

California Mastitis Test in Relation to Subclinical Mastitis

M.El Sagheer Ahmed; Laila Ali and A.G. Hegazi

National Research Center, Dokki, Cairo, Egypt

A TOTAL of 716 individual milk samples from clinically normal udder quarters of 98 and 81 dairy buffaloes and cows were examined microbiologically as well as by using California Mastitis Test (CMT) for detection of subclinical mastitis.

The microbiological findings revealed that 31.89% and 28.39% of the examined buffaloes' and cow's milk samples proved to be mastitic. Coagulase-negative and positive staphylococci and *Escherichia coli* were the most prevalent infective agents and were isolated from 38.04%, 28.53% and 15.34% cases respectively.

The highest percentage of organisms were isolated from milk showing 3+ scores by CMT.

Key words: California Mastitis Test, subclinical mastitis, buffaloes and cows.

Mastitis is the most frequent disease responsible for early culling of milking animals. This culling may be at times takes place before the animal reaches the age of maximum production and once the disease set in, the inflammatory reaction continues for several days regardless of any further effect by the causative agents.

Mastitis in its chronic form is an insidious disease causing small daily losses that are not readily apparent but are cumulative and irreplaceable.

In 1970 Reichmuth, *et al.* reported that, subclinical mastitis leads to a reduction of 4.3% of milk yield and this equivalent to a loss of 369 million DM/Yr. while in 1971, Szajko and kosa measured milk yields from 6 cows suffering from subclinical mastitis for 2 months and they found that, milk yield was reduced by 0.1-0.6 kg per milking and the magnitude of the decrease was significantly ($p < 0.05$) correlated with the degree of severity of mastitis. In 1978 Meyer found that, milk yields from udder quarters with subclinical mastitis were 20.8% lower than that from healthy parallel quarters and the yield reduction in 216 mastitic cows was 13.2% when compared with 254 healthy cows.

Regarding public health, mastitis is considered of quite vital importance due to its association with many zoonotic diseases in which milk acts as a source of infection "APHA" (1978).

The aim of this work is to study the incidence of subclinical mastitis among dairy buffaloes and cows in El Katta village and to find the relationship between CMT and the microbiological results.

Material and Methods

For conducting this work 716 fore-milk samples were aseptically collected from clinically normal quarters of 98 and 81 dairy buffaloes and cows randomly selected from El katta village at Giza Governorate. Collected samples were examined using the following tests :-

A. California Mastitis Test (CMT) was done according to the procedure described by American public health Association "APHA" (1978) .

B. Microbiological Examination Which Includes :

1. Cultivation of milk sediment. The milk sediment (obtained by centrifugation of 10 ml of the sample for 20 minutes at 3000 r.p.m) was seeded onto plates of nutrient agar; blood agar; Edward's medium; Mac Conkey's agar and Sabouraud's Dextrose agar containing chloramphenical (0.05 mg /ml).
2. Examination of incubated milk; loopfuls from the incubated samples (over night at 37°C) were streaked on the same forementioned media. Inoculated plates were incubated at 37°C for 48 hours except Sabouraud's agar plates which were incubated at 25°C and checked daily for the growth of fungi for 3 weeks. suspected colonies appearing on different media were examined microscopically and isolated in pure culture for further identification according to Ajello, *et al.* 1966 and Sonnenwirth and Jarett, 1980.

Results

Results in table (1) showed that the incidence percentage of subclinical mastitis among lactating animals was 34.69 in lactating buffaloes and 28.39 in dairy cows, while the incidence of the disease among udder quarters was 31.89% and 28.39% for buffaloes and cows respectively and all were due to specific mastitogenic organisms.

TABLE 1. Incidence of subclinical mastitis .

	Total number examined	Mastitic No.	Cases %
Buffalo	98	34	34.69
Individual quarter samples	392	125	31.89
Cow	81	23	28.39
Individual quarter samples	324	92	28.39

Table (2) shows that 97.38% of the examined normal buffaloes milk samples reacted negatively to CMT, while similarly 98.28% of normal cows milk samples gave the same results. Normal samples giving a score(+) and (2+) constituted 2.62% and 1.72% of buffaloes and cows respectively (false positive results). Also from this table, it is evident that, out of 125 mastitic buffaloes milk samples 123 (98.4%) reacted positively, while the accuracy of the test was 94.56% in cases of cows milk samples.

TABLE 2. California Mastitis Test (CMT) of normal and mastitic samples.

Animal	Number of samples	CMT			
		-	+	2+	3+
Buffalo	Normal	267	260 97.38%	5 2.62%	2 -
	Mastitic	125	2 1.6%	13 98.4%	20 90
Cow	Normal	232	228 98.28%	3 1.72%	1 -
	Mastitic	92	5 5.43%	7 94.56%	17 63

It is evident in table (3) that, scores (+) and (2+) were in agreement with the microbiological findings in 72.22% and 90.90% respectively in buffaloes milk samples, while in case of cows milk the percentage was 70 and 94.44.

All samples of both species which showed a positive reaction (Score 3+) proved to be microbiologically positive (100% agreement). Thus, it can be concluded that, scores 2+ and 3+ could be relied upon as indicative of udder troubles, while samples giving score(+) should be considered suspicious and further confirmatory tests should be applied or follow up is recommended.

Table (4) shows that, the frequency distribution of microorganisms implicated in mastitic cases. coagulase negative and coagulase positive staphylococci and *Escherichia coli* were the most prevalent microorganisms (38.04%, 28.53% and 15.34% respectively), While *Streptococcus uberis*, *Kelbsiella pneumoniae*, *Corynebacterium pyogenes*, *Pseudomonas aeruginosa* and *Aspergillus fumigatus* could be isolated in varying percentages.

TABLE 3. Correlation between positive CMT and microbiological results.

Animal	Score	Number of samples	Microbiological results agreement		
			+ve	-ve	%
Buffalo	-	262	2	260	
	1+	18	13	5	72.22
	2+	22	20	2	90.90
	3+	90	90	-	100.00
	Total	392	125	267	
Cow	-	233	5	228	
	1+	10	7	3	70.00
	2+	18	17	1	94.44
	3+	63	63	-	100.00
	Total	324	92	232	

TABLE 4. Frequency distribution of isolated microorganisms from mastitic milk samples.

Isolated microorganisms	Frequency	
	No.	%
Coagulase-negative staphylococci	124	38.04
Coagulase-positive staphylococci	93	28.53
Escherichia coli	50	15.34
Streptococcus uberis	22	6.75
Kelbsiella pneumoniae	13	3.98
Corynebacterium pyogenes	9	2.76
Aspergillus fumigatus	8	2.45
Pseudomonas aeruginosa	7	2.15
	326	100.00

Discussion

Mastitis is the most important disease affecting mammary glands of dairy animals, and the serious effect created by mastitis are mostly due to its subclinical form, in which the infected quarters show no obvious signs and secreted milk looks apparently normal for a long time during which the causative organism acts as an invisible potential source of spreading infection in the herd without the farmer being aware of it, thus infection becomes difficult to eradicate.

In this study, it is clear that, the incidence of subclinical mastitis among examined dairy animals is high (table I) and the disease is usually associated with a considerable reduction in milk yield which caused a heavy economic losses.

In 1982 Narendra, *et al.*, found that, subclinical mastitis in 258(18%) out of 1400 cows and 67 (13%) out of 508 buffaloes causing a total loss of milk up to 320 kg per day in cattle and 178 kg per day in buffaloes.

The colonization of mammary gland by pathogenic microorganisms results in a series of events which lead to major alterations in the composition of milk secreted from the tissue cells. These compositional changes reflect the degree of damage caused to the cells. Some of these changes are more marked than others, thus can be used as a basis for designing rapid diagnostic tests for udder infection (*i.e.* CMT) suitable for use on large scales monitoring programmes.

It is clear from this study, that all the examined milk samples of the two species of animals which showed a score (3+) proved to be infected by the mastitogenic microorganisms (100% agreement), table (3), while score (2+) was in agreement with the microbial findings in 90.90% and 94.44% for buffaloes and cows milk samples respectively. Thus it can be concluded that, scores (2+) and (3+) can be relied upon, while samples which give score (+) should be considered suspicious and follow up must be recommended weekly for these animals.

From table (4) it is clear that, the prevalence isolates from infected quarters were coagulase negative and coagulase positive staphylococci, and *Escherichia coli*, 38.04%; 28.53 % and 15.34% respectively.

The presence of mycotic cases in this study may be attributed to the intensive haphazard use of antibiotic in the treatment or as a prophylactic measure against mastitis.

The results obtained in this study are nearly simulate to those reported by El Rasheedy, *et al.* (1988); El Sagheer, *et al.* (1988); Quinones, *et al.* (1989) and Verma (1988)

In conclusion, the application of CMT leads to early detection of subclinically infected quarters and aid in the selection of dairy animals for either segregation or therapy, As the cost of a sound mastitis control program is normally less than the costs of the disease including the large losses in milk production, therefore preventive practices and hygienic measures for mastitis control should be highly recommended.

References

- Ajello, L; George, L.K.; Kaplan, W. and Kaufman, L. (1966) *Laboratory Manual for Medical Mycology*. U.S. Department of Health Education and welfare, Georgia.
- American public Health Association "APHA"(1978)*Standard Methods for the Examination of Dairy products*. 14th Ed., Amer, public Health Ass., washington.
- El Rasheedy, A.A.; Amin, A.; Mostafa, A.S. and Tawfik, M.S.(1988) Evaluation of mastitis screening tests in diagnosis of subclinical cases compared with the conventional bacteriological method. *Veterinary Medical journal*. 36 (1) 91.
- El sagheer, M.A.; Laila, A.and Nagi, G.M. (1988) Teat shape and size in relation to subclinical mastitis in friesian cow. *Egypt., Vet., Sci.*, 25 (1),77.
- Meyer, B.(1978) production losses due to subclinical mastitis measured using on impulse cytophotometer. Thesis, Zurich Univ., Switzerland. 42pp. *Dairy Sci . Abst.*42, 2926(1980).
- Narendra,S.; Sharma, V.K.; Rajani, H.B. and Sinha, Y.R. (1982) Incidence, economy and test efficacy of subclinical mastitis in dairy animals. *Indian veterinary journal*, 59(9)693.
- Quinones, J.; Demo, M.; pajaro, C.; Martin, V. and Bagni, G. (1989) Results obtained with the California Mastitis Test, Somatic cell count, and bacteriological analysis in the evaluation of udder health in dairy cows. *Veterinaria Argentina* 6 (51)60.
- Reichmuth, J.;Zeldler, H.;Tole, A.and Heeschen, W.(1970) The influence of subclinical mastitis on bovine milk production. XVIII Int. *Dairy Congr.* IE: 636. *Dairy Sci.Abst.*32, 4936.
- Sonnenwirth, A.C. and Jarett, L.(1980) *Gradwohl's Clinical laboratory Methods and Diagnosis* Vol.II, 8th Ed. The C.V. Mosby Co. st. Louis ,Toronto, London 2980.
- Szajko, L. and Kosa, L.(1971) Effect of subclinical mastitis on milk yield. *Mosonmgy mezogazda sagtudom, Kar Kozl.* 14, (5), 5 c.b *Dairy Sci. Abst.* 34, 5172. (1972).
- Verma, R. (1988) Studies on clinical and subclinical bovine mastitis. *Indian journal of comparative Microbiology, Immunology and Infectious Diseases.* 9 (1) 28.

العلاقة بين التهاب الضرع الغير سريري واختبار كاليفورنيا

محمد الصفير احمد - ليلى على محمد - احمد جعفر حجازى

المركز القومى للبحوث - الدقى - الجيزة

أجريت هذه الدراسة على ٧١٦ عينة لبن من ٩٨ جاموسة و٨١ بقرة لمعرفة مدى كفاءة اختبار كاليفورنيا فى اكتشاف التهاب الضرع الغير سريري لهذه الحيوانات ومقارنة نتائج هذا الاختبار بالفحوص الميكروبيولوجية، وقد أثبتت النتائج أن كفاءة اختبار كاليفورنيا فى اكتشاف التهاب الضرع الغير سريري ٩٨٪ فى حالات الجاموس و٩٤.٥٦٪ فى حالات الأبقار ، كما أوضحت الفحوص الميكروبيولوجية أن أغلب هذه الاصابة كانت بالمكورات العنقودية سالبة أنزيم الكواجيلولاز (٢٨.٠٤٪) ، ثم المكور العنقودية موجبة أنزيم الكواجيلولاز (٢٨.٥٢٪) ثم ميكروب القولون الاسريشيا كولاى (١٥.٢٤) بينما أمكن عزل ميكروب المكور السبحى والكيليبسيلا وسيدمونس اريجنوزا والكوريني والاسبيراجلس من هذه الحيوانات بنسب متفاوتة .

ويوصى الباحثون باستخدام اختبار الكاليفورنيا فى الاكتشاف المبكر لمرض التهاب الضرع الغير سريري وذلك لسهولة استخدامه وسرعة نتائجه حتى يمكن عزل وعلاج الحيوانات المصابة بهذا المرض . لكى نتجنب الخسائر التى تنتج عن فقد اللبن أو فقد الضرع كوحدة إنتاجية.