of penicillin and number of treatments given, with favorable results as to the number of recoveries. They found that the effectiveness of the treatment did not appear to be dependent upon the lactation period, the week of lactation, the duration of the infection prior to treatment, the degree of induration present at the time of treatment or the degree of microscopic alteration of the secretion present at the time of treatment.

Klein and associates (9) found that after rather intense administration of penicillin (eight injections of 500 ml. each containing 22,800 Oxford units per dose, at six hour intervals) in the treatment of staphylococcic mastitis, all quarters treated became negative following the first infusion, but five out of

the seven quarters treated again showed the infection in from three to six days following the last infusion.

The Study

Four farms here-after known as farms A, B, C, and D were selected for this study. A picture of the prevailing conditions present on each farm is as follows:

Farm A is a 100 acre farm with an old basement barn and a poorly drained barnyard. There is no running water in the barn. The platforms behind the old wooden stanchions drain fairly well and there is a deep gutter, but there are no partitions between the cows. The cattle are good grade Jerseys, totaling about 25 head, of which there are generally eight to ten in the milking line. The owner is inherently a good cow man and takes pride in his cattle. Premature drying up of a quarter giving gargety milk resulted in the original call.

Farm B is a 120 acre farm with a "drive-through" type of barn and a well-drained barnyard. The stanchion, platform and gutter installation is average and there are no partitions

between the cows. Drinking cups are provided and there is cold water at a faucet in the barn. The cattle are mostly pure-bred Guernseys, high producers, and there are generally ten to twelve in the milking line. The farmer is a good cow man. Flare-ups of acute mastitis in two quarters following the use of milk tubes in "crushed" teats resulted in the original call.

Farm C is approximately of the same size as farm A. The cow barn has just been remodeled with all new steel stanchions, new concrete work, and everything designed to facilitate cleanliness and thus enhance a mastitis control program. The cattle are mixed grades, recently purchased, with eight in the milking line. The owner is a recently retired vocational agriculture teacher who took over the former herd of fourteen milking Holsteins, all positive to Strep. agalactiae, and all showing markedly indurated udders. They were shipped to market for beef. Heifers raised from the mastitis positive herd are now freshening from time to time. Very low production plus inability to get the milk secretion through the strainer pad resulted in the first call to this farm.

Farm D is a 220 acre farm with a good basement barn and a well drained barnyard. The milking line installation is average, with drinking cups and cold water at the faucet. As on farm C, there are partitions separating the cows. A milk room adjoins the cow stable, with hot and cold water and tubs for washing utensils. The herd totals about fifty good Holsteins, mostly grades, of which there are about 18 in the milking line. The owner is a former factory worker who has gone back to the farm after twenty-five years in town. He is interested in doing anything recommended provided he can see future financial gain. An acute flare-up in a dry cow three weeks pre-parturient resulted in a mastitis check of the entire herd.

Milking machines are used on all four farms. Farm A does not have any box stall or other place available to isolate a cow from the milking line, and the other three farms do not have isolation facilities suitable for winter weather. A boy of school age assists at milking time on each of the first three farms, and a full time hired man who does most of the milking is kept on farm D.

The Control of Mastitis

The control of mastitis is dependent upon three phases of medicine: diagnosis, therapy, and prevention.

a. Diagnosis -- The diagnosis of mastitis in this series of studies was made by considering the findings of a strip-cup examination of the fore-milk, the physical examination of the udder and the microscopic examination of the properly collected and incubated milk samples by the Breed smear method (10). Milk samples were obtained from every lactating cow at the beginning of the study. The collection of uncontaminated samples is of utmost importance for accurate diagnosis. The samples used in this study were drawn just before the regular milking time in the following manner:

1. The udder was washed with a chlorine solution (250 parts per million in water quite warm to the hand). The teats were re-wiped with a freshly wrung cloth.

2. Two streams of milk were drawn from each quarter into the strip cup and any abnormality of the secretion noted.

3. Composite or individual samples were

Table I. The age and number of cows examined in the four herds of this study.

Herd	Age in years							Total cows in herd
	2	3	4	5	6	7	8	
A	0	3	1	1	1	4	0	10
B	5	1	2	2	0	0	2	12
C	7	1	0	0	0	0	0	8
D	6	3	4	2	3	0	0	18
Total cows	18	8	7	5	4	4	2	48

Table II. The age and number of cows in which a diagnosis of infectious mastitis was made.

Herd	Age in years								Total infected cows
	2	3	4	5	6	7	8		
A	0	3	1	0	1	4	0	9	
B	2	1	1	1	0	0	1	6	
C	1	1	0	0	0	0	0	2	
D	0	1	0	2	2	0	0	5	
Total infected	3	6	2	3	3	4	1	22	

Table III. The percentage of the total number of cows, of each age group, in which a diagnosis of infectious mastitis was made.

	Age in years							Totals
	2	3	4	5	6	7	8	
Number of infected cows	3	6	2	3	3	4	1	22
Total number cows examined	18	8	7	5	4	4	2	48
Percentage of cows infected	16.6%	75%	28.5%	60%	75%	100%	50%	45.8%

drawn into sterile prepared vials.

Following the regular milking procedure, the udders were examined for symmetry, wounds, induration, and any abnormal findings recorded.

The microscopic examination of the milk was made by the stained smear method following 24 hours incubation at 37°C. Smears were placed on a glass slide with an inoculating loop, dried, and stained by immersing for one minute each in xylol, alcohol, and methylene blue stain. The slide was washed, dried, and examined under the oil immersion lens.

A total of 48 cows were examined in the four herds. The range of ages of these cows was from two to eight years (Table I), and includes most of the ages commonly found in the milking line of farm herds. Cows of all the ages represented were found to be harboring the long chain streptococcus commonly associated with infectious mastitis (Table II). The incidence of infectious mastitis in each age group (Table III) varied, ranging from 16.6% of the two year old cows, to 100% of the seven year old cows. In all, twenty-two or 45.8%, of the forty-eight lactating cows examined were found to be harboring the long chain

Table IV. The stage of lactation of the cows treated with penicillin.

Herd	Month of lactation										Totals
	1	2	3	4	5	6	7	8	9	10	
A	0	0	0	0	0	1	1	3	1	1	7
B	0	2	1	0	2	0	1	0	0	0	6
C	1	1	0	0	0	0	0	0	0	0	2
D	2	1	0	0	0	0	0	0	1	0	4
Totals	3	4	1	0	2	1	2	3	2	1	19

streptococcus.

Of these twenty-two infected cows, three were removed from the herds and sold for slaughter for various reasons. Cow A2 had a poor production record and a non-gravid uterus in the sixth month of lactation in addition to infectious mastitis. Cow A5 had a poor production record and infectious mastitis. Cow D4 was a hard milker and a confirmed kicker in addition to infectious mastitis. In each case, the culling out of the cow was the owner's wish and in line with the herd improvement program.

The remaining nineteen infected cows were in all stages of lactation, ranging from less than one month to ten months, with the one exception that there were not any cows in the fourth month of lactation. (Table IV) One cow (A4) was treated during the tenth month and retreated twenty-seven days later after having been dry two weeks.

b. Therapy -- Chemo-therapy consisted of the infusion of a penicillin solution into all four quarters of each infected cow within a few days of the time of diagnosis of infectious mastitis. The solution was made by dissolving

the contents of one vial of penicillin (85,000 Oxford units)* in 500 ml. of sterile tap water. The dosage used was approximately 25 ml. (4250 Oxford units) per quarter; the equipment for infusion consisted of a gravity flow intravenous outfit, a hemostatic forcep used as a stop clamp, and a supply of sterile, one and three quarter inch, blunt tapered, small diameter, teat tubes. Aseptic technique is of utmost importance if results are to be satisfactory.

In this trial, the teats and lower part of the quarters were carefully wiped with a cloth wrung out of chlorine solution, after which the quarters were stripped out. Following cleansing of the end of the teats again with the freshly wrung chlorine cloth, the teat tube was attached to the infusion tubing from which the air had been exhausted, and the tube inserted into the teat canal far enough so that the teat tube orifice was free within the teat, and the infusion was made. The teat tube was withdrawn, still attached to the tubing, and then detached and discarded into the pail of chlorine solution. A separate, sterile tube was used for infusing each quarter.

* The penicillin used in this experiment was furnished by Parke, Davis & Co., Detroit, Michigan

Table V. Schedule of the therapy of infectious mastitis in the four herds studied.

Cow	Age in years	Month of lacta- tion.	Diag- nosis for strep.	Time lapse, diag- nosis to treat- ment	Time lapse, treat- ment to re- test	Diag- nosis for strep.	Time lapse before retreat- ment	Time lapse before retest	Diag- nosis for strep.	Time lapse before retest
A1	7	8	+	3 da.	4 da.	-	24 da.	42 da.	-	
A3	3	9	+	3 da.	4 da.	-				
A4	7	10	+	3 da.	4 da.	+				
A6	3	8	+	3 da.	4 da.	-				
A8	3	8	+	3 da.	4 da.	-				
A9	4	6	+	3 da.	4 da.	-				
A10	7	7	+	3 da.	8 da.	+	0 da.	4 da.	-	
B3	3	5	+	0 da.	3 da.	-				
B4	3	5	+	9 da.	3 da.	-				
B9	2	7	+	9 da.	3 da.	-				
B10	8	2	+	9 da.	3 da.	-				
B11	2	2	+	7 da.	3 da.	-				
B12	4	2	+	0 da.	1 da.	+	0 da. 26 da.	2 da. 4 da.	+	16 da. 5 da.
C7	5	3	+	9 da.	3 da.	-				
C8	2	1	+	3 da.	3 da.	-				
D5	3	2	+	3 da.	19 da.	-				
D8	3	9	+	0 da.	3 da.	-				
D13	6	1	+	0 da.	3 da.	-				
D15	5	1	+	3 da.	5 da.	-				

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

Any tube accidentally contaminated before insertion into the teat was discarded.

Following infusion, each quarter was gently massaged by lifting five or six times with the palm of the hand.

The time lapse between the collection of milk samples for diagnosis and the treatment of infected cows is shown in Table V, as is also the time lapse between treatment and the collection of samples for determining if recovery was effected by the treatment. In each case where there was no time lapse between diagnosis and treatment, the cow was suffering from a sub-acute flare-up in one or more quarters, as evidenced by tenderness and swelling of the udder and an abnormal secretion as noted in the strip cup, so a treatment by infusion was given immediately following the collection of a milk sample and the stripping out of the udder.

Cow B11 had suffered a teat injury seven days prior to the udder infusion and the milk had been removed twice daily with a teat tube. The sub-acute flare-up evident at the time of infusion was further treated by the use of a camphor soap liniment, with moderate massage, at two hour

intervals for the period between infusion and milking out of the quarter.

Definite arrangements were made beforehand for the infusion treatment of the cows other than those with sub-acute flare-ups. The grain ration was reduced at least 50% for one or two feedings prior to infusion, and the cow was kept in the barn overnight. No cows were turned out before mid-afternoon of the day they were infused, and then only if the weather was warm and dry. They all were housed the first night following infusion.

All cows were milked out ten to twelve hours following infusion with the exception of the RR/4 of cow A4, which had not been lactating for approximately six months. Lactation was apparently normal in this quarter following parturition.

Cow C7 showed considerable reaction within 8 to 10 hours following infusion, with an acute flare-up in all quarters, feverishness characterized by tremors, rapid and shallow respirations, complete anorexia and body weakness characterized by incoordination. Seventy-two hours later the appetite and the milk secretion were normal

Table VI. Summary of the results of the diagnosis and therapy of infectious mastitis in the four herds studied.

Herd	Total cows	No. of infected cows	No. of cows sold for slaughter	Cows treated	Negative following treatments		
					1st.	2nd.	3rd.
A	10	9	2	7	5	2	0
B	12	6	0	6	4	0	2
C	8	2	0	2	2	0	0
D	18	5	1	4	4	0	0
Total	48	22	3	19	15	2	2

and the microscopic test of milk samples was negative for infectious mastitis. This occurred during August when the herd was being fed moldy ensilage. Although the largest clumps of moldy ensilage were being discarded and not used as feed, the cattle were ingesting considerable mold. The results obtained in this cow suggest the possibility that cattle ingesting moldy feed might become sensitized to penicillin.

With the one exception mentioned, the infusions did not result in an appreciable inflammation or tenderness of the quarters, or in any undesirable change in the character of the milk produced.

Nineteen, or 100% of the cows treated, recovered, with fifteen, or 78.9% of the recoveries being with one treatment, and two, or 10.5% of the recoveries being with two treatments, and two or 10.5%, of the recoveries being with three treatments. (Table VI).

All cows treated in this study were negative to the microscopic test for infectious mastitis at approximately three and eight week intervals following the negative test noted in Table V.

Without exception, owners did not report any

marked drop in production following treatment, and in several instances even reported slight increase in production. Accurate daily production records are not available on the average farm.

c. Prevention -- The preventive measures used on these farms were designed to eliminate as many of the sources of udder injury and infection as possible; they included:

1. Immediate treatment of infected cows.
2. Routine washing of udders, and dipping of teat cups between cows, in two pails of chlorine solution.
3. Milking in the order of cows negative to infectious mastitis, cows under suspicion, and cows just treated.
4. Fast milking with elimination of hand stripping as soon as possible.
5. Wiping teats with a cloth wrung out of chlorine solution after milking.
6. Routine testing of all newly-freshened heifers and newly purchased additions to the milking line.
7. Periodic testing of all lactating cows.

Conclusions

Penicillin is a satisfactory chemotherapeutic agent for intra-mammary infusion in treating infectious mastitis caused by streptococci. It fulfills the requirements of being economical, non-irritating -- during all stages of lactation -- and is effective.

An infusion of 4250 Oxford units in 25 ml. of sterile tap water per quarter resulted in recovery from infectious mastitis in fifteen of nineteen cows with one treatment, while two required two treatments, and two required three treatments to effect recovery.

Attention to details of the mastitis problem on each farm is an essential part of the program in successful mastitis control.

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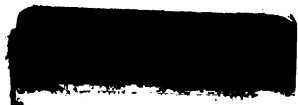
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