A STUDY OF EXPERIMENTAL STREPTOCOCCIC MASTITIS IN DAIRY CATTLE

A STUDY OF EXPERIMENTAL STREPTOCOCCIC MASTITIS IN DAIRY CATTLE

Thesis

Submitted to the Faculty of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of Doctor of Philosophy

C. S. Bryan

June, 1937

TABLE OF CONTENTS

/

A STUDY OF EXPERIMENTAL STREPTOCOCCIC MASTITIS IN DAIRY CATTLE

Streptococcic mastitis is a major dairy problem from both the economic and hygienic viewpoints. Shaw and Beam(27) reported that quarters infected with streptococci produce approximately 22 per cent less milk and 24 per cent less butterfat than the streptococcus-free quarters. Since many cows are infected in all four quarters a similar decrease in milk and butterfat production may be expected and has been observed among dairy cows. Minnett and Martin (24) studied the milk yield of mastitis infected cows and reported that the reduction of milk yield due to mastitis was 954 pounds per lactation period. More recently, White et al. (32) reported the reduction of milk yield of mastitis-infected cows as being 425 to 562 pounds per lactation period. It is obvious that the milk production of mastitis-infected cows is reduced as compared to the production of mastitis-free cows, even though investigators do not exactly agree on the extent of reduction.

Bryan and Trout (8) found that milk produced by cows with streptococcic mastitis was inferior in quality as determined by flavor, chloride content, leucocyte content, bacteria count, and methylene blue reduction time. Ninety per cent of the milk samples from infected cows in one herd were criticized as having a salty flavor, while only 14 per cent of the milk samples from a herd free of streptococcic mastitis were so criticised. These

latter animals were near the end of their lactation period when such changes may normally occur. Only 50 per cent of the streptococcus-infected cows produced a class one (good) milk as judged by the methylene blue reduction test, as compared with 98.5 per cent of the non-infected cows. Similarly, 68.5 per cent of the infected cows produced milk with a standard plate count of more than 1000 as compared with 5.8 per cent of the non-infected cows. The decreased production and lowered quality of milk produced by cows with streptococcic mastitis are sufficient reasons for emphasizing the economic side of the problem. In addition, the hygienic aspects of the problem are important.

The streptococci that cause mastitis have been found to be the cause of human infection. These include the streptococci of human and bovine origin that cause mastitis. The human infection usually takes the form of septic sore throat epidemics when human strains are concerned and isolated cases of sore throat together with further complications when streptococci of bovine origin are concerned. Brooks and Tiedeman (5) present evidence that milk from mastitis infected cows may cause human "distress", especially in babies, as a result of the toxins present in the milk. The economic and hygienic aspects of the problem are greatly emphasized when the incidence of this cattle disease is considered.

Eighty-six per cent of the herds and 26.2 per cent of all milking cows were found to be infected with streptococcic

mastitis in a survey conducted in a typical milk shed. (6). Figures dealing with the incidence of streptococcic mastitis, as reported by other investigators, are presented in the above paper. These data, together with the human health problems involved, make this one of the, if not the, most important dairy cattle disease at the present time.

The importance and wide spread of streptococcic mastitis create a big problem with respect to the control or eradication of the infection. It is essential that such recommendations be based on an understanding of the following:

1. The manner of spread of the streptococcus from cow to cow or from some other source to the cow.

2. The portal of entry of the streptococcus into the udder.

3. The time when most spread of infection occurs. A great deal of this information can only be gained from a study of experimentally induced streptococcic mastitis.

The study was undertaken to determine: (1) the portal of entry of the streptococcus into the udder, (2) the possibility of controlling streptococcic mastitis by segregation of the infected cows and the employment of sanitary procedures in handling the herd, (3) the variation in composition of the milk following infection by the streptococcus, (4) the rapidity of the reduction in the quality of the milk after infection, (5) the value of the various tests in the detection of experimentally induced streptococcic mastitis (a) direct-microscopic test(7) (b) indirect tests (20) and (c) physical examination of

the udder (28), (6) the normal cellular and hemoglobin content of the blood and variations following exposures and infection, and (7) the histopathology of the udders when the duration of infection is definitely known.

Methods

This study covered a period of two years involving two groups of three cows, two cows and a control being studied each year. All cows were free of tuberculosis, Bang's disease and streptococcic mastitis when placed on experiment and remained free of tuberculosis and Bang's disease throughout the period of the study. The cows were housed in a good barn with concrete floors in metal stanchions. A sufficient number of windows provided adequate light in the barn.

The control cow was separated from the two experimental cows by a three foot wide concrete walk. The barn was thoroughly cleaned by ~~chemical~~ mechanical means and whitewashed after removal of one group of animals and prior to being used for the second group.

The routine sanitary measures employed in the barn were: 1. to milk the control cow before the two cows exposed to the streptococcus, 2. to remove manure from and give clean bedding to the control cow first each time, 3. to keep the barn clean at all times, and 4. to keep a film of lime on the floor at all times.

As already indicated two cows were exposed and the third cow was the control. The exposures and frequency of exposures in addition to the order of exposures follow:

(a) Fed - five cubic centimeters of a 24-hour broth culture twice daily for one month.

(b) Subcutaneous - 10 cubic centimeters of a 24-hour broth culture injected at a point just posterior to the joint of the withers, three times a week for one month (a total of 12 injections).

(c) Intravenous - 10 cubic centimeters of a 24-hour broth culture three times a week for one month (a total of 12 injections) into the jugular vein.

(d) Dip teats - into a 24-hour broth culture three times a week for one month (a total of 12 exposures).

(e) Injure teats - lacerate teats with scalpel (sufficient to draw blood) then dip teats for one month(a total of 12 exposures). The first year, following infection of the two cows exposed, the control cow was exposed by dipping the teats without previous exposure to the streptococcus by the oral, subcutaneous and intravenous routes. During the second year the natural exposure in the barn resulted in infection of the control cow, therefore she was not experimentally exposed.

The quanitative determination of the milk for per cent of casein nitrogen and per cent of whey protein nitrogen were made following the technic of Van Slyke and Bosworth (29). The Kjeldahl-Gunning method (1) was used to determine the total nitrogen content of milk. The casein was precipitated in the undiluted milk at its isoelectric point and separated from the whey by filtration. An aliquot portion of the filtrate was

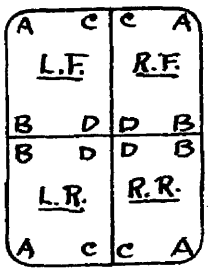
analyzed, in duplicate, for the per cent of whey nitrogen. The per cent of casein nitrogen was obtained by difference; subtracting the whey nitrogen from the total nitrogen content of the milk.

The curd tension of the milk was determined according to the technic of Cole (9). The leucocyte content, pH (thybromol test), and per cent chlorides were determined by the methods of Hucker (20). The methylene blue reduction test and standard plate count were made according to standard methods of the American Public Health Association (2). The presence of the streptococcus was determined by the microscopic test (7). The technic of Udall (28) was followed in making physical examination of the udder. Blood cell counts were made by following the technic of Kolmer and Boerner (22). A Sahli hemoglobinometer was used for making hemoglobin determinations.

The hemoglobinometer tube was calibrated on the basis of 14 grams of hemoglobin per 100 cubic centimeters of blood as 100 per cent of hemoglobin.

In each case the above determinations were made three times a week throughout the experiment. The chemical determinations were made in duplicate. All figures were averaged to obtain the figures presented in the Tables. In most cases the spread of the figures averaged was very small; in some cases, especially after infection, the spread of figures averaged was greater. On this account, the spread of such figures is indicated by giving the average together with the outer limits of spread of the figures.

The three cows of the first year were slaughtered one to two and one-half months after infection and the udders examined in gross and histologically. The udder was divided into planes and quadrants of each quarter for convenience in recording the location of the pathological conditions observed on gross examination and in identifying tissues removed for microscopic study. Plane one refers to the upper part of the udder or that nearest to the body of the cow, plane two refers to the middle part and plane three to the lowest portion of the udder. The quarters of the udder are designated R.R. for right rear, R.F. for right front, L.F. for left front, and L.R. for left rear as viewed from behind the cow. Imaginary quadrants were designated in each plane according to the following scheme:



Thus RR1B refers to a part of the right rear quarter of plane one and quadrant B. The tissues for microscopic study were fixed in Zenker's solution, embedded in paraffin and stained by eosin and hematoxylin.

Results and Discussion

1. The streptococcus employed in this study - Streptococcus agalactiae.

The streptococcus used in this study was isolated from a

chronic case of mastitis three months prior to its use in inducing mastitis and identified as Streptococcus agalactiae. Its characteristics can best be given as stated by Hansen (16). "It has been concluded that the preferable name for this streptococcus is Strep. agalactiae, Lehmann and Newmann (1896). Its most significant characteristics which differentiate it from other closely related species of streptococci are its acid production from maltose, sucrose, and dextrin with no acid production from mannitol, sorbitol, arabinose, xylose, raffinose, inulin, and amygdalin. It attacks sodium hippurate, does not slipt esculin, and produces either a viridans or narrow-zone type of hemolysis on blood agar plates. It curdles milk before reduction, reduction progressing afterwards slowly from the bottom upwards." This culture was of the gamma type on blood agar. These characteristics are essentially those reported by Englebrecht (14) and Williams (35) for Strep. agalactiae.

2. The portal of entry of the streptococcus into the udder.

Exposure of the experimental cows were made using the above organism. All cows were at the beginning of a lactation period; in all cases being either in their first or second lactation periods. The first month of study was a control period (no exposure) followed by exposures for one month per os, followed in turn by subcutaneous, intravenous, dipping teats and if not yet infected, the teats were lacerated and then dipped into a broth culture of the organism. The results of this work are presented in Table I.

Table I. The portal of entry of the streptococcus into the cows udder as indicated by either infection or no infection following experimental exposure.

COW	MONTH OF STUDY 1	2	3	4	5	6	7
	Method of Exposure						
	None	Fed	Subcu-taneous	Intra-venous	Dip Teats	Injure Teats	Dip Teats
G 1935-36	-	-	-	-	-	+	0
H 1935-36	-	-	-	-	+	0	0
1 1936-37	-	-	-	-	+	0	0
2 1936-37	-	-	-	-	+	0	0
GH(control)1935-36	0	0	0	0	0	0	+[1]
3(control) 1936-37	0	0	0	0	0+[2]	0	0

Legend: + = infection, - = no infection, 0 = no experimental exposures.

1. Control of 1935-36 remained negative throughout period of exposure of the two experimental cows, after their infection the control cow was exposed by dipping the teats.
2. Control of 1936-37 became infected as a result of exposure to the streptococcus due to being stabled in the same barn with the experimental cows.

Three of the exposed animals (H, 1 and 2) developed streptococcic mastitis as a result of dipping the teats into the culture. Infection followed seven to nine experimental exposures made three times a week. One other cow (G) developed streptococcic mastitis only after dipping of the lacerated teats. In this case infection

followed one exposure. This indicates the part played by injuries of the udder on teats in the spread of streptococcic mastitis and the necessity for properly caring for such injuries.

These results confirm the work of Kitt (21) and Maass (23) in which mastitis could not be produced by inoculation per os or the subcutaneous and intravenous routes into experimental animals. Kitt (21), Rosell (26), Edwards,(13), Davis and Capps (10), Maass (23) and Hadley and Frost (15) produced streptococcic mastitis by introducing the streptococcus into the teat canal or into the udder. It is significant, concerning the results here reported, that the exposure of the uninjured and the injured teats resulted in infection, for there are many opportunities in the routine stabling and handling of cows for such contaminations and injuries to occur.

3. <u>The control of streptococcic mastitis by sanitary measures and segregation of infected cows.</u>

After the two experimental cows were infected, cow GH, which served as a control cow during the first year, was exposed to the streptococcus by dipping the teats into the culture, and became infected following three such exposures. The fact that the control cow (G) of the 1935-36 period was protected from the streptococcic mastitis present in the herd by the pursuance of sanitary procedures seems significant. The data on Table I show that cow 3, control cow for the 1936-37 period, became infected without experimental exposure even though sanitary procedures were routinely employed to prevent

the spread of streptococcic mastitis. These results indicate the difficulty of controlling the infection by segregation of the infected cows apart from the negative cows in the same barn and the employment of sanitary procedures in handling the dairy herd. That they are of value is demonstrated by the freedom of infection of the control cow of the 1935-36 period prior to her exposure by dipping of the teats.

4. The variation in composition of milk following infection.

The presence of mastitis streptococci in the udder is not a normal condition, therefore one might expect variations in the composition of milk as a result of interference with the normal function of the udder. The data of the casein nitrogen, whey nitrogen, and curd tension, leucocyte count, pH and per cent of chlorides prior to and after infection of each cow are presented in Table 2. In each case these values were normal, with slight variations, prior to infection. In four of the six animals studied, the whey nitrogen increased and the casein nitrogen decreased after infection. At the same time the curd tension decreased from its normal value to a point where frequently no curd was formed. Hill's (18) standard 30 grams or less indicating a soft curd and more than 30 grams a hard curd was used to classify the curd character of the milk. The curd character was fairly uniform during the period prior to streptococcus infection, and agrees with the results reported by Berry (4) and Ridell et al. (25). Weisberg et al. (30) report that the concentration of whey

constituents wholly or separately exhibits little or no influence in differentiating a soft curd from a hard curd milk. They did find that a high concentration of casein was associated with a hard curd milk and a low concentration with soft curd milk. Doan and Welch (12) showed that the curd tension values of milk from infected udders are lower, compared with the casein content, than a normal milk. Welch and Doan (31) summarize their work thus — "soft curd is not evidence that milk comes from an udder showing disease but udder infections do cause a lowering of curd tension due to a lower casein content." The data of Table 2 indicate that the decrease in hardness of curd from its normal value in each case developed coincident with a decrease in casein nitrogen and an increase in whey nitrogen.

The pH, chloride content, and leucocyte content of the milk produced by the cows after infection varied considerable from time to time; not all of these conditions were abnormal at any one time unless the milk was flaky or gargety. Some of these test reactions were abnormal continuously after infection by the streptococcus. On account of the variation of these test results from cow to cow or from time to time on the same or different cows, the pH, chloride and leucocyte contents of the milk cannot alone be used accurately to detect cases of streptococcic mastitis.

Table 2. The composition of milk prior to infection and after infection for each one of the six cows of the experiment.

	Month of Study						
	1	2	3	4	5	6	7
	Cow G						
	Prior to Infection					After Infection	
Percent of casein N	0.43	0.44	0.44	0.445	0.43	0.41	0.36
Percent of whey N	0.105	0.11	0.11	0.11	0.11	0.112	0.18±0.07
Curd tension	40±10	45±8	48±11	40±14	38±12	45±10	20±20
Leucocyte content	50T	85T	70T	70T	75T	100T±30T	40M± 40M
pH	6.2	6.6	6.7	6.6	6.6	6.6	7.3
Percent chlorides	0.14	0.14	0.14	0.14	0.14	0.14	0.19
	Cow H						
	Prior to Infection				After Infection		
Percent of casein N	0.31	0.32	0.33	0.32	0.32	0.33	0.30±0.04
Percent of whey N	0.09	0.08	.079	0.08	0.08	0.08	0.10±0.02
Curd tension	28±5	32±10	30±8	24±12	20±12	28±10	10±20
Leucocyte content	100T	100T	60T	100T	64T	75T	5M±4M
pH	6.6	6.7	6.7	6.7	6.7	6.6	7.1±2.0
Percent chlorides	0.14	0.14	0.14	0.14	0.15	0.15	0.18
	Cow I						
	Prior to Infection				After Infection		
Percent of casein N	0.38	0.40	0.388	0.40	0.40	0.38	0.36
Percent of whey N	0.095	0.10	0.105	0.10	0.11	0.115	0.11
Curd tension	40±15	35±10	48±12	45±9	32±10	30±12	36±7
Leucocyte content	100T	70T	110T	60T	10M±10M	8M± 3M	4M± 4M
pH	6.6	6.6	6.7	6.7	7.1	6.9	6.7
Percent chlorides	0.14	0.14	0.14	0.14	0.19	0.18	0.18
	Cow 2						
	Prior to Infection				After Infection		
Percent of casein N	0.31	0.305	0.32	0.34	0.34	0.30	0.30
Percent of whey N	0.10	0.09	0.10	0.10	0.11	0.116	0.11
Curd tension	28±5	28±8	35±10	38±12	18±12	5±15	12±10
Leucocyte content	100T	70T	95T	130T	2M±2M	800T±600T	200T
pH	6.6	6.6	6.7	6.6	6.9	6.7	6.6
Percent chlorides	0.14	0.14	0.14	0.14	0.17	0.16	0.15

Table 2 (continued)

	Cow GH						
	Prior to infection						After Infection
Percent of casein N	0.32	0.35	0.33	0.32	0.315	0.32	0.30
Percent of whey N	0.10	0.105	0.10	0.10	0.102	0.103	0.09
Curd tension	25±8	30±12	30±8	33±4	28±11	33±15	18±17
Leucocyte content	60T	80T	120T	100T	85T	120T	2M±2M
pH	6.7	6.7	6.6	6.6	6.7	6.6	6.7
Percent chlorides	0.14	0.15	0.14	0.15	0.15	0.15	0.15
	Cow 3						
	Prior to infection				After Infection		
Percent of casein N	0.40	0.39	0.395	0.388	0.38	0.37	0.38
Percent of whey N	0.105	0.108	0.11	0.11	0.118	0.12	0.114
Curd tension	35±10	28±15	30±10	39±18	20±18	5±10	20±12
Leucocyte content	100T	70T	120T	80T	6M±5M	4M±2M	5M±4M
pH	6.6	6.6	6.7	6.6	7.4	7.2	7.0
Percent chlorides	0.14	0.14	0.14	0.14	0.19	0.20	0.18

5. The reduction in the quality of milk produced by infected cows.

The chemical and cellular composition of milk are important because they influence the bacteriological quality and the flavor and odor which are important from the consumer's standpoint. The standard plate count of the milk from each cow was low prior to infection as indicated by the data of Table 3; in each case within from one to three months after infection the count increased to well over 1000. This demonstrates the desirability of having mastitis free cows for high quality milk production. The methylene blue test rating was class one in each case prior to infection; during this time the leucocyte content of the milk was less than an average of 130,000 per cubic centimeter. Following infection four of the cows exhibited a poor methylene blue test rating (class 2, 3 or 4) and at the same time had an increased leucocyte content of the milk ranging from 2,000,000 to 8,000,000 per cubic centimeter. An increase in leucocyte content has been found to be responsible for the reduction of the methylene blue, thereby causing such milk to be classed as two, three or four (11). In two of the six cows the decrease in quality of milk, as measured by the standard plate count and methylene blue reduction test, did not occur until from one to three months after infection by the streptococcus while the other four cows exhibited the decrease in quality within the first month. Therefore, a cow with streptococcic mastitis will produce milk of a lower quality as

Table 3. The Quality of Milk Produced by Cows before and after Infection.

	Month of Study						
	1	2	3	4	5	6	7
	Cow G						
	Prior to Infection					After Infection	
Standard plate count	100	150	150	150	200	150	1200±400
Meth. blue class	1	1	1	1	1	1	3
Leucocyte content	50T	85T	70T	70T	75T	100T±30T	40M±40M
	Cow H						
	Prior to Infection				After Infection		
Standard plate count	110	125	100	150	150	150	5000±1000
Meth. blue class	1	1	1	1	1	1	3
Leucocyte content	100T	100T	60T	100T	64T	75T	5M±4M
	Cow I						
	Prior to Infection				After Infection		
Standard plate count	200	230	200	300	2000±1700	2200±500	2800±200
Meth. blue class	1	1	1	1	4	3	3
Leucocyte content	100T	70T	110T	60T	10M±10M	8M±3M	4M±4M
	Cow 2						
	Prior to Infection				After Infection		
Standard plate count	150	200	280	280	400	3000±1000	2800±600
Meth. blue class	1	1	1	1	3	2	2
Leucocyte content	100T	70T	95T	130T	2M±2M	800T±600T	200T
	Cow GH						
	Prior to Infection						After Infection
Standard plate count	100	150	170	150	150	100	900±200
Meth. blue class	1	1	1	1	1	1	2
Leucocyte content	60T	80T	120T	100T	85T	120T	2M±2M
	Cow 3						
	Prior to Infection				After Infection		
Standard plate count	200	300	250	200	850±200	1800±400	2000±180
Meth. blue class	1	1	1	1	3	2	2
Leucocyte content	100T	70T	120T	80T	6M±5M	4M±2M	5M±4M

measured by the above tests and the decrease may not immediately follow infection by the streptococcus.

6. The value of the various tests in the detection of experimentally induced streptococcic mastitis.

The variations in the composition of milk resulting from infection have been utilized as the basis of the indirect tests for mastitis. Similar variations in leucocyte content, chloride content, pH (thybromol test) and physical appearance have been noted in the milk of normal cows near the beginning or the end of the lactation period, the cows remaining negative to the microscopic test and giving no evidence of mastitis on physical examination of the udder. Such variations in the milk of normal cows make difficult the interpretation of the indirect tests for mastitis.

The data of Table 4 present the results of the various tests employed, i.e. the direct test-microscopic, indirect tests-leucocyte and chloride content, pH and the physical appearance of the milk and the physical examination of the udder of each cow following infection. In all cases the test results were negative or normal prior to infection by the streptococcus. The first evidence of infection was a positive microscopic test, which detected the presence of the streptococcus. In the six cows of this study detectable scar tissue developed during the first month of infection in three cows and in the second month in the remaining three cows. These were followed in decreasing order of efficiency in detecting streptococcic mastitis by the

Table 4. The efficiency of the various tests in detecting experimental streptococcic mastitis in six cows.

Examination	Months following infection when test reaction became positive		
	1	2	Not in 2*
Microscopic test	6	0	0
Physical exam or udder	3	3	0
Leucocyte content	4	1	1
Chloride content	3	1	2
Thybromol test (pH)	3	1	2
Physical exam of milk	2	0	4

*The cows were either slaughtered or study on them was discontinued after the two months period.

leucocyte content, chloride content, thybromol (pH) and the physical appearance of the milk. The microscopic test and the physical examination of the udder are of greatest value in the early detection of streptococcic mastitis, and the results of these two tests remain constant after infection while the results of the indirect tests vary, between negative or positive from time to time.

7. The cellular and hemoglobin content of bovine blood.

Erythrocyte and leucocyte counts were made according to the methods listed by Kolmer and Boerner (22) on citrated blood from each animal. Blood smears were made on cover glass and stained with Hastings stain for differential leucocyte counts. The counts did not vary significantly from time to time or during the different exposures to the streptococcus. Typical counts are presented in Table 5 for each one of the six cows. These normal counts agree with those reported by Hayden and Fish (17) and Holm (19). Hemoglobin determinations, were made by these investigators, using the Tallquist method; on this basis they found from 75 to 92 per cent of hemoglobin in cow's blood. The Sahli hemoglobinometer was used to determine hemoglobin content of the blood in this investigation; the amount was lower as indicated by an average of from 10.25 to 10.5 grams per 100 cubic centimeters of blood and from 73 to 75 per cent as compared with the values reported by Hayden and Fish, and Holm. The data confirm the work of Holm that bovine blood remains unaltered by mastitis infection and in addition indicate that experimental exposures do not significantly alter

Table 5. The cellular and hemoglobin content of the blood before and after infection (no marked variation).

	COWS					
	G	H	I	2	GH	3
Erythrocytes per cu. mm.	6,300,000	6,800,000	6,000,000	6,500,000	6,500,000	6,300,000
Leucocytes " " "	8,800	8,600	8,800	9,000	8,500	8,400
Differential -						
Eosinophiles	3	4	3	3	2	4
Basophiles	2	2	2	3	2	4
Stab neutrophiles	5	4	5	5	3	2
Neutrophiles	29	30	24	27	31	28
Lymphocytes	57	57	62	58	58	59
Monocytes	4	3	4	4	4	3
Grams hemoglobin per 100 c.c. of blood	10.5(75%)	10.25(73%)	10.5(75%)	10.5(75%)	10.25(73%)	10.25(73%)

its cellular and hemoglobin content.

8. Gross pathology and histopathology of the udders.

Gross examinations and histopathological studies were made of the udders of the three cows used in the experiment in 1935-36. They are designated as G (gurnsey), GH (gurnsey-holstein) and H (Holstein). All three lactating cows were free or tuberculosis, Bang's disease and streptococcic mastitis when placed on experiment, and remained free of the first two diseases throughout this study. They were of age three and one-half to four and one-half years. The experiment was conducted during the first lactation period of G and during the second lactation period of cows GH and H. The cows were slaughtered three hours after being milked in the seventh month of their lactation period. The udders were examined in gross and materials removed and placed into Zenker's solution within three hours after slaughter.

Cow G Gross examination - The left half measures 35.5 centimeters anteroposteriorly, 12.5 centimeters medio-laterally and 20,3 centimeters dorso-ventrally. Upon incision into the three planes the tissue of L R 2 D and C is fleshy or fibrotic. The appearance of L F 2 A and B indicates the presence of very little functional parenchyma. In the third plane the interlobular connective tissue is very prominent.

The right half measures 35.5 centimeters antero-posteriorly, 11 centimeters medio-laterally and 20.3 centimeters dorso-ventrally Upon cutting, the secretion that exudes is like a watery milk. The interlobular septa are uniformly prominent in all planes of

the R R and R F quarters. The appearance or R R 2 B and R F 1 B indicate the presence of either considerable fat or products of exudation.

Microscopic findings - In the first plane; though mostly functional, there are many foci of polymorphonuclear infiltration. Also there are numerous mononuclear leucocytes in the interstitial tissue. There are many areas showing polymorphonuclear exudation into the alveoli completely filling them in places. Moderate fibrosis is evident, with greatest amount in this plane on the left side.

The second and third planes are largely non-functional and contain marked productive changes. Marked fibrosis of the interlobular and intralobular connective tissue is uniformly distributed. There are numerous small foci of acute infection characterized by fibrinous exudate, polymorphonuclear, and lymphocytic response. The foci showing exudative changes are very numerous in planes two and three of the right side.

Cow GH Gross examination - The left half measures 33 centimeters anteroposteriorly, 12.1 centimeters medio-laterally and 24.1 centimeters dorso-ventrally. Quadrants A and C of L F quarter in plane one have prominent interlobular septa. In the second plane areas of non-functional parenchyma are evident. The changes are progressively more pronounced in the third plane; here the lobules are small, interlobular septa prominent, and very little normal functional parenchyma are found.

The right half measures 33.3 centimeters antero-posteriorly, 12 centimeters medio-laterally, and 25 centimeters dorso-ventrally. The secretion that drains out upon cutting into the udder is normal in appearance. The interlobular septa of all quadrants in the first plane are prominent, parenchyma apparently normal, in the second plane very few, if any, lobules appear normal but give the appearance of marked exudation. The changes in the third plane do not differ from those noted in the second plane.

GH - Microscopic findings - In the first plane are a few scattered foci of exudation of polymorphonuclear cells. There is also infiltration of interstitial tissue by polymorphonuclear leucocytes and lymphocytes. No degenerative changes of the parenchyma are evident.

As noted on gross examination, the changes are progressively more pronounced in the second and third planes. In many lobules one-half of the alveoli are involved in acute exudate processes while the other one-half of the alveoli are not yet involved. Many of the alveoli are small and apparently inactive. The foci of interstitial infiltration and exudation of polymorphonuclear leucocytes are more numerous in the second plane with greatest number of involved areas in plane three. Moderate to marked fibrosis is present.

Cow GH - Gross examination - The left half measures 32.5 centimeters anterolaterally, 12.5 centimeters medio-laterally,

and 23.7 centimeters dorso-ventrally. Upon incision into the left half an abscess about the size of a hazel nut is found at the base of the L F teat. In L R 3 D is found a yellowish gray film homogenous area about 6 millimeters in diameter. There is evidence of necrosis, but no distinct encapsulation. Very little apparently functional tissue is found in any plane of either front or rear quarters. The interlobular septa are very prominent and the glandular tissue is discolored. The left supra mammary lymph node is hyperemic or edematous.

The right half - measures 34 centimeters antero-laterally 12.5 centimeters medio-laterally, and 28.5 centimeters dorso-ventrally. The tissue of plane one is uniform in appearance except in R R 1 B where an area 3 millimeters in diameter is found or yellowish gray color with prominent interlobular septa. In the second plane the appearance is not as uniform as the first, except near the zone separating the front and rear quarters. Otherwise, the interlobular septa are prominent. The cut surface of the udder in the third plane indicates that proliferation of connective tissue has taken place at the expense of the secretory tissue. In addition much of the tissue is discolored, giving rise to a patchy yellowish-gray appearance.

H. Microscopic findings- In the first plane are localized areas of polymorphonuclear infiltration together with a local lymphocytic response. Numerous foci of exudation of polymorphonuclear cells are present. The section from R R 1 B

represents sub-acute mastitis. The pathological picture of plane one indicates a limited focal interstitial mastitis with areas of acute exudative mastitis.

The alveoli of plane two are small and the alveolar walls are greatly thickened. Numerous small foci of infection are present, which are characterized by a fibrinous exudate with localized areas of fibrinous exudate and polymorphonuclear infiltration together with a lymphocytic response. Degenerative changes are evident throughout involving destruction of epithelium. Marked fibrosis of interstitial tissue is evident. Similar changes are present in the third plane.

The processes in plane two and three are of longer duration than those of the first plane as evidenced by the areas of fibrosis and degeneration of the epithelium. The areas of fibrosis in the alveolar walls indicate healed lesions. In addition there are several small foci of infection involving anywhere from two to approximately 20 alveoli. These are characterized by a fibrinous exudate with localized areas of polymorphonuclear infiltration with definite lymphocytic and macrophagic cellular reaction.

All 12 quarters or the three cows were infected with Strep. agalactiae for periods varying from two weeks to two and one-half months. Exudative processes are present in all 12 quarters, with productive processes in 10 and fibrosis in five quarters. The quarters not yet showing fibrosis or with no productive processes in evidence are of recent infection.

Six of the 12 quarters show sub-acute interstitial mastitis. In all quarters the processes of plane three are of longer duration than those of plane two and similarly those of plane two are of longer duration than of plane one as evidenced by the areas of fibrosis, productive processes, and degeneration of the epithlium. The alveolar walls in the udder of cow H are fibrotic; this indicates healed lesions. At the same time all four quarters of this cow show acute, exudative processes. The Strep.agalactiae infection was of two and one-half months duration.

These findings are in harmony with those reported by the Animal Pathology department of Michigan State College (3) "The results of this study indicate that, when streptococcic infection becomes established in the udder, evidence of acute and recent injury can usually be found in the udder regardless of the duration. Streptococcic mastitis may then be interpreted as a chronic but progressive process."

Summary

A typical strain of Strep. agalactiae, recently isolated from a case of chronic mastitis, was used in experimentally inducing streptococcic mastitis in normal cows, i.e. cows that were free from tuberculosis, Bang's disease, and streptococcic mastitis.

The cows subjected to experimental exposure became infected only after repeated exposure by dipping the uninjured teats into the culture or after dipping of injured teats into the culture. When the teats were injured, sufficiently to draw blood, infection developed as a result of one exposure. Repeated exposure of the cows to the streptococcus per os, or by the subcutaneous or intravenous routes did not result in streptococcic mastitis. Of the six cows used in these experiments four became infected by dipping the teats into the culture, one by dipping the lacerated teat into the culture and one (the control cow or the second year) with no experimental exposure but exposed to the streptococcus as a result of being stabled in the same barn with the infected cows.

These results indicate the difficulty of controlling streptococcic mastitis within a herd even though the infected cows are segregated from the noninfected cows and sanitary procedures are employed in handling the dairy herd. Such procedures are of value in the control of infection since the control cow of the first years study was protected from infection by these means.

The casein nitrogen, whey nitrogen, and curd tension, leucocyte content, pH and chloride content of the milk did not vary greatly prior to infection. In general, following infection the casein nitrogen and curd tension of the milk were decreased, while the other determinations gave increased values with respect to the composition of the milk.

The quality of the milk produced, following infection, was greatly reduced as measured by the standard plate count, and the methylene blue reduction test. Three of the cows gave milk with a bacteria count of greater than 1000 per cubic centimeter during the first month after infection, two in the second month and one in the third month. The methylene blue test rating of the milk dropped from class one to class two, three or four within one month after infection in four cows, within two months in one cow and within three months in one cow.

The value of the various tests in detecting experimental streptococcic mastitis, in decreasing order of efficiency are: 1. microscopic test, 2. physical examination of the udder. 3. leucocyte count of milk, 4. chloride content of milk, 5. thybromol test (pH) and 6. physical examination of the milk.

Exposures of cows to Strep. agalactiae and infection of the udder by this organism did not alter the cellular or hemoglobin content of the blood. The normal values, for the cows studied, are presented in this paper.

Exudative processes were present in all twelve quarters of the three experimental cows slaughtered, with productive processes in 10 and fibrosis in five of the quarters. In all

quarters the processes of plane three were of longer duration than those or plane two and of plane two than those of plane one. The evidence presented indicate that streptococcic mastitis may be considered as a chronic but progressive condition.

LITERATURE CITED

1. The American Association of Official Agricultural Chemists Methods of Analysis. p. 20 1930.

2. The American Public Health Association, New York City Standard Methods of Milk Analysis 1934.

3. The Annual Report of the Division of Veterinary Science, Michigan State College page 39 1936

4. Berry, M. H. Soft Curd Milk Studies 1935 Maryland Agricultural Experiment Station Bul. 388

5. Brooks, Paul B. and Walter Von D. Tiedeman. Relations of Bovine Mastitis to Human Disease, Amer. Jour. Pub. Health 27(4):338-341. 1937

6. Bryan, C. S. The Incidence of Streptococcic Mastitis Among Dairy Cattle. Vet. Med. 32(2): 70-74 1937.

7. Bryan C. S. The Microscopic Diagnosis of Infectious Mastitis Vet. Med. 30(10): 149-156 1935

8. Bryan, C. S. and G. M. Trout. The Influence of Streptococcic Infection of the Udder on the Flavor, Chloride Content and Bacteriological Quality of the Milk Produced. Jour. of Dairy Sci. 18(12): 777-792 1935

9. Cole, Alden P., New Apparatus for Determining the Curd Character of Milk. Milk Plamt Monthly (24(1): 24-25 1935

10. Davis, David J. and Joseph A. Capps. Experimental Bovine Mastitis Produced with Hemolytic Streptococci of Human Origin. Jour. of Inf. Dis. 15(1): 135-140 1914

11. Devereux, E. D. and C. S. Bryan. The Effect of Streptococcic Mastitis on the Methylene Blue Reduction test. Mich. Agr. Exp. Sta. Quarterly Bul. 18(3):161-163

12. Doan, F. J. and R. C. Welch. Soft Curd Milk Penna. Agr. Exp. Sta. Tech. Bul. 312 1934

13. Edwards, S. J. Studies on Bovine Mastitis. VI The Non-hemolytic Streptococci of Bovine Mastitis and Their Relationship to Certain Saprophytic Streptococci from Cattle. Jour. Comp. Path. and Ther. 45: 43-57 1932

14. Engelbrecht, Mildred. The Biological Characters of the Streptococcus Commonly associated with Mastitis (Strep. agalactiae)
Cornell Vet. 25(4) 313-333 1935

15. Hadley, F. B. and W. D. Frost. Experimental Bovine Mastitis.
Cornell Vet. 23: 40-46 1933

16. Hansen, P. Arne. Mastitis III. The Identity of Streptococcus agalactiae.
N.Y. Agri. Exp. Station Tech. Bul. 232 1935.

17. Hayden, C. E. and Fish, P. A. Normal Blood of Some Domestic Animals.
Cornell Vet. 18(3): 197-201 1928

18. Hill, R. L. Soft-Curd Milk
Utah Agri. Exp. Station Bul. 227 1931

19. Holm, G. E. Blood Picture in Relation to Bovine Mastitis.
Thesis for M.S. Degree Univ. of Idaho Library. 1933

20. Hucker, G. J. Mastitis: The Incidence and Detection of Sub-Clinical Streptococcus Mastitis
N. Y. Agri. Exp. Station Tech. Bul. 199 1932

21. Kitt, Th. Bacterienkunds und Pathologische Mikroskopie.
Wien, Perles, Ed 2:1899, Ed. 3. 1893. Ref. in N.Y. Exp. Station Tech. Bul. 232 1935

22. Kolmer, John A. and Fred Boerner. Approved Laboratory Technic pages 56-93. D. Appleton and Co. New York 1931.

23. Maass, Wilhelm. Lassen sich Menschenpathogene Haemolytische Streptokokken auf das Euter von Rindem Ubertragen?
Thesis for D.V.M. degree from Vet. School of Berlin 1932

24. Minett, F. C. and W. J. Martin. Influence of Mastitis and of Brucella abortus Infection upon the Milk Yield of Cows.
Jour. Dairy Res. 7(2): 122-145 1936

25. Riddell, W. H. et al. Normal variations in the Curd Tension of Milk.
Jour. Dairy Sci. 19(3): 15-7-164 1936

26. Rosell, J. M. The Bacteriology of Chronic Streptococcic Mastitis.
Cornell Vet. 21:317-333 1931

27. Shaw, A. O. and A. L. Beam. The Effect of Mastitis Upon Milk Production.
Jour. Dairy Sci. 18(6): 353-357. 1935

28. Udall, D. H. and S. D. Johnson. The Diagnosis and Control of Mastitis.
Cornell Agr. Exp. Station Bul. 579 1933

29. Van Slyke, L. L. and Bosworth, A. W. Preparation of Casein
N. Y. Agr. Exp. Station Tech. Bul. 65 1918
and Chem. Age. 32, 163 1924

30. Weisberg, S. M. et al. Laboratory Studies on the Chemistry of Soft Curd Milk
Jour. Dairy Sci. 16(3): 225-247 1933.

31. Welch, R. C. and F. J. Doan. Soft Curd Milk and Mastitis
Milk Plant Monthly. November, 1933.

32. White, G. C. et al. Chronic Bovine Mastitis and Milk Yield.
Jour. Dairy Sci. 20(4): 171-181 1937.

33. Williams, W. L. The Identification of Streptococci of Mastitis in Routine Milk Samples.
American Journal Public Health 27(5): 453 1937

ACKNOWLEDGMENT

I wish to acknowledge my indebtedness to Dr. Ward Giltner, Dr. E. T. Hallman, and Professor C. D. Ball for their interest and cooperation in this problem.