THE CANADIAN VETERINARY JOURNAL
LA REVUE VETERINAIRE CANADIENNE

Volume 2 March 1961 Number 3

THE USE OF THE CALIFORNIA MASTITIS TEST FOR THE DETECTION OF BOVINE MASTITIS*

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(*Received for publication on August 16, 1960*)

In 1957 Schalm and Noorlander (6) reported the development of a rapid, simple test for mastitis which they called the California Mastitis Test (CMT). They indicated that the degree of precipitation and gel formed by a mixture of the reagent and milk reflected the cell count of the milk. They showed that when milk from a normal gland was mixed with abnormal milk and tested, a gel or precipitate was produced. Jensen (4) reported that the reaction was due solely to the formation of a gel of leucocyte proteins and found that the test was negative until the count exceeded 500,000 cells per ml. He also applied the test to bulk milk samples. In 1958 Frank and Pounden (2) compared the reaction of the CMT and Whiteside Test, while Easterday et al (1) used the two tests in conjunction with culture and leucocyte counts.

In order to determine the relationship of the results of the CMT on quarter milk samples to the health of the gland, as well as the relationship of the results of the test on bulk milk to the degree of mastitis in the herd, the following investigations were made.

Material and Methods

The first set of samples was collected in 1958. The driver of the milk transport took specimens from the bulk milk tanks at 130 farms at the same time as samples were taken for other tests. The samples were kept refrigerated until examined.

For the second series in 1959, 28 herds that were shipping milk to two city dairies were used. Fourteen were chosen at random from 30 bulk milk shippers that supplied one dairy and 14 from 30 can shippers that supplied the other. Samples of herd milk were collected from the bulk tanks at the farms two weeks prior to and at the time of herd sampling, while herd milk samples from the can shippers were collected from the weigh vat at the dairy once weekly for three weeks during the survey period. On one occasion individual quarter samples were drawn from each milking cow in the herd at the regular milking period before the machine was applied. Aseptic precautions were exercised in the collection of the samples, which were kept cool until laboratory examinations were made.

The pooled herd samples were tested by the CMT, Whiteside, sodium teepol and resazurin tests, as well as by direct culture, total leucocyte count, differential leucocyte count, total plate and coliform counts. The individual quarter samples

*Presented in part at the Twelfth Annual Meeting of the Northeastern Mastitis Conference, October 14 1959, Dover, Delaware.

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were examined by the CMT, Whiteside test, leucocyte count and direct culture. All quarters were classified as positive if the milk sample *a* yielded Streptococcus agalactiae on culture, or *b* showed a cell count of over 500,000 together with an organism in excess of 1,000 colonies per ml, or *c* showed a cell count of over 2,000,000 together with a CMT reaction 3+ or more and a negative culture.

The procedures of the tests are as follows:

California Mastitis Test

The test was performed by pouring each sample of well-mixed milk to a level of 3 ml in one quarter of a partitioned plastic paddle, then adding 3 ml of the commercial CMT reagent by means of an automatic pipette. The paddle, which held four samples, was rotated quickly by hand 10 times and graded as follows:

- – Negative—no change in consistency.
- T Trace —no visible change in consistency, but when paddle is tipped a slime is momentarily seen on the bottom.
- 1+ —a gel or thick slime forms, but when the paddle is swirled the solution does not move into the centre.
- 2+ —a thick lumpy gel forms, which, when swirled, quickly moves toward the centre.
- 3+ —a distinct gel forms which tends to adhere to the bottom of the paddle, and during swirling a distinct central peak forms.

Sodium Teepol Test

The method described by Jensen (4) was followed using a 10% solution of sodium teepol in distilled water. Ten drops of the solution was added to 10 drops of milk on a glass slide and stirred for 10–15 seconds. During and after stirring, the test was read according to the instructions.

Whiteside Test

The modified test was made by adding one drop of a normal solution of NaOH to 5 drops of milk on a glass plate and stirring with an applicator for 10 seconds. The test was graded as follows:

- – the mixture remains opaque and free from particles.
- + no apparent reaction occurs during stirring but upon close inspection the mixture is opaque and contains finely dispersed particles.
- 1+ a slight thickening occurs during stirring with little or no tendency for the mass to adhere to the stick; on continued stirring, separation into a milky whey and well defined particles takes place.
- 2+ the mixture thickens almost as soon as stirring is started, the mass follows the movement of the stick and finally, when separation occurs, the particulate matter is arranged in thread-like whorls in a clear whey.
- 3+ a tenacious coagulum forms immediately and adheres to the stick; upon continuous stirring, the mass separates into a clear whey and thready, clumped, opaque material.
- 4+ a tenacious coagulum forms which shows little or no tendency to break down; a gummy mass remains in the centre with a peripheral ring of clear whey.

Leucocyte Count

A Breed smear was made from each sample and stained by the Newman-Lamport method for the leucocyte count. A total leucocyte count was estimated by counting 40 fields. For the differential count, the smears were stained using hemotoxylin-eosin, and cells were classified as granular leucocytes, lymphocytes and epithelial cells.

Culture

All samples were cultured by streaking 0.01 ml of milk over one-third of a 5% sheep blood agar plate which was incubated for 18 hours at 37°C. The approximate number of colonies of each organism was recorded. All streptococci that produced a positive CAMP reaction were classified as Streptococcus agalactiae.

Other Tests

The resazurin test, total plate count and coliform count were performed by standard methods.

Results

In the 1958 series, 21 samples from the 130 farms produced a 1+ reaction by the CMT and 19 of these had a leucocyte count of 500,000 or more per ml. Eighty samples produced a trace reaction; of these 53 had a cell count of less than 500,000. Two of the 29 negative samples had a cell count of 750,000, while the others were below 500,000. There was no relation between CMT reaction and resazurin test, or plate counts.

A summary of the mastitis test on individual quarter samples of the second series is given in Table I. Although none of the herd owners felt he had a problem, the percentage of infected quarters was higher than that found in the mastitis diagnostic laboratory (5); 30.7% of the quarters were positive in herds where bulk tanks were used, and 25% in those where milk was shipped in cans. The grouping of herds in relation to percentage of infected quarters (Table V) indicated that only 4 herds had less than 10% infected quarters, and 10 of the 28 herds had over 30%.

Table II shows the relationship between infection and the CMT reaction on individual quarter samples. Over 97% of the samples that were negative to the CMT came from uninfected quarters. The false negative reaction obtained on 23 infected quarters represents 3.8% of the total number of infected quarters. It is difficult to assess the significance of the trace reactions as 75.5% of these were from uninfected quarters. In Table III and Figs 1 to 4, the reactions of the CMT and Whiteside test with the leucocyte count in the quarter samples are compared.

TABLE I

Incidence of Mastitis in 28 Herds

Comprising 533 Cows

Quarters	Number	% of total tested	% of total infected
Tested	2,122		
Blind	10		
Negative	1,524	71.4	
Positive	598	28.6	100
Pos—Str agalactiae	273	12.9	45.6
Pos—Hem staphylococcus	172	8.1	29.1
Pos—other organisms	88	4.1	14.5
Pos—no organisms	65	3.0	10.8
Pos—strip-cup	21	1.0	

TABLE II

CMT ON QUARTER SAMPLES RELATED TO INFECTION

	Number	%
Reaction on Pos Qts		
*Pos CMT	405	65.6
Trace CMT	170	28.5
Neg CMT	23	3.9
Reaction on Neg Qts		
Pos CMT	160	10.5
Trace CMT	523	34.3
Neg CMT	841	55.2
Pos CMT		
Pos Qts	405	71.7
Neg Qts	160	28.3
Trace CMT		
Pos Qts	170	24.5
Neg Qts	523	75.5
Neg CMT		
Pos Qts	23	2.7
Neg Qts	841	97.3

*All reactions of 1+ and over

A 1+ reaction or higher in both tests indicated a cell count in excess of 500,000 leucocytes per ml. The same relationship between reactions and leucocyte counts in herd milk is evident from Table IV.

In Table V the tests on the bulk tank milk, taken at or near the time of herd sampling, are related to the percentage of infected quarters in the herd. A 1+ reaction with either test was recorded only in herds with more than 10% infected quarters. Trace reactions occurred in all herd groupings. A comparison of the two tests on herd milk is given in Table IV. Discrepancies occurred between trace and negative, or trace and positive, but not between negative and positive. From bulk tank shippers 37% of the herd samples gave a trace reaction to the CMT and a negative Whiteside reaction, against 3.5% from can shippers. There was complete agreement between the two tests in 48.2% of the bulk-tank samples as compared to 71.9% of the can shippers' weigh-tank samples.

The differential leucocyte count on samples of herd milk showed the range of granular leucocytes to be from 62 to 95%, with an average of 86.6; the lymphocytes to be from one to 28%, with an average of 12.8; and the epithelial cells to be 0 to 20%, with an average of 4.6. There appeared to be no relationship between percentage of different types of cells and CMT reaction. Twenty-two of 29 samples of bulk milk, and 15 of 39 can samples were grade I by the resazurin test. All except three of the poor grades had sufficiently high total plate counts to account for the poor quality; the three exceptions had a positive CMT.

The sodium teepol plate test was conducted on pooled milk samples and compared with the CMT. While the results were similar, the plate test is not as convenient to perform.

TABLE III

QUARTER SAMPLES: RELATIONSHIP OF LEUCOCYTE COUNT TO CMT AND WHITESIDE REACTIONS

	Leucocytes (in thousands)						
	0–250	251–	501–	1,001–	2,001–	5,001–	10,001+
CMT							
Neg	777	90	25	5	–	–	–
T	137	192	225	106	33	–	–
1+	2	12	53	126	104	11	–
2+	–	–	8	30	87	53	6
3+	–	–	–	–	11	26	46
Whiteside							
Neg	814	165	110	24	3	–	–
T	100	110	148	109	38	1	–
1+	3	20	48	120	110	14	4
2+	–	–	4	14	69	53	13
3+	–	–	–	–	13	22	36

FIGURE 1

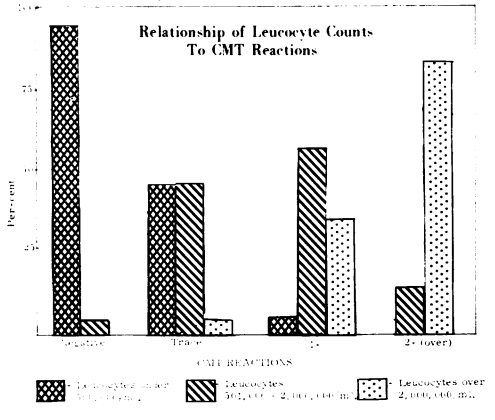


FIGURE 2

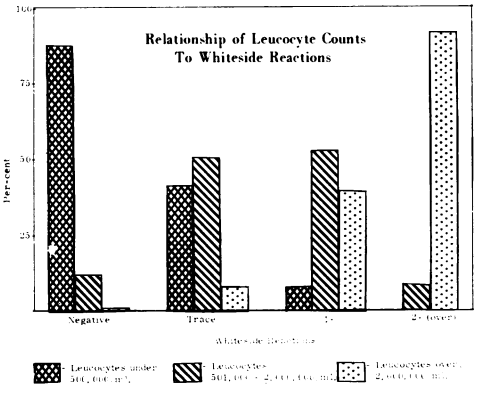


FIGURE 3

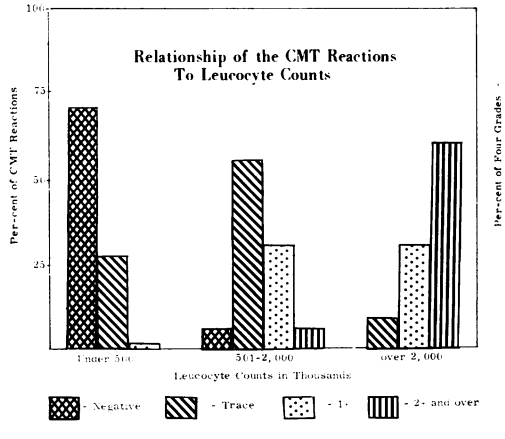


FIGURE 4

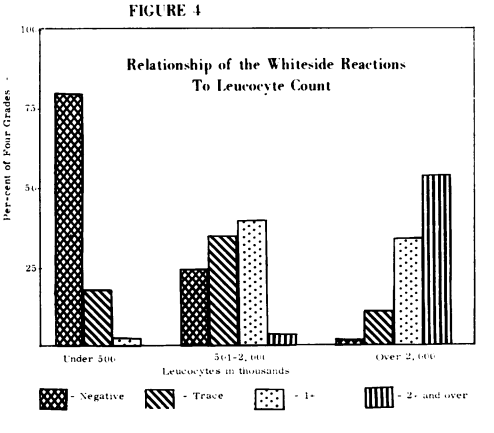


TABLE IV

HERD MILK: RELATIONSHIP OF LEUCOCYTE COUNT TO CMT AND WHITESIDE REACTION

		Leucocytes (in thousands)									
		0–250		251–500		501–1000		1000+		Total	
	Reaction	CMT	Wh	CMT	Wh	CMT	Wh	CMT	Wh	CMT	Wh
Bulk-Tank Shippers	Neg	1	3	1	6	–	1	–	2	2	12
	Trace	2	–	9	4	7	6	5	2	23	12
	1+	–	–	–	–	1	1	3	4	4	5
Can Shippers	Neg	3	3	3	3	–	–	–	–	6	6
	Trace	2	2	17	17	20	19	6	2	45	40
	1+	–	–	–	–	3	4	3	7	6	11

DISCUSSION AND CONCLUSIONS

The results presented here support the findings of other workers (3,4,6) on the place of the CMT on milk from individual quarters. As the test is an indication of leucocyte count, the interpretation of the test must be considered in this relationship. It has been shown (3) that foremilk has a higher leucocyte count than middle milk, and that if the first streams are discarded the next 15 to 20 ml are representative of the total milk in the quarter in so far as the CMT reaction is concerned. Where strict foremilk is tested one would therefore expect a greater sensitivity and a larger number of false positives. Since only 2.7% of negative reactions were from quarters classed as positive due to the presence of mastitis pathogens, it appears that the CMT is a useful test to screen fresh quarter samples in both field and laboratory. It is suggested that the CMT be applied to milking cows that are to be purchased, with the assumption that negative reactors could be safely introduced into the herd, while reactors (1+ or greater) should be rejected. Further tests, such as culture, would be required on those quarters showing a CMT trace reaction before a decision to purchase could be made.

The place of the Whiteside test and CMT as platform tests is not clearly defined by these results. A negative reaction on bulk herd milk is a useful achievement for the dairyman, for although it does not guarantee freedom of the herd from mastitis it does indicate that the incidence of infection is low. A positive (1+) reaction was associated with over 30% infection in 7 of the 9 herds. Thus it would seem logical that herds whose bulk milk is in this category may have a high incidence of either sub-clinical or clinical infection.

At the present time, the diagnostic significance of a trace reaction is not clear. The majority of herd samples tested fell into this group; two trace reactions by the CMT occurred in herds where the infection was below 10%, while in two others more than 50% of the quarters were infected.

The CMT, by the paddle method, is a convenient test to perform but the test must be read as outlined. Further swirling makes the test difficult to read as the slime observed in trace reactions disintegrates considerably. Experience is required in both the method of testing and interpretation of the reactions if uniformity and accurate results are to be obtained.

TABLE V

RELATIONSHIP OF CMT AND WHITESIDE TEST ON HERD MILK SAMPLES TO INFECTION IN HERD

		No of Herds	% Infected Quarters			
			0–10	11–30	31–50	51+
Bulk-Tank Shippers	CMT					
	Neg	–	–	–	–	–
	Trace	12	1	8	1	2
	1+	2	–	–	2	–
	Whiteside					
	Neg	1	1	–	–	–
	Trace	8	–	7	–	1
	1+	5	–	1	3	1
Can Shippers (from weigh tank)	CMT					
	Neg	3	2	1	–	–
	Trace	9	1	4	4	–
	1+	2	–	1	1	–
	Whiteside					
	Neg	3	2	1	–	–
	Trace	10	1	5	4	–
	1+	1	–	–	1	–

SUMMARY

1 Herd milk and quarter milk samples from 28 herds were tested by CMT and Whiteside tests as well as by culture, leucocyte counts, resazurin and plate counts.

2 Both the CMT and the Whiteside test reflect closely the leucocyte count. More samples containing less than 500,000 leucocytes per ml gave a trace reaction with the CMT than with the Whiteside test.

3 71.7% of samples giving a positive CMT were from quarters classified as positive by the criteria stated. Only 2.7% of samples giving negative CMT were classified as positive, and 75.5% of samples giving a trace CMT were from quarters classified as negative.

4 Positive reactions to both CMT and Whiteside tests were found in samples of milk from herds where there was a high incidence of sub-clinical mastitis. An interpretation of the trace CMT reactions on herd milk could not be made due to variation in the percentage of infected quarters found.

RÉSUMÉ

1 On a fait l'épreuve d'échantillons d'un troupeau entier et d'échantillons de quartiers provenant de vingt-huit troupeaux, au moyen des tests CMT (California Mastitis Test) et Whiteside par les cultures, le comptage des leucocytes, la résazurine et le comptage en plaques.

2 Les tests CMT et de Whiteside ont révélé un comptage assez juste des leucocytes. D'autres échantillons contenant moins de 500,000 leucocytes par ml ont manifesté une légère réaction, plus marquée avec le test CMT qu'avec celui de Whiteside.

3 71.7% des échantillons dont le test CMT était positif provenaient de quartiers classés comme positifs d'après la norme établie. Seulement 2.7% des échantillons dont le test CMT était négatif étaient classés comme positifs, et 75.5% des échantillons manifestant une légère réaction au test CMT provenaient de quartiers classés comme négatifs.

4 Des réactions positives aux tests CMT et Whiteside ont été révélées dans des échantillons de lait provenant de troupeaux où la proportion des cas de mammite sous-clinique était très élevée. L'interprétation des faibles réactions au CMT sur du lait de troupeau n'a pu être révélatrice par suite de la variation du pourcentage des quartiers infectés.

ACKNOWLEDGEMENT

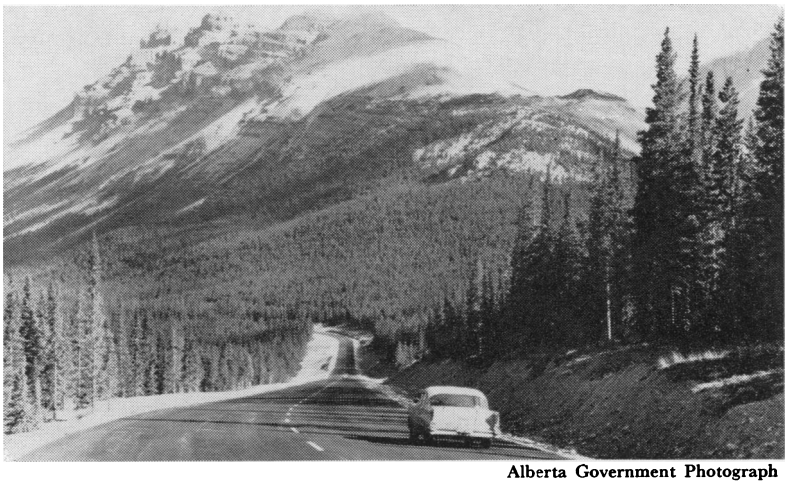
The technical assistance of Mrs E Plagemann and Mrs P Schroder is acknowledged.

REFERENCES

1 EASTERDAY B C, SIMON J and HANSON R P. The use of the modified Whiteside test as a screen for bovine mastitis. JAVMA 133:470–473. 1958.
2 FRANK A and POUNDEN W D. A comparison of the California and Whiteside mastitis tests. JAVMA 132:98. 1958.
3 GRAY D M and SCHALM O W. Interpretation of the California Mastitis Test from individual mammary quarters, bucket milk and bulk herd milk. JAVMA 136:195–198. 1960.
4 JENSEN P T. Investigations into the Whiteside Test and the CMT for the detection of pathological secretions in herd milk samples. Nord Ved Med 9:590–608. 1957.
5 Report of the Ontario Veterinary College p 61. 1958.
6 SCHALM O W and NOORLANDER B S. Experiments and observations leading to development of the California Mastitis Test. JAVMA 130:199–204, 1957.

"YOU'LL TAKE THE HIGH ROAD"

To BONNIE BANFF



Alberta Government Photograph

The 2,560 square miles of Banff National Park lie wholly in Alberta and are traversed by the new Trans Canada highway that reaches British Columbia. Many fine drives for the sightseeing traveller are routed around Banff. They may be taken in a private car or in a tourist bus that serves both Jasper and Banff parks. One fine drive is to Johnson's Canyon. Another is to Radium Hot Springs.