LABORATORY METHODS FOR THE DETECTION OF SUBCLINICAL MASTITIS

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Mastitis is a term that has been used roughly to describe all types of pathological conditions of the udder in cattle. This use of the term has caused considerable confusion but for this paper the terms will be defined.

The clinical or acute type of mastitis is relatively common, the etiological agent being a particular type of streptococcus. These cases have distinct symptoms including toxemia, a highly inflamed and swollen udder, and prostration of the cow, while little or no milk can be drawn from the infected quarters. A purulent discharge is usually noted and also a rise in temperature.

The more common, subclinical, type or chronic mastitis is in direct contrast to the acute type. In this case the individual may appear practically normal. There is no evidence of toxemia. Little or no rise in temperature is noted, and the udder may not be swollen or inflamed. The milk may not show any curd when strained through a fine gauze or screen, but on the contrary a watery condition may be noted. The animal may show irregular acute symptoms or flare-ups, which are cused by abnormal conditions such as high protein feeding, chilling, incomplete milking, injury to the udder, etc.

The streptococcus causing mastitis is an animal strain, and may not cause infection in humans. The animals may become infected by human strains from the milkers and when such infection does occur the symptoms are identical with the animal variety.

Within the last few years public health officials have given this problem greater attention due to the number of epidemics of septic sore throat which have been traced to infected cows. The organism responsible for these epidemics is **Streptococcus epidemicus.**

The detection of subclinical mastitis has been a problem of considerable importance for the last twenty years. Prescott and Breed, 1910, presented a method for determining body cells in milk. Breed, 1911, developed a method for direct microscopic examination.

Since this date numerous workers, Breed, Udall, Baker, Van Slyke, and notably Hucker, have investigated this problem. The accepted standard for cells in milk as given by these men was set at under 500,000 cells per cc. and other correlated factors, such as a pH above 6.8, presence of curd in strip cup, presence of Beta hemolytic streptococci and abnormal udders indicated the cow as infected.

This problem was suggested to the authors when in routine class work on market milk, samples were found to contain approximately 600,000 cells per cc. From this one would conclude that some animals were excreting large numbers of cells into the milk.

The following methods were used in the study of the milk. Two special racks were constructed, each to hold 48 test tubes. They were 12 test tubes long and 4 deep. Each rack would accommodate the milk from 48 quarters or 12 cows. Large 1"x 8" test tubes were capped with aluminum foil and sterilized. The samples were taken in the evening and examination started immediately.

Chart No. 1.

1. Milk was drawn aseptically from the cow, each quarter separately. (Chart I legend.)

CHART I

TYPICAL CROSS-EXAMINATION OF LABORATORY EXAMINATION

1 Cow No.	2 pH C	2 pH E	3 Leucocytes	4 Strip Cup	5 Pred.	6 Hemo. Orgs.	7 Bact. B. A.	8 Bact. P. A.	9 Cond. Milk	10 Remarks
1a	6.8-6.7		4,825,000	—	Strep	5,600B	12,000	5,600	O. K.	
b	6.4-6.6		none	—	Rod	100B	2,400	12,500	O. K.	
c	6.6-6.7		13,075,000	—	Strep	600B	1,500	1,500	O. K.	
d	6.6-6.7		none	—	Rod	none	100	100	O. K.	
2a	6.8-7.0		1,512,000	—	Rod	100B	100	3,100	O. K.	
b	6.9-7.1		768,000	++	Rod	100B	100	none	Th. Blue	
c	6 6-6.8		720,000	—	N. G.	none	none	1,500	O. K.	
d	7.0-7.1		1 392,000	++	Strep	100B	100	300	O. K.	
3a	7.0-6.9		4,940,000	—	Strep	1,500B	3.600	1,800	O. K.	
b	6.8-6.8		9.800,000	—	Micro	100B	700	400	O. K.	
c	6.6-6.7		16,300,000	—	Strep	1,100B	5,700	3,200	O. K.	
d										No Teat
4a	6 7-6 7		150.000	—	N. G.	none	200	none	O. K.	
b	6 8-6.8		816,000	—	Micro	100B	400	500	O. K.	
c	6.6-6.7		15.580 000	—	Strep	none	none	200	O. K.	
d	6.9-6.9		920,000	—	Strep	400B	1,700	700	O. K.	
5a	6.8-6 8		85.200	—	N. G.	none	none	none	O. K.	
b	6 7-6.7		63.000	—	N. G.	none	300	none	O. K.	
c	6.6-6.6		21,250	—	N. G.	none	none	none	O. K.	
d	6 7-6.7		127,500	—	N. G.	none	none	200	O. K.	
6a	6.7-6 8		2,380,000	+	Staph	600B	2,200	1,700	O. K.	
b	7.0-6 8		1 68.000	+	Strep	120 000AB	123,000	*	O. K.	
c	6.8-6.9		4.450 000	—	Strep	6.900B	6 900	5 800	O. K.	
d	6 6-6.9		1,679,000	—	Micro	5,000B	5,900	4,100	O. K.	

* Plate not poured

Cow No.	Dairy name or T.B. tag
a	Right Front Quarter
b	Left Frant Quarter
c	Right Rear Quarter
d	Left Rear Quarter
pH C	Colormetric
pH E	Electrometric
Leucocytes	Breeds microscopic
Strip Cup	+ =Slight curd ++ =Medium curd +++=Heavy curd

Pred. Org.	Predominating Flora
Hemo. Org.	Count and type from blood agar
Bact. B. A.	Total count on blood agar
Bact. P. A.	Total count on plain agar
Cond. Milk	Physical condition of milk, microscopic appearance.

2. The pH of the milk was ascertained immediately after milking. "C" is the colormetric determination, using bromothymol blue, and "E" the electrometric.
3. Leucocytes were determined by Breed's microscopic method. 1/100cc. milk was smeared over 1 sq. cm. and stained with methylene blue. Counts over 75,000,000 cells were indicated as "countless".
4. Examination of the fore milk was made by using the strip cup. The strip cup top used was made of dark cloth with approximately 40 threads per inch. Three or four streams of the fore milk were used in determining this test. A separate clean pad was used for each quarter.
5. Predominating enrichment flora was determined from an enrichment culture incubated for 24 hours at 37°C. and examined microscopically. The enrichment method plus the types of colonies found on the blood agar plates were used to determine the final predominating flora.
6. Hemolytic streptococci: Milk was plated on 10 per cent blood agar using a 1:100 dilution of the milk. This was incubated 24 and 48 hours at 37°C. The number of hemolytic bacteria was observed and the type of hemolysis noted. Typical hemolytic colonies were fished off into veal brain-heart infusion broth and incubated. Further examination was made to determine if the culture was pure and if so the culture was put on a blood agar slant and stored to be identified later.
7. The total bacteria count on blood agar was made after 48 hours incubation at 37°C.
8. The total bacteria count on plain agar was determined after 48 hours incubation at 37°C.
9. Physical condition of the milk was noted after standing two hours or less. Precipitation of the casein and any changes in the color and consistency of the milk were noted. The appearance of this milk was that of a yellow, serous fluid with the casein precipitated and no cream layer at all. The fore milk from these cases showed a heavy curd. Other samples were thin, watery, and blue with a precipitation of the casein.

10. Remarks included any general notations such as physical conditions of the udder, absent test, etc.

Columns 4, 9, and 10 were determined in the field, and the others in the laboratory.

The source of the samples in this work was several of the dairy herds of southeastern South Dakota. There were seven herds totaling 178 cows.

The results of the laboratory examination are given in Chart No. 1. This is a typical cross-section of the results, showing good, suspicious, and definitely infected animals. Cow No. 1 was a typically infected animal. The pH of the "a" quarter was suspicious, indicating a slight inflamation. The number of leucocytes was eight times greater than the limit for good milk. The results in columns 5 and 6 show that both Beta and Alpha prime hemolytic streptococci were present. Cows numbers 3, 4, and 6 were also infected. pH 6.5 is considered normal and 7.0+ indicates definite inflamation.

Cow number 2 was suspicious, as the pH of the "a" and "d" quarters, 6.9 and 7.0 respectively, was indicative of inflamation. The leucocyte count was not exceedingly high and the bacterial count did not show a large number of organisms,

Chart II

LEUCOCYTE PARTITION

Herd No.	Under 500,000 Cells	500,000 to 1,000,000	1,000,000 to 3,000,000	3,000,000 to 5,000.000	5,000,000 to 10,000,000	Over 10,000,000 Cells	Total No. Cows
1	9	7	11	14	8	19	68
2	2	4	3	5	7	8	29
3	6	2	7	7	3	2	27
4	6	2	2	1	0	2	13
5	12	4	7	3	4	2	32
6	4	1	3	0	3	1	12
7	10	1	2	1	0	0	14
Total	49	21	35	31	25	34	195
Per cent	25	10.8	18	16	12.8	17.4	

Chart III

pH, ABNORMAL MILK and INJURY as INDICATOR of INFECTION

Herd No.	Total Cows	pH				Abnorm. Milk	Injured Quarter	Number Infected
		Acid 6.2	Normal 6.5	Susp. 6.9	Infect. 7.0+			
1	68	8	23	21	16	7	9	37
2	29	3	11	8	7	6	2	15
3	33	2	23	5	3	7	4	8
4	13	0	11	1	1	1	0	2
5	32	1	22	5	4	0	3	9
6	12	2	10	0	0	0	1	0
7	14	4	10	0	0	0	0	0
Total	201	20	110	40	31	21	19	71
Per cent		9.5	54.7	19.9	15.4	10.4	9.45	35

Chart IV

SUSPICIOUS and INFECTED ANIMALS

Herd No.	No. Cows	Over 500,000 Cells	Strep	Suspicious Animals	Infected Animals	Per Cent Infected
1	45	35	28	4	31	69
2	29	27	19	5	24	83
3	33	21	29	4	25	76
4	13	7	10	1	8	61.5
5	32	20	18	3	20	62.5
6	12	7	7	0	7	58
7	14	4	4	1	3	20
Total	178	121	115	18	118	66.2

and only 100 Beta streptococci per cc. The strip pad showed that the fore milk was not normal due to the presence of a medium curd.

Cow number 5 was typical of a good animal, but the pH was slightly high. This may be attributed to a high protein feed. The number of leucocytes was well within the limits, and the enrichment culture gave no growth. Columns 7 and 8 show that the milk was practically sterile.

Chart II shows the leucocyte partition for all the animals milked including duplicates.

The cows from each herd are grouped according to the number of leucocytes per cc. It is interesting to note that the largest percent of the total number of cows, exclusive of those which were normal, fell into that group over 10,000,000 cells per cc.

Chart III. The relative efficiency of the various tests and the determination of infected animals can be seen in this chart. Each herd is taken separately and the pH, production of abnormal milk, and injured quarters are used to determine the number infected. The pH shows those animals which produced acid milk and which was of no significance in this work, normal milk, suspicious, and definitely infected. Herds numbers 6 and 7 showed no animals infected but in **Chart IV** there will be other factors brought out which will show infeced animals.

Conclusion

Chart IV. Taking all the laboratory results into consideration we find that in the dairy herds tested 118 cows out of 178 were infected or had been infected to such an extent that the cell count was raised and the udder had been damaged. 76 per cent of the cows were infected or suspicious. 155 cows were harboring streptococci which may or may not be injurious to man.

From the public health stand point there are several factors which must be emphasized in view of our findings.

First: A rigid control of milk production that will eliminate any cows showing diseased udders. A competent veterinarian must make these physical examinations.

Second: Each cow must pass a final laboratory examination at regular intervals.

Third: All definitely infected cows must be eliminated and suspicious animals should be isolated from the herd until laboratory examination can confirm the field results.

Last and most important: The milk must be pasteurized to eliminate any possibility of the streptococci excreted by the cows causing an infection in the human consumers.