

South Dakota State University
**Open PRAIRIE: Open Public Research Access Institutional
Repository and Information Exchange**

Bulletins

South Dakota State University Agricultural
Experiment Station

6-1-1948

Bovine Mastitis: Treatment with Penicillin and Herd Practices which aid in its Control

D. F. Breazeale

P. L. Kelly

E. Bartle

A. B. Hoerlein

Follow this and additional works at: http://openprairie.sdstate.edu/agexperimentsta_bulletins

Recommended Citation

Breazeale, D. F.; Kelly, P. L.; Bartle, E.; and Hoerlein, A. B., "Bovine Mastitis: Treatment with Penicillin and Herd Practices which aid in its Control" (1948). *Bulletins*. Paper 392.

http://openprairie.sdstate.edu/agexperimentsta_bulletins/392

This Bulletin is brought to you for free and open access by the South Dakota State University Agricultural Experiment Station at Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange. It has been accepted for inclusion in Bulletins by an authorized administrator of Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange. For more information, please contact michael.biondo@sdstate.edu.

Bovine Mastitis

TREATMENT WITH PENICILLIN AND HERD PRACTICES WHICH AID IN ITS CONTROL

Bulletin 392
JUNE 1948



**DAIRY HUSBANDRY DEPARTMENT
AND
VETERINARY SCIENCE DEPARTMENT**

**S. Dak. Agricultural Experiment Station
SOUTH DAKOTA STATE COLLEGE ❖ BROOKINGS**

Table of Contents

Introduction	3
Literature Cited	3
Procedure	4
General considerations	4
Diagnostic methods	5
Treatments with penicillin	6
Herd management practices and milking parlor sanitation	7
Results	8
Terms defined	8
Survey of mastitis in the herd at the beginning of the experiment	9
Bacteriological examinations	9
Results of penicillin treatments for mastitis caused by various organisms	10
Penicillin treatments for mastitis caused by <i>Str. agalactiae</i>	11
Infections by other organisms after the quarters were freed of <i>Str. agalactiae</i>	12
Early treatments	13
Relationship of physical conditions of the quarters to previous mastitis infections	13
Udder damage due to mastitis	14
Bacteriological observations	16
Housing of dairy cows as a factor in the control of mastitis	17
Discussion	19
Summary	21
References	23

Bovine Mastitis: Treatment With Penicillin and Herd Practices Which Aid In Its Control

D. F. BREAZEALE, P. L. KELLY and EMERY BARTLE
Dairy Husbandry Department

A. B. HOERLEIN* and G. S. HARSHFIELD
Veterinary Science Department

Introduction

All of the work reported herein was conducted with the College dairy herd at Brookings. Incomplete records and earlier publications from this station indicate a considerable history of mastitis in this herd. Various treatments including the use of Tyrothricin, Phemerol, Novoxil, sulfanilamide and sulfamerazine had been used. When this project was started (May 1945), more than half of the cows in this herd had mastitis in one or more quarters. There were many chronic cases of long duration.

Penicillin had been found to be very effective in human medicine for infections of streptococci and staphylococci. Small quantities of it were becoming available for animal use and it seemed reasonable to assume that it might be an effective means of eliminating streptococci and staphylococci from mastitis udders. The purposes of these experiments were (1) to determine the effectiveness of penicillin treatments for mastitis and (2) to compare pens with stanchions as a means of controlling this disease. Other herd management practices, including sanitation in the milking parlor, were to be considered, although it was not planned to study them in detail.

Literature Cited

The literature pertaining to bovine mastitis is too extensive to be reviewed in this publication. A symposium edited by Little and Plastridge (5) gives a comprehensive review of the subject. Only a few articles pertinent to the two phases of the problem considered in the experimental part will be mentioned here.

At the time that this experimental work was started, limited supplies of commercially produced penicillin sodium were just becoming available for veterinary use. A few reports had appeared in the literature on the use of penicillin infusions for mastitis.

Kakavas (3) observed undesirable tissue reactions, following infusion of crude penicillin produced in a medium containing corn steeping liquor. Less reaction resulted from raw penicillin in whey broth filtrate. With this product 21 of 23 quarters were freed of *Streptococcus agalactiae* and 16 of 27 quarters were freed of *Staphylococcus aureus*. Using the sodium salt of penicillin which he prepared, 10 of 15 quarters were freed of *Streptococcus agalactiae* and 4 of 7 quarters were freed of *Staphylococcus aureus* following infusion of from one to eight doses of 1,200 to 15,900 Oxford units each. No unfavorable reactions were observed with this product.

*Resigned January 1947; now at Iowa State College, Ames, Iowa.

Bryan, Horwood and Huffman (1) treated 32 cows infected with chronic streptococcic mastitis with penicillin sodium. Three doses of from 1,000 to 20,000 units dissolved in sterile distilled water were administered into each quarter. All of the cows thus treated became free of the bacteria causing mastitis. In these trials no animal with marked fibrosis of the udder was treated.

Murphy and Pfau (6) reported a higher percentage of bacteriological cures in mastitis due to *Streptococcus agalactiae* when five infusions of 20,000 units each of penicillin sodium were administered, than when single doses of 5,000 to 200,000 units were given. In one experiment 32 quarters were given the five doses, and all were freed of the bacteria as compared to 5 of 15 quarters receiving the single treatment. In another experiment, treatment was successful in 6 of 10 quarters, in which five doses of 10,000 units each were given after regular milking intervals. The animals were unselected, and several with well advanced cases of mastitis were included.

The United States Bureau of Animal Industry in cooperation with the University of Maryland (7) carried out experimental treatment, with penicillin, of 46 cows. The causative bacteria were eliminated from 58.8 per cent of the 76 quarters infected with *Streptococcus agalactiae*, 48.5 per cent of the 19 quarters infected with other streptococci and 60 per cent of five staphylococcus infected quarters. A filtrate containing 68 to 95 Oxford units per ml. was used. The total dosage was not mentioned in this report.

Slanetz and Allen (8) reported 93.2 per cent bacteriological cures of 55 quarters infected with *Streptococcus agalactiae* and 78.5 per cent of 11 quarters infected with staphylococci. They did not consider a quarter cured unless quarter milk samples tested at weekly intervals for a period of 4 to 5 weeks, following treatment, remained free of the causative bacteria. Twenty-five of the *Streptococcus agalactiae* infected quarters and five quarters infected with staphylococci became free of the bacteria following one injection. Each injection contained 100,000 or 200,000 units of penicillin in 100 ml. of distilled water. They reported that all cows which were producing discolored or stringy milk before treatment gave normal milk following treatment.

In trials to determine the efficacy of penicillin in staphylococcic mastitis, Klein, Crisman and Moor (4) eliminated the bacteria from only two of the seven quarters treated. Both of these had received eight injections, each containing 22,800 units.

Herd management practices which are important in a mastitis control program are considered to some extent in these experiments, but the only phase in which a direct comparison was made was that of the pen barn vs. stanchions. Heizer *et al.* (2) reported that an experiment over a four-year-period failed to show any definite advantage for stalls or pens in the control of mastitis. Tie chains were used in the stalls and the stalls were wider than is common for stanchions, thus giving the cows somewhat more freedom of movement.

Procedure

General considerations. The College dairy herd at Brookings, consisting of from 45 to 53 lactating cows, was used for these experiments. Four breeds were represented, namely, Holstein, Brown Swiss, Guernsey and Jersey. These cows

were machine-milked twice daily in the milking parlor. During the course of the experiments, the milking routine was changed to three times daily.

The cows were kept in stanchions in the main part of the barn, adjacent to the milking parlor. During cold, windy days this part of the barn was cold and drafty. The stanchions were not adjustable, and consequently some were not the proper length for the cows. These conditions and some others which will be mentioned later, may have been contributing causes to the large number of mastitis cases in the herd.

Since more than half of the cows had mastitis at the beginning of these experiments, an extensive culling program did not seem feasible. The general plan was to keep as many of the potentially good cows in the herd as long as possible to obtain additional calves. Keeping these infected cows in the herd increased the possibility of further spread of mastitis, but it was hoped that the use of penicillin treatments plus good management practices would minimize this danger.

Diagnostic methods. Since accurate diagnosis is necessarily the foundation to the study or control of any disease, special emphasis was placed on this phase. A combination of the most accurate diagnostic means was employed in order to obtain as complete a picture of the herd as possible.

The strip-cup was used at each milking for the purpose of determining the presence of any visible abnormalities in the milk. Reports of these tests were obtained daily from the milkers. Also recorded on these reports were any injuries, illnesses, udder abnormalities, breeding dates, calving dates and drying-off dates.

One complete physical examination by udder palpation was made during April of 1946. The quarters were classified according to the system devised by Udall (9).

Quarter milk samples for laboratory examination were collected monthly during the first year of the study and every three months during the second year. During the second year, milk samples were also collected for bacteriological examination at intervals of three to four weeks following treatments with penicillin.

Extraneous contamination from the outside of the udder was minimized by first cleaning the udder with paper towels dipped in a warm solution containing approximately 200 parts per million (p.p.m.) of available chlorine. The teats were then wiped with pledgets of cotton moistened with alcohol, and finally the ends of the teats were painted with a tincture of metaphen on cotton swabs. The first streams of milk, amounting to about 15 milliliters, were collected in sterile test tubes with rubber stoppers. Generally, the sampling was done just prior to a regular milking period.

The milk samples were incubated overnight at 37° C. A loopful of each incubated sample was streaked onto tryptose blood agar plates. After 24 to 48 hours of incubation at 37° C. the plates were examined for bacterial growth and suspicious colonies were isolated for further identification.

Procedures used in the identification of streptococci included colony characteristics, hemolytic action on the blood agar, microscopic examination of smears from 24-hour broth cultures, and growth reactions in sodium hippurate, esculin, and differential carbohydrate broths. A large group of streptococci cultures were carried through additional tests in an attempt to classify those which did not conform to the usual reactions for *Streptococcus agalactiae*. These included growth

at 10° C. and 45° C. and in the presence of 6.5 and 9.6 per cent sodium chloride. Precipitin tests were carried out using Lancefield Type B and Type C antisera, as well as four antisera prepared by immunizing rabbits with strains representing other groups of streptococci isolated in these studies.

Methods used for the identification of staphylococci were: colony characteristics, hemolytic action on blood agar, microscopic examination of broth cultures and coagulase reactions. Usual bacteriological methods were used for the preliminary identification of other groups of bacteria.

After the milk samples were incubated overnight, smears were made for direct microscopic examination. The types of bacteria and the numbers of leucocytes were noted. The numbers of leucocytes were recorded in these groups: (1) less than 1,000,000, (2) from 1,000,000 to 5,000,000, (3) from 5,000,000 to 10,000,000 and (4) more than 10,000,000 per ml. of milk.

Treatments with penicillin. Solutions of penicillin sodium in sterile distilled water were used for all of the work herein reported. This antibiotic was purchased in the usual vials containing either 100,000 or 200,000 Oxford units. It was dissolved in about five ml. of sterile water and then transferred to a sterile eight-ounce bottle containing sterile water. The penicillin solution was infused into the teat canal by gravity using a gravity flow apparatus. A separate sterile teat tube was used for each quarter injected.

For nearly all of the treatments reported, 100,000 units of penicillin were contained in 100 ml. of water. During the first few weeks of the experiment when supplies of penicillin were limited, a few dosages as low as 5,000 to 25,000 units per quarter were used. With these few exceptions the dosages were standardized at 100,000 units per quarter. Usually only single treatments were given; however, a series of three 100,000 unit dosages were given in a very few cases which had not responded to the single treatment.

The udders and teats were prepared for the penicillin infusions in the same manner as previously described for the collection of samples. This care was deemed necessary to prevent possible infections by organisms not sensitive to penicillin. For the most part only infected quarters were treated. In a few instances all of the quarters were treated when two or more were infected and the leucocyte count indicated a slight disturbance in the others, or when the rate at which the infection was spreading indicated the advisability of treating uninfected quarters.

In these experiments no attempt was made to select certain udders or quarters for treatment. It seemed desirable to obtain information on the effectiveness of penicillin in the treatment of mastitis in its various stages of development and with different degrees of udder damage. Hence the range in cases treated, varied from those of very recent infection with no apparent damage, to those of long duration, with one or more quarters showing marked fibrosis* and giving small amounts of milk with abnormal characteristics.

At the beginning of the experiment, a group of nine cows with advanced cases of chronic mastitis was isolated in another barn away from the main herd. This was done to help prevent further spread of the disease. About three months later when more penicillin was available, these cows were treated and were returned to the main herd.

*The replacement of functioning mammary tissue by fibrous or scar tissue.

It was the initial plan to treat all cases of mastitis caused by streptococci or staphylococci, and during the first year of the experiment this plan was followed as closely as available supplies of penicillin permitted. At the end of this period the total infection in the herd had been reduced greatly and, for this and other reasons, a number of cases were observed for a period of about six months without treatment. Again, these were not selected cases, but included all of those which developed during this period, as well as some which had not responded to previous treatment. Thus, these untreated animals served, at least partially, as a control group, and some information was obtained to show what may be expected to take place in a mastitis control program which is interrupted or not carried out completely.

Herd management practices and milking parlor sanitation. A survey of the herd practices and sanitary procedures which had been used with this herd indicated that a number of changes should be helpful in the control of mastitis. These were put into effect as rapidly as possible. The general floor plan of the barn is shown in Figure 1.

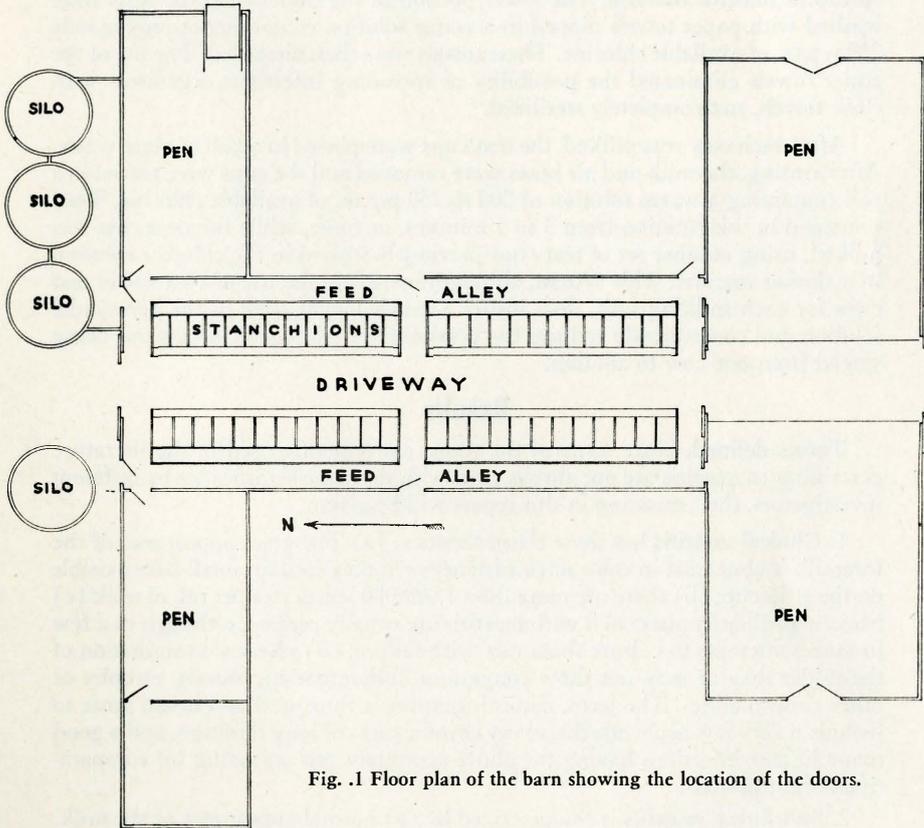


Fig. .1 Floor plan of the barn showing the location of the doors.

One of the most important problems pertaining to the barn was the cold, drafty conditions existing around the stanchions. Before the winter season of 1945-46, close-fitting doors were placed at the entrances to the silos, and new doors were placed at the corners of the stanchion part of the barn to minimize drafts from the wings of the barn. Non-lactating heifers were placed in the stanchions nearest the doors in an attempt to give added protection to the lactating cows. These efforts were only partially successful, for the barn still seemed drafty. During cold weather the humidity in the barn was so high that moisture dripped from the ceiling and walls.

The milking order was checked very frequently to make certain that mastitis-free cows were first in the line, those with probable infections next, and those with definite and most severe infections last. The cows were trained to respond to the "fast-milking" procedure and hand stripping was used to check on the completeness of milking, the importance of which was emphasized to the milkers.

As a result of some preliminary bacteriological tests, the following procedures were adopted and used consistently during the course of this investigation, in an effort to establish a routine as nearly fool-proof as possible in preventing the spread of mastitis bacteria. The lower portion of the udders and the teats were washed with paper towels dipped in a warm solution containing approximately 200 p.p.m. of available chlorine. These towels were then discarded. The use of the paper towels eliminated the possibility of spreading infectious organisms with cloth towels, not completely sterilized.

After each cow was milked, the teat cups were placed in a pail of rinse water. After rinsing, the milk and air hoses were removed and the cups were placed in a pail containing a warm solution of 200 to 250 p.p.m. of available chlorine. They remained in this solution from 3 to 5 minutes, or more, while the next cow was milked, using another set of teat cups previously soaked in the chlorine solution in a similar manner. This system, although involving the use of two sets of teat cups for each milking unit, does insure a much longer time in the germicidal solution and consequently reduces the possibility of pathogenic organisms being spread from one cow to another.

Results

Terms defined. Since some of the terms conveniently used in the literature pertaining to mastitis are not always used with the same significance by different investigators, their meaning in this report will be given.

1. **Clinical mastitis** has these characteristics: (a) The gross appearance of the foremilk is abnormal in color or consistency or it may contain small flakes visible on the strip-cup, (b) there are more than 1,000,000 leucocytes per ml. of milk (c) bacteria ordinarily associated with mastitis are usually present, although in a few instances attempts to culture them met with failure, (d) physical examination of the udder may or may not show congestion, inflammation, fibrosis, atrophy or other abnormalities. The term, clinical mastitis, is thus used in a broad sense to include a very few acute attacks, many chronic cases of long duration, and a good many lighter infections having the above symptoms but persisting for comparatively short periods.

2. **Subclinical mastitis** is characterized by (a) normal appearance of the milk,

(b) the presence of bacteria ordinarily associated with mastitis and (c) more than 1,000,000 leucocytes per ml. of milk which are not attributable to the stage of lactation.

3. **Bacteriological cures, freed of bacteria, or became negative to the causative bacteria**, are terms used to indicate that the bacteria involved in the reported case of mastitis were not found on bacteriological examination of milk samples taken at varying intervals during the month after the treatment. The quarter apparently remained free of the bacteria for at least one month. In the case of those receiving no treatment, the use of these terms means that the bacteria were not found in the next regular examination. In these cases the leucocyte count frequently remained above 1,000,000 for several weeks; however, no abnormalities were apparent in the milk on the strip-cup. The use of these terms is not intended to imply that a damaged quarter returned to a normal condition even though the infection was removed.

Survey of mastitis in the herd at the beginning of the experiment. There were 51 cows in the milking herd when the first bacteriological examinations of the milk were made. These examinations were spread over a period of three months and included all of the lactating cows in the herd. The data from these tests, along with the reports of strip-cup tests and the palpation of some of the udders, revealed that 28 of the cows (55 per cent) had mastitis (clinical and subclinical) in one or more quarters. Similarly, these data show that 32.3 per cent of the quarters were affected with mastitis. More than half of these were chronic cases of long duration.

Bacteriological examinations. During the period of observation (May 1945 to June 1947) the milk samples from a total of 334 quarters were examined bacteriologically. A summary of the results is presented in Table 1.

Table 1. Types of bacteria found in mastitis quarters

Description	Numbers	Percent of total quarters	Percent of mastitis quarters
Total quarters examined	334	-----	-----
Total non-mastitis quarters	146	43.7	-----
Total mastitis quarters	188	56.3	-----
<i>Streptococcus agalactiae</i>	60	18.0	31.9
Streptococci, not <i>Streptococcus agalactiae</i>	46	13.8	24.5
Staphylococci	58	17.4	30.9
Coliforms	11	3.3	5.8
Miscellaneous bacteria	3	0.9	1.6
No causative organism found	10	3.0	5.3

At some time during this period, 188 (56.3 per cent) of these quarters had mastitis. The organisms found in the majority of these cases were *Streptococcus agalactiae*, staphylococci and streptococci (other than *Streptococcus agalactiae*) listed in the order of their importance. It will be noted that there was no great difference in the numbers of mastitis quarters caused by each of these groups of organisms. This is perhaps somewhat unusual since *Streptococcus agalactiae* has

been found to be the causative organism in the majority of cases reported in the literature. Three possible explanations are offered:

(1) Clinical and subclinical cases are considered together, which gives a somewhat different picture than would result if only clinical cases of long duration (chronic) were presented (compare with Table 2).

(2) Since many of the cases of mastitis were of very long standing, it is possible that *Streptococcus agalactiae* was the organism causing the initial damage and that other organisms found conditions opportune for growth in the weakened udder.

(3) Only one herd is involved. The distribution of causative organisms varies among different herds.

Table 2. Results of penicillin infusions in mastitis quarters infected with different organisms vs. no treatment.

Causative bacteria	Type of mastitis	Penicillin infusions	Freed of bacteria %	Not treated	Freed of bacteria %
<i>Streptococcus agalactiae</i>	Clinical	37	62.2	17	5.9
	Sub-clinical	47	76.7	18	16.6
	Total	84	70.3	35	11.4
Streptococci (not <i>Streptococcus agalactiae</i>)	Clinical	6	100.0	4	25.0
	Sub-clinical	2	100.0	34	70.6
	Total	8	100.0	38	65.8
Staphylococci	Clinical	3	0.0	5	0.0
	Sub-clinical	16	18.8	50	60.0
	Total	19	15.8	55	54.5
Coliforms	Clinical	4	25.0	2	100.0
	Sub-clinical	1	0.0	4	100.0
	Total	5	20.0	6	100.0
Miscellaneous	Clinical	2	50.0	---	---
	Sub-clinical	1	0.0	---	---
	Total	3	33.3	---	---

Results of penicillin treatments for mastitis caused by various organisms. Penicillin infusion treatments were given for mastitis caused by streptococci, staphylococci, a few coliforms and other organisms. The results are summarized in Table 2. These data are given in terms of separate treatments rather than quarters treated. In some cases these are numerically equal, but in others, notably those caused by *Streptococcus agalactiae*, some quarters were given two or more treatments for infections at different times.

The value or success of these treatments was measured by the absence of the causative bacteria in the milk samples and a normal appearance of the milk (daily strip-cup tests). These values are listed in column 4 under the caption "freed of bacteria." The last column of the table has the same heading and is used in the

same way with the exception that it applies to those cases which were not treated, and it is assumed that the bacteria found were merely carried in the udder for a short time and were not able to set up an infective process.

Although it is recognized that the untreated cases are not "controls" in a strict sense, it is believed that a comparison of them with the treated cases gives valuable information on the treatment phase of a mastitis control program. Some of the clinical cases not treated were of much shorter duration and less severe than those treated. As a result of the thorough diagnostic procedures used, subclinical cases were frequently encountered which were transitory in nature, i.e., observed at one time and not found in the next examination.

It will be noted that 70.3 per cent of the mastitis cases treated for *Streptococcus agalactiae* became negative to these bacteria, while only 11.4 per cent of the non-treated cases became negative. With both groups, recoveries from the bacterial infections were greater with the mild, subclinical cases than with the clinical cases of longer duration.

Only eight cases were treated for various species of streptococci other than *Streptococcus agalactiae* and all of these treatments were effective in eliminating the bacteria. Of the subclinical cases 70.6 per cent became negative without treatment, while 25.0 per cent of the clinical cases became negative; however, there were only four cases in this group.

Treatments for mastitis caused by staphylococci were less effective, with none of the three clinical cases showing any response and only 18.8 per cent of the subclinical cases becoming negative to the bacteria. Many of these were well established cases having been in the quarter for several months before treatment. The untreated clinical cases also remained infected, but 60.0 per cent of the subclinical group became negative.

A comparatively few cases of coliform mastitis were observed. One was the result of a serious injury, and other pathogenic bacteria were found in addition to coliforms. Penicillin treatments were used in five cases, but only one was effective. Six cases were observed, but not treated; all of them became negative.

Three cases of mastitis caused by unidentified rod-shaped bacteria were treated with penicillin; only one became negative as a result of the treatments.

Penicillin treatments for mastitis caused by *Streptococcus agalactiae*. Some interesting relationships were observed in the results of the penicillin treatments for mastitis caused by *Streptococcus agalactiae*, and it is believed that sufficient data were obtained to warrant further subdivision and discussion of these parts. Section A of Table 3 shows the percentages of the quarters which became negative to *Streptococcus agalactiae* following the first treatment of the quarter. As would logically be expected, the treatments of mild, subclinical cases gave a higher percentage of bacterial cures than was obtained with the more severe, clinical cases. These values were 90.3 per cent and 66.7 per cent respectively. When both groups are considered together, 80.8 per cent became negative. Although it would require observations over several years to determine an average time that these cows would remain negative, it is known that half of them were still free of *Streptococcus agalactiae* at the end of the two year observation period.

Of the 14 clinical cases which became negative to *Streptococcus agalactiae* after the penicillin infusion, 10, or 71.4 per cent, became reinfected with the same

Table 3. Results of penicillin infusions for mastitis caused by *Streptococcus agalactiae*

Description	Clinical Mastitis	Subclinical Mastitis	All Cases of Mastitis
A. First treatments of quarter	21	31	52
Quarters freed of <i>Streptococcus agalactiae</i>	14	28	42
Per cent freed of <i>Streptococcus agalactiae</i>	66.7	90.3	80.8
Recurrence of <i>Streptococcus agalactiae</i> , No.	10	16	26
Recurrence of <i>Streptococcus agalactiae</i> , per cent...	71.4	57.1	61.9
B. Treatments for reinfections	16	16	32
Cases freed of <i>Streptococcus agalactiae</i>	9	8	17
Per cent freed of <i>Streptococcus agalactiae</i>	56.3	50.0	53.1

species of bacteria during the observation period. For convenience in this discussion, these cases are assumed to be reinfections, although it is recognized that there is the possibility that the organisms were never completely eliminated from the quarter. In this case they were not being shed when subsequent samples were taken, or if they were shed in small numbers, they were not detected. These reinfections were first observed at time intervals ranging from 2 to 11 months, and averaging 6 months after the quarter was reported negative to *Streptococcus agalactiae*.

There were 28 subclinical quarters which became negative to *Streptococcus agalactiae* after the first treatment. Reinfections were observed in 16, or 57.1 per cent, of this group. These occurred from 1 to 18 months (average 10 months) after the quarter was freed of these bacteria by treatment. It is thus evident that a higher percentage of bacteriological cures was obtained with mild cases of mastitis, than with the more severe cases; also these subclinical cases remained free of the infection for a somewhat longer average period of time. These data indicate the advisability of treating the quarter as soon as possible after the *Streptococcus agalactiae* infection has been diagnosed.

There were 32 penicillin infusions administered for reinfections of *Streptococcus agalactiae*, divided equally between the clinical and subclinical classes. (Section B of Table 3.) There were 26 quarters involved, which means that a few quarters had more than one reinfection and treatment during the period. The results of these treatments show that nine clinical and eight subclinical cases became negative to *Streptococcus agalactiae*. The experiment was not carried long enough for these values to have much significance, but there does seem to be some indication that cows which had mastitis due to *Streptococcus agalactiae* and were freed of this infection by penicillin infusions, did not develop immunity towards the organism, but on the contrary, they were more susceptible to it. This statement is based principally upon the fact that the reinfections occurred at an average time of 8.5 months following the first successful treatments and 4.9 months after the successful treatments for reinfections.

Infections by other organisms after the quarters were freed of *Streptococcus agalactiae*. The data show that following 59 successful treatments in which *Streptococcus agalactiae* was no longer found in the milk samples, other types of bacteria were found in 25 (42.4 per cent) cases. These bacteria caused clinical masti-

tis in six (10.2 per cent) cases and subclinical mastitis in 19 (32.2 per cent) instances. Causative bacteria involved were chiefly staphylococci, streptococci and a few coliforms.

Early treatments. A total of 12 penicillin infusion treatments were made in which the only evidence of mastitis was the presence in the milk samples of *Streptococcus agalactiae* (seven quarters); streptococci, not *Streptococcus agalactiae* (one quarter) and staphylococci (four quarters). Since these organisms were not found in the next examination, bacteriological cures may be claimed for these treatments.

Comparable, although larger, groups of the same types of organisms were found, but not treated. These included 17 *Streptococcus agalactiae*, 67 streptococci not *Streptococcus agalactiae*, and 67 staphylococci. The percentages of these cases which were negative at the next examination were 89.5, 88.1 and 74.6 respectively. Such high percentages of spontaneous bacteriological cures suggests (1) that these bacteria were contaminants from the exterior of the teats and were not actual infections, or (2) that they were very light, transient infections easily overcome in most cases by the natural defense mechanism of the cow.

Relationship of physical conditions of the quarters to previous mastitis infections. The udders of all the lactating cows were palpated during April of 1946 and the quarters were classified on the degree of fibrosis according to the system of Udall (9). Records of bacteriological examinations of milk samples for the previous six months (or slightly longer in some cases in which the lactation period began earlier) were used to determine the effects of previous infections on the present physical condition of the quarters. A total of 132 quarters were palpated. Of these, 58 had records of mastitis. The results are presented in Table 4.

Table 4. Physical condition of quarters as influenced by previous mastitis infections

Description	All quarters	Normal, no fibrosis	Slight fibrosis	Distinct fibrosis	Marked fibrosis
Total in groups	132	45	60	16	11
No mastitis observed %*	56.1	84.4	51.7	31.2	0.0
Subclinical mastitis %	21.2	13.4	28.3	31.2	0.0
Clinical mastitis %	22.7	2.2	20.0	37.6	100.0
Causative bacteria total	58	7	29	11	11
<i>Streptococcus agalactiae</i> %	51.7	14.3	51.7	54.5	72.7
Other streptococci %	10.3	42.8	10.4	0.0	0.0
Staphylococci %	24.2	28.8	27.6	27.3	9.1
Coliforms %	6.9	14.3	3.4	18.2	0.0
Miscellaneous and no bacteria %†	6.9	0.0	6.9	0.0	18.2

*Refers to the percentage within each group.

†No bacteria found in two cases, one was *C. pyogenes* and one unclassified.

The data in the upper portion of the table show some very definite trends. As the degree of fibrosis increased, there was a sharp increase in the percentage of quarters having had clinical mastitis and a corresponding decrease in the percentages of those in which no mastitis had been observed. All of the quarters classified

with marked fibrosis had a record of clinical mastitis. On the other hand, there was no record of mastitis in 84.4 per cent of the quarters classified as normal with no fibrosis.

The lower portion of the table gives the kinds of bacteria which were found in the 58 mastitis quarters. *Streptococcus agalactiae* was involved in slightly more than half of these cases. As the degree of fibrosis increased, the proportion of *Streptococcus agalactiae* increased over the other organisms. Staphylococci accounted for the second largest group (24.2 per cent); however, these organisms were found in only 9.1 per cent of the quarters showing marked fibrosis.

A study of the data in Table 4 indicates that udder palpation is very useful in the complete diagnosis of mastitis. Considerable information relative to the advisability of penicillin infusions can be obtained with this technique when used by the experienced veterinarian.

Udder damage due to mastitis. The amount of damage caused by mastitis infections may vary from slight to extensive. Extreme cases show marked fibrosis, as was indicated in the preceding section. In order to obtain additional information concerning the nature of this damage, several udders affected with mastitis were returned from the slaughter house for study in the laboratory. The milk was removed from the udders and a dye solution was forced with slight pressure into them through the teat canals in order that the milk ducts and secretory tissue would be stained. After removal of the dye solution, the right and left halves of the udders were separated. Longitudinal sections were then made through these halves at the center of the teats.

The photograph on the front cover shows cow No. 472 as she appeared a few days before freshening. The udder was large and distended giving the appearance of considerable milk producing capacity. However after freshening she produced only five pounds of milk daily. In an earlier lactation she had averaged 55 pounds of milk per day for the entire period. The amount of the gland which could still function normally is shown in Fig. 2.

There are essentially two types of cell structures in the udder which come in contact with the milk. These are the alveoli, grape-like structures in which the milk is produced, and the ducts which drain the milk from the alveoli down into the gland cistern located in the lower part of each quarter. A close inspection of the left front quarter will show a large amount of fibrous tissue lining the gland cistern which had been much reduced in size. The dye did not penetrate into the upper portions of this quarter, indicating a stoppage in all of the ducts at the point where they entered the gland cistern. Even though the alveoli were probably functioning, as the enlargement of the gland at freshening time would indicate, there was no possible drainage from them to the gland cistern. The left rear quarter still had some ducts which were draining normally, as was evidenced by the dye penetrating into some of the upper portions of the gland. The damage done by the organisms causing mastitis is especially serious if it affects the ducts in the lower portion of the gland. These are the ones which connect the gland cistern to the largest number of alveoli. As would be expected, after these permanent fibrotic changes were present, penicillin infusions were totally ineffective in these quarters.

A much different type of mastitis udder is shown in Figure 3. This cow, No.

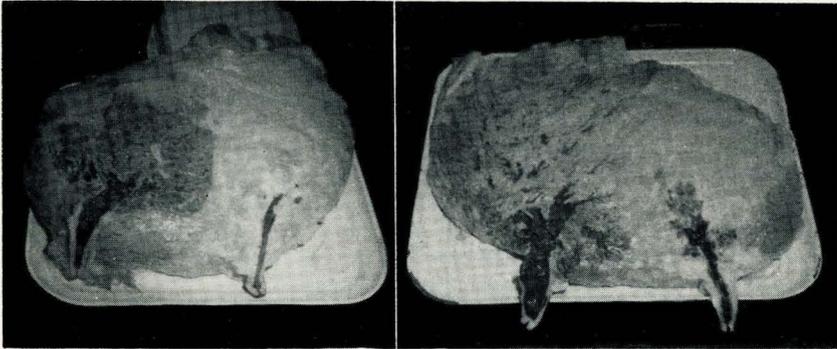


Fig. 2. Longitudinal section of left and right halves of udder from cow No. 472.

616, had a history of mild but frequent flare-ups in which flaky milk was produced for periods of a few days. Both *Streptococcus agalactiae* and staphylococci had been cultured from the milk. Penicillin infusions were not effective in removing these organisms from the quarters. Little information was obtained by palpation except for a slight hardness of the tissue at the base of the teats and a rather meaty type of udder. The photographs show that the dye solution penetrated almost to the top of the left side indicating very little damage. The right half shows some white, fibrous tissue extending from the top and intermingling with secretory tissue, covering the upper one-third of the rear quarter and, to a lesser extent, in the front quarter. Milk ducts leading to the teat cisterns were not affected, and although the infection was present for two or more years, the volume of milk produced was not greatly reduced. The fact that the fibrosis was rather high in the udder (and presumably the causative bacteria were there also), suggests a possible explanation for the failure of the penicillin infusions to free the udder of these bacteria: the penicillin in sufficient concentration may not have penetrated this far.

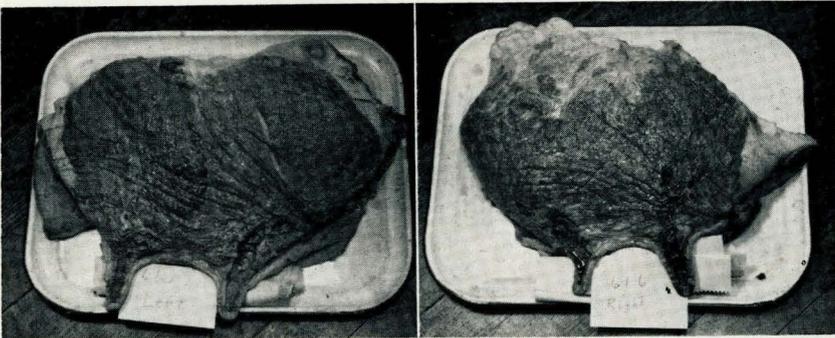


Fig. 3. Longitudinal section of udder through teats (cow No. 616) Upper portion of right half shows some fibrous tissue.

Bacteriological observations. During the course of the investigation, identification studies were made on 322 cultures of streptococci. These cultures were obtained by isolating hemolytic colonies developed from streaked, incubated milk samples on blood agar plates. The colonies were transferred to tryptose phosphate broth and after 24 hours of incubation at 37° C., smears were prepared for microscopic examination. Length of chains was observed, and those containing typically more than six cells were recorded as long chains. The last two columns of Table 5 show the percentages of cultures of each species having long and short chains. It will be noted that 93.4 per cent of the *Streptococcus agalactiae* cultures existed in long chains and that 74.1 per cent of the comparatively non-pathogenic *Streptococcus bovis* were also in long chains.

Approximately half of the 322 cultures of streptococci were accumulated during the first six months before the identification studies were begun. During this time they were preserved on agar slants and were transferred periodically. Unfortunately, records were not kept of chain length of the streptococci in the incubated milk samples until the identification work was started. Records which are available for a direct comparison of chain lengths in broth cultures and in incubated milk samples show that 58.6 per cent of the 82 cultures of *Streptococcus agalactiae* occurred in long chains in milk and 98.8 per cent in broth. The 35 cultures of *Streptococcus fecalis* showed 65.7 per cent long chains in milk and 42.8 per cent in broth. In the case of *Streptococcus bovis* 15.4 per cent of the 13 cultures were observed as long chains in milk and 84.6 per cent in broth. This evidence indicates that the criterion of long chains is unreliable as a sole means of identifying *Streptococcus agalactiae*.

The data in Table 5 also show the influence of the different species of streptococci on the numbers of leucocytes in the milk. These values are expressed as the percentage of streptococci cultures of each species, associated with each of the leucocyte number groups. As has been mentioned previously, leucocyte numbers of less than 1,000,000 per ml. of milk were considered normal and indicative of the absence of udder infections. It will be noted that within this group the percentages of streptococcus species varied from 16.4 for *Streptococcus agalactiae* to 37.1 for *Streptococcus fecalis*. Although these two species were undoubtedly more pathogenic than the others listed (and for this reason lower percentages might logically be expected to be associated with normal leucocyte numbers) it is probable that many of these cultures represented transient infections or possible contaminants.

Table 5. Relationships of species of streptococci to length of chains and numbers of leucocytes in quarter milk samples.

Species	Number of cultures	Number of leucocytes per ml. of milk				Per cent of*	
		Less than 1,000,000	1,000,000 to 5,000,000	5,000,000 to 10,000,000	More than 10,000,000	Long chains	Short chains
All streptococci	322	116	36	69	101	64.9	35.1
		Each species as per cent of group					
<i>Streptococcus agalactiae</i>	167	16.4	50.0	50.9	87.1	93.4	6.6
<i>Streptococcus fecalis</i>	72	37.1	22.2	15.9	9.9	29.2	70.8
<i>Streptococcus bovis</i>	27	17.2	0.0	8.7	1.0	74.1	25.9
Other species	56	29.3	27.8	14.5	2.0	21.4	78.6

*24-hour broth cultures.

There was a sharp increase in the percentages of *Streptococcus agalactiae* cultures with increasing numbers of leucocytes. In the group containing more than 10,000,000 leucocytes per ml. of milk 87.1 per cent of all the streptococci found were *Streptococcus agalactiae*, while 9.9 per cent were *Streptococcus fecalis*. If increasing numbers of leucocytes may be assumed to indicate an increase in the extent or severity of the infection, it is evident that *Streptococcus agalactiae* was responsible for the great majority of mastitis cases due to streptococci found in this herd and that *Streptococcus fecalis* was the next worst offender.

The ability of staphylococci to coagulate plasma was determined on 98 cultures. Of this group 59 were positive and 39 negative. Only 10 were involved in clinical mastitis; however, nine or 90 per cent, of these were coagulase positive. There were 40 cultures which had been obtained from subclinical mastitis. In this group 55 per cent were coagulase positive and 45 per cent negative. The remaining 48 cultures were obtained from milk samples in which there was no other evidence of mastitis; 58.4 per cent of these were coagulase positive and 41.6 per cent negative. From the limited number of mastitis cases caused by staphylococci, it appears that those cultures which coagulated plasma were more pathogenic than those which did not.

Housing of dairy cows as a factor in the control of mastitis. It was well known from previous experience that the main barn, in which the cows were kept in stanchions, was cold and drafty on windy days during the winter months. It was considered that this condition may have been an important contributing factor to the mastitis history of the herd. To determine the influence of these drafty conditions on the cases of mastitis already present and on the development of new cases, about one-third of the herd was removed from the stanchions and placed in pens in wings of the same barn. An effort was made to select these cows for the pens in such a manner that they would be as nearly comparable in every respect as possible to those left in the stanchions. An examination of the records shows that the ratio of mastitis quarters to normal quarters was 1:10 for the cows in the pen barn and 1:9.6 for those in the stanchions, at the beginning of this phase of the experiment, Nov. 15, 1945.

It was somewhat colder in the north wings of the barn where the cows were kept in pens, than in the main part of the barn where the other group was stanchioned. The cows in the pens were free to move about and thus it was possible to avoid drafts to some extent, at least. Manure was allowed to accumulate in the pens which kept the temperature of floors warmer than those in the stanchions, which were kept clean. Bedding was placed in the pens at least twice daily, but it was not possible to keep these cows as clean as those in the stanchions. Ventilation was not adequate in either the main part of the barn or in the wings, and humidity was excessive much of the time.

This experiment was carried through the winter season until March 15, 1946. The principal results are summarized in Table 6. The data pertaining to the development of new cases of mastitis and improvement of existing cases, includes a comparison of 10 cows in the pens and 25 in the stanchions. Cows treated with penicillin from September 1945 to March 15, 1946, are not included in this portion of the table. With regard to the development of new cases of mastitis the data indicate a greater proportion of the mild, subclinical cases in the pen barn group. Likewise, although not shown in the table, there was an increase in the

Table 6. Comparison of pens and stanchions in the control of mastitis.

Description	Cows in pens	Cows in stanchions
Development of new cases		
Clean quarters to subclinical mastitis	5	5
Clean quarters to clinical mastitis	0	7
Total of new cases	5	12
Ratio of new cases to total quarters	1:8	1:8
Improvement of existing cases		
Clinical to clean	0	4
Clinical to subclinical	0	1
Subclinical to clean	5	6
Total improvements	5	11
Ratio of improvements to total quarters	1:8	1:8.7
Number of flaky milk samples (strip-cup)		
following sudden decreases in temperature	4	22
Ratio of flaky samples to quarters	1:12	1:6

number of cultures of bacteria (usually associated with mastitis) obtained from this group of cows. Perhaps these findings may be explained on the basis of poorer sanitation in the pens and the freedom of the cows to move around and spread the bacteria from one place to another in the bedding. On the other hand, all of the more severe clinical types of mastitis developed among the cows kept in the stanchions.

In November when the cows were separated and this experiment was begun, only one had clinical mastitis; she was placed in the pen group. During the winter, seven quarters developed clinical mastitis among the stanchioned cows (none in the pen-barn group); however, infection was overcome in four of these and one showed improvement to the extent that flakes were no longer found (sub-clinical).

It was a frequent observation, during this season, that when the temperature dropped suddenly, usually accompanied by strong, northerly winds, that the reports of the strip-cup tests for the following days showed increased numbers of flaky milk samples. The data in the lower portion of Table 6 give a comparison of the strip-cup tests obtained following 12 periods in which the minimum daily temperatures dropped from 20° to 42° F. (within 1-3 days). Those cows which gave flaky milk almost continuously were excluded. Included were 12 cows in the pens and 33 in the stanchions. The results substantiate the above mentioned observation, namely, that a greater ratio of flaky samples was produced by the cows in the stanchions—in fact, twice as many. This is attributed to the conditions prevailing in the stanchion part of the barn and to the fact that the cows in this group could not move away from drafts.

Since it was not possible to keep cows in the pen barn during the winter of 1946-47, this phase of the experiment was discontinued. The daily strip-cup tests from the cows in the stanchions, did not, however, show increased numbers of flaky samples following sudden decreases in minimum daily temperature. The only explanation that can be offered for this difference is that there was less clin-

ical mastitis in the herd during the latter season. A comparison of the strip-cup tests at the beginning of each season shows that flakes were found in the samples from 11 quarters in the month of November 1945, and in six quarters during the corresponding month of 1946.

Discussion

The experimental work herein reported was concerned primarily with only two phases of the mastitis problem. The recent book, *Bovine Mastitis*, edited by Little and Plastridge (5) gives a good over-all picture of the disease.

Although a comparison of diagnostic procedures was not one of the main purposes of this investigation, some different methods were used and these results have been presented. They agree in general with those of other investigators, in that a combination of methods gives a more nearly accurate diagnosis than any one used singly. It is considered that satisfactory diagnoses were obtained with these procedures: (1) daily strip-cup tests, (2) udder palpations, (3) microscopic examination of incubated fore milk samples from each quarter and (4) bacteriological examination of milk samples on blood agar plates.

Penicillin was used to treat mastitis in practically all stages of the disease. The majority of these cases were caused by streptococci and staphylococci although a few were caused by coliforms and other species. Nearly all treatments were given to lactating udders. Very few infusions were made during the dry period. The data show that *Streptococcus agalactiae* was found in 31.9 per cent of all mastitis cases observed in this herd from May 1945 to June 1947. Penicillin infusions were effective in 70.3 per cent of all the *Streptococcus agalactiae* cases treated. It is probable that this value would have been higher if only those cases usually deemed suitable for treatment had been selected. Since many of the infusions were administered to quarters showing marked fibrosis, it is not surprising that some were ineffective.

The results of treatment, by infusion of penicillin into infected quarters, show that some of the apparently hopeless cases were freed of *Streptococcus agalactiae* as well as a much higher percentage of those with comparatively little udder damage. Some of these treatments which seemed to be successful were of a temporary nature; that is, within a few months the quarter became reinfected with the same kind of bacteria. These reinfections were more prevalent, and occurred sooner, when the original treatments had been given to udders showing considerable damage, than to those showing less damage. There were some exceptions to this general observation just mentioned, the most notable being the case of cow No. 497. Herd improvement records for five consecutive lactations of this cow are given below:

Age at beginning of lactation		Times milked	Length of record days	Actual production		Holstein mature equivalent (2X, 305 days)
Years	Months			Milk lbs.	Fat lbs.	Fat lbs.
2	11	3X	361	11,249	427.5	416.2
4	1	2X	299	11,109	355.6	393.0
5	1	2X	365	10,855	336.3	291.9
6	10	3X	365	20,186	702.9	615.0
8	1	3X	114	7,924	296.1	255.4*

*Partial lactation period.

Herd records available prior to this study, show that this cow had some mastitis during her second lactation. Early in her third lactation the two front quarters became infected with *Streptococcus agalactiae*. They produced very little milk and this was thick and stringy. These quarters shrunk to about half their normal size. They were treated with penicillin, and the bacteria causing the mastitis were eliminated. During the remainder of this lactation period very little milk was produced by these quarters, but it was normal in appearance. This cow was given a rest period of four months and when she freshened these quarters had a normal appearance, and judging from the production records, they produced at a normal rate. Also, these quarters have remained free of mastitis infection to date (approximately three years after treatment).

During these experiments some cows with clinical mastitis of long duration and with extensive udder damage (but high producers prior to these infections) were treated and re-treated even though the treatments were effective for only a few months. It was thus possible to keep them in the herd long enough to get one or two calves and, by the use of treatments, to minimize the danger of spreading the disease, although the reduction of mastitis proceeded at a slower rate than would have been true with more severe culling. Potentially less valuable animals, as well as those with extensive udder damage, were culled first. During the two years of the experiment 15 cows were culled because of mastitis. At the end of the period eight cows remained in the herd which had shown symptoms of mastitis throughout the experiment. Although this culling was not as severe as would be recommended in the usual mastitis control program, it played an important part in the reduction of the total infection in this herd and allowed more calves to be raised for replacements.

Penicillin infusions against staphylococci and coliform bacteria were relatively ineffective. Treatments for species of streptococci other than *Streptococcus agalactiae* were 100 per cent successful (only eight cases). On the other hand, 38 cases of mastitis caused by this group of organisms were observed, but not treated; 65.8 per cent of these recovered spontaneously. With only a few exceptions, the mastitis cases caused by these streptococci were mild and subclinical in nature.

No adverse reactions were observed at any time as a result of the penicillin infusions. Production records for the three days previous to the treatment and the three days following it were obtained for 95 cases. There was a decrease of only 0.27 pounds in the daily milk production following the infusions.

Throughout this investigation all of the cows were machine-milked in a milking parlor with comparable conditions for each cow and group. Special efforts were made to devise a sanitary milking procedure which would be as nearly fool-proof as possible. The udders and teats were washed with paper towels dipped in a warm solution containing 200 p.p.m. of chlorine. The towels were used only once, thus reducing the possibilities of carrying the bacteria from one cow to another. Two sets of teat cups were used at each unit which permitted a much longer exposure time for the cups in the antiseptic solution without adding appreciably to the total time of milking. No data were obtained to show whether or not these methods were completely satisfactory. A few preliminary trials indicated that the total numbers of bacteria on the surfaces of the teat cup liners were

sharply reduced when immersed three minutes in a chlorine solution of 200 p.p.m. as compared to one-half minute immersion.

The comparison of pens and stanchions as they affect the control of mastitis has several points of interest. Cold, drafty conditions seemed to contribute to the development of clinical mastitis among the stanchioned cows to a greater extent than among those kept in the pens. Reports of the daily strip-cup tests indicated that twice as many cases of flaky milk were observed from the stanchioned group (following sharp drops in the minimum daily temperature) as compared to the group in the pens. The explanation for these results seems to be the fact that the cows in the stanchions could not escape the drafts while those in the pens had freedom of movement and could find the least drafty places to lie down. Manure was allowed to accumulate in the pens which tended to keep the floor temperature comparatively higher than that of the stanchions. This was probably an important factor in minimizing the effects of sudden changes in outside temperature.

Sanitation in the pens was not as good as in the stanchions and the data showed that mastitis bacteria, particularly *Streptococcus agalactiae*, were spread to a slightly greater extent among the cows in the pens as compared to those in the stanchions. This, of course, indicates a greater potential danger for the spread of mastitis among the cows in the pens, if environmental conditions should become unfavorable and lower their resistance to the bacteria. However, all of the cases which developed in this group were mild and subclinical and would not be noticed in the absence of bacteriological examinations.

It is always difficult to obtain an accurate estimate of the loss in production of milk due to mastitis in the herd. The production records of this herd give some indication of the seriousness of the disease. This mastitis control program was started in May of 1945. There were 53 cows in the herd during the year ending April 14, 1946. They were milked twice daily. For the year ending April 14, 1948 there were 48 cows in the herd on three-times-a-day milking. Taking into consideration the difference in number of milkings per day, the records show an increase of more than 20 per cent in fat production for the latter period. Better management practices and more comfortable quarters have undoubtedly played a part in this increased production. Another important factor is that it is now no longer necessary to cull for disease control, only. In other words, mediocre animals can be culled without depleting the herd, because it is now necessary to cull few cows for mastitis.

Summary

1. Treatment of bovine mastitis by penicillin infusion of infected quarters was carried out in the College dairy herd over a two-year period.
2. Several herd management practices, including milking machine sanitation, were given consideration as preventive measures. A comparison was made of the relative merits of pen-type barns and stanchions in the control of mastitis.
3. Diagnostic procedures included (1) classification of quarters by palpation, (2) daily use of the strip-cup, and (3) microscopic and bacteriological examination of quarter milk samples.
4. A survey of the mastitis present in the herd at the beginning of these experiments showed that 28 of 51 cows (55.0 per cent) had infections in one or more quarters. At the end of the two-year period this condition had been reduced to

31.2 per cent. During this time 15 cows with extensive udder damage were culled. Eight cows remained in the herd which had shown mastitis symptoms almost continuously during the entire period. They were kept in the herd for additional calves since they were originally high producers. If this group is excluded, only 14.6 per cent remained with mastitis.

5. Penicillin was much more effective against streptococci than against staphylococci and some other organisms which caused mastitis.

6. In 70.3 per cent of all cases (84) treated with penicillin for mastitis caused by *Streptococcus agalactiae*, the quarters were freed of these bacteria.

7. Treatments with penicillin were more effective for mild cases of short duration than for well advanced infections. Quarters treated for the first time were freed of *Streptococcus agalactiae* in 90.3 per cent of the subclinical cases and in 66.7 per cent of those in the clinical group.

8. Penicillin infusions were somewhat less effective for reinfections of *Streptococcus agalactiae* than were the first treatments. Also, those re-treated quarters did not remain free of the bacteria for as long a period as they did following the first successful treatments.

9. The comparison involving pen-barns and stanchions showed that more flare-ups of clinical mastitis occurred among the cows in the stanchions than among those in the pens.

References

1. BRYAN, C. S., R. E. HORWOOD, and C. F. HUFFMAN. A preliminary report on penicillin in the treatment of chronic streptococci mastitis. *Vet. Med.* **40**: 87-89. 1945.
2. HEIZER, E. E., GEORGE BARRETT, I. W. RUPEL, and ERNEST ZEHNER. Pen barns have certain advantages for dairy cows. *Wisc. Agr. Exp. Sta. Bul.* **469**: 57-58. 1946.
3. KAKAVAS, J. C. Penicillin in the treatment of bovine mastitis. A Preliminary Report. *North Am. Veterinarian* **25**: 408-412. 1944.
4. KLEIN, LOUIS A., DAVID W. CRISMAN, and JOHN W. MOOR. Effect of local injections of penicillin on staphylococci in the cow's udder. *Am. J. Vet. Research* **6**: 3-8. 1945.
5. Bovine Mastitis, A Symposium. Edited by RALPH B. LITTLE and WAYNE N. PLASTRIDGE. McGraw-Hill Book Company, Inc., New York. 1946.
6. MURPHY, J. M. and K. O. PFAU. The value of local injections of penicillin sodium in the treatment of *Streptococcus agalactiae* infection and mastitis of cows. *Cornell Vet.* **35**: 88-103. 1945.
7. Report of the Chief of the Bureau of Animal Industry, Agricultural Research Administration, 1944.
8. SLANETZ, L. W. and F. E. ALLEN. Treatment of bovine mastitis with penicillin. *J. Am. Vet. Med. Assoc.* **107**: 18-20. 1945.
9. UDALL, D. H. *The Practice of Veterinary Medicine*. 4th Edition. The Author, Ithaca, New York. 1943.