Cairo University
Fac. of Vet. Med., Beni-Suef
Head of Dept. Dr. A.M. El-Kholy

CHEMICAL AND CYTOBACTERIOLOGICAL STUDIES FOR DETECTION OF SUBCLINCAL MASTITIS

(With 4 Tables)

By

A.M. El-KHOLY; H.I. HOSEIN* and A.EL-R. THABET**

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دراسات كيميائيه وسيتوبكتريولوجيه للكشف عن
التهاب الضرع الكامن

عادل الخولى ، حسين ابراهيم
عبد الراضى ثابت

أجرى هذا البحث على عدد ١٠٠ عينة من الألبان جمعت من أرباع حلوب أبقار سليمه ظاهرياً تحت ظروف معقمه وقد تم فحص هذه العينات كيميائياً وسيتوبكتريولوجياً للكشف عن التهاب الضرع الكامن وأظهرت النتائج ما يلى :-

باستخدام عدد كوستلر وجد أن ٥٠٪ من عينات الألبان التى كانت أكبر من العدد ٣ كانت ايجابيه للفحص البكتريولوجى .

ان نسبة التوافق بين اختبار هوايت سايد والفحص البكتريولوجى كانت ٨٢ ر ٨١ ٪ ، ٣٣٠ ر ٨٣ ٪ ، لل ٢ ، ٣٠ + على التوالى بينما كانت النتيجه السلبيه الزائفه ٢٤ ر ٣٩ ٪ .

وجد أن نسبة التوافق بين العدد الكلى للخلايا الجسميه الداله على الالتهاب لكل ١ مللى من الالبان والفحص البكتريولوجى كانت ٩٥ ر ٨٠ ٪ حيث وجد أن ٢١٠ عينه كان بها عدد من الخلايا أكثر من ٠٠٠ ر ٥٠٠ خليه لكل ١ مللى منهم ٧١ عينه تم عزل المسببات البكتيريه للالتهاب الضرع منها .

أمكن عزل البكتريا التاليه من عينات الالبان وهى :- (الميكروب السبحى أجالاكتى (٤٢ ر١٠٪) والميكروب السبحى ديجالاكتى (١٧ ر ٤٪) ، الايشيريشياكولاى (٥٨ ر ١٤٪) ، الميكروب العنقودى الذهبى (٩١ ر ٢٢٪) ، الميكروب العنقودى الابيديرميدس (٢٥ ٪) ، الكورينى بكتريم بيوجينيز (١٧ ر ٤٪) وذلك من ناحية العدوى الفرديه بينما أمكن عزل الميكروب السبحى اجالاكتى مع الميكروب العنقودى الذهبى (٥ ر ١٢٪) ، الميكروب السبحى اجالاكتى مع الايشيريشيا كولاى(٢٥ ر ٦ ٪)من ناحية العدوى الزوجيه .

هذا وقد نوقشت الاهميه الصحيه للميكروبات المعزوله وانتهينا إلى أن الكشف المبكر للأرباع المصابه يعتبر من أهم العوامل فى الوقايه من التهاب الضرع وذلك باستخدام الاختبارات الاستبيانيه المدعمه بالفحص البكتريولوجى المؤكد .

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*: Depts. of Food Hygine and Animal Medicine, Fac. of Vet. Med., Beni-Suef, Cairo Univ.

**: Animal Health Research Insttute, Assiut.

SUMMARY

A total of 100 apparently normal quarter cow's milk samples were collected under aseptic condition and examined cytobacteriologically and chemically for detection of subclinical mastitis. The koestler No. could detect subclinical mastitis in 42 out of 84 milk samples (50%) which showed >3. The agreement percentage between modified white side test (MWT) and bacteriological examination were 81.82% for 2+ve and 3+ve respectively, while the false negative results was 39.24%. On the other hand, the agreement percent between somatic cell count/ml and bacteriological examination was 80.95% in which 21 samples showed a count more than 500.000 cells/ml. Out of them 17 samples were positive for bacteria. The isolated bacteria were found to be Str. agalactiae (10.42%), Str. dysgalactiae (4.17%), E.coli (14.58%); Staph. aureus (22.91%); Staph. epidermidis (25%) and Cory. pyogenes (4.17%) for the single infection, while Str. agalactiae with Staph. aureus and Str. agalactiae with E.coli were found to be 12.5% and 6.25. Respectively for the double infection. In conclusion, it is evident that early detection of infected gland is the most important factor in the control of mastitis by applying screening tests supported by confirmatory bacterialogical examination.

INTRODUCTION

Mastitis is considered to be one of the most important destructive disease producing abnormal milk and monetary losses to dairy industry (*SCHALM* et al., *1971*). Since most mastitic cases occur in subclinical forms in which the infected quarters show no obvious symptoms and secreted milk looks apparantly normal for long time, hence the diseased animals continues for a time to be a dangerous source of infection in the herd and milk acts as a vehicle of infection among consumers for many zoonotic diseases (A.P.H.A., 1978).

Most surveys in different countries have shown that up to 50% of lactating animals, at any time, may suffer from chronic latent mastitis after suffering from subclinical mastitis (*GRUNERT and WEIGHT, 1979*). It is evident that subclinical mastitis accounts for a reduction of milk yield at

a varying percentage ranging from 4.3%-23% (*REICHMUTH et al.,* *1970; MILLER, 1973; MEYER, 1987 and NARENDRA et al., 1982).* This mean lower return to the producers and problems in processing due to lower levels of casein and subsequently poor product stability and quality (*KIELWEIN, 1976*).

Various field and laboratory tests including bacteriological examination were carried out by many specialists for detection of subclinical mastitis (*TIELEN et al.,1983; BRAMLEY et al., 1984; BAKR, 1986 and AFIFI and MOUSTAFA, 1991*).

Therefore, the aim of this work was to evaluate some tests currently used as compared with cytobacteriological methods to spot out an efficient simple scheme for detection of subclinical mastitis under the present environmental conditions.

MATERIAL and METHODS

Collection of samples:

After disinfecting teat orifice cf each quarter with 70% ethyl alcohol, a total of 100 apparently normal quarter cow's milk samples were collected in sterile screw capped bottles which subjected for the followings examination.

A- Chemical examination:
1- Modified whiteside test was performed according to Atherton and *NEWLANDER (1977)*.
2- Cholrine test was carried out according to the method described by *LING (1963)*.
3- Lactose content was determined as described by *LING (1963)*.
4- Koestler number was applied according to *DAVIS (1955)*.

B- Cytological examination:

The somatic cell count (SCC) was carried out according to *IDF (1984)*.

C- Bacteriological examination:

The milk sediment obtained by centrifugation of 10ml of the sample for 20 minutes at 3000 rpm., was seeded onto Blood and MacConkey's agar plates. The inoculated plates were incubated at 37°C for 48 hours. Suspected colonies were isolated in pure culture and identified according to *KOWALSKI (1977)* and *SONENWIRTH* and *JARETT (1980)*.

RESULTS

Results are shown in tables (1,2,3 and 4).

DISCUSSION

From the data recorded in table (1) it is evident that 14 out of 100 examined apparently normal quarter milk samples showed normal Koestler No. out of which 5 samples (35.71%) were considered false negative as causative bacteria were isolated from them. The No. 2-3 could detect one case from 2 samples (50%) while samples showed > 3 could detect subclinical mastitis in 42 out of 84 quarter milk samples (50%). Thus, it is evident that Koestler No. can not be considered as decisive or even reliable test for diagnosis of subclinical mastitis due to low percentage of agreement between the number and positive bacteriological findings as well as comparatively high percentage of false negative reaction. It is found that not only mastitis, but stage of lactation, age, feed etc. can increase the chloride content and subsequently decrease the lactose content (*DAVIS, 1955; BAKER 1986 and EL-RASHIDY, 1986*).

Results of modified whiteside test given in table 2 show that 79 out of 100 milk samples gave negative result, out of which 31 samples are false negative (39.24%) as they were bacteriologically positive. MWT showed positive reaction in 21 out of 100 milk samples. Out of them 17 samples were bacteriologically positive (80.95%). The agreement percent constituted 81.82% and 83.33% with a score 2+ve and 3+ve respectively. Therefore, a 2+ve and 3+ve to a certain extent can be taken as indication for udder troubles, while a score 1+ve should be considered suspicious and further confirmatory tests should be applied. Nearly similar findings have been reported by *ABDEL-KARIM and EL-ASHMAWY (1979); BAKER (1986) and EL-RASHIDY (1986)*.

The results of somatic cell count as compared with bacteriological examination were recorded in table 3 from which it is evident that 79 out of 100 milk samples showed a count less than 500.000. Out of them 31 (39.24%) samples were bacteriologically positive. This may attribute to latent infection (*REICHMUTH, 1968 and ROSENBERGER, 1979*). On the other hand, 21 samples showed a count more than 500.000. Out of them 17 (80.95%) samples were bacteriologically positive and 4 (19.05%) samples were false positive. It is obvious from the results obtained that there is no sharp limit regarding the total cell count between mastitis and normal milk samples and this may be attributed to physiological condition and some other factors. (*SCHALM, 1960 and ROSENBERGER, 1979*). FOX et al (1985) and SABREEN (1992) noticed a positive correlation between somatic cell count and bacteriological status in quarter milk samples.

From Table 4 it is evident that the prevalence of single mastitogenic bacteria from examined quarter milk samples were Str. agalactiae (10.42%), Str. dysgalactiae (4.17%), E. coli (14. 58%), Staph. aureus (22.91%), Staph. epidermidis (25%), Corynebacterium pyogenes (4.17%), while double mastitogenic bacteria were Str. agalactiae with Staph. aureus (12.5%) and Str. dysgalactiae with E. coli (6.25%). Similar causative organisms in different percentage were reported by *ABDEL-KARIM and EL-ASHMAWY (1979); NARENDRA* et al., *(1982); MILLER* et al., *(1984); EL-RASHIDY (1986); MAHMOUD (1988)* and *AFIFI* and *MOUSTAFA (1991)*.

Although results of some screening tests often show good correlation with the bacteriological findings, yet no single test was completely statisfactory for detection of subclinical mastitis.

In conclusion, the control measures including the hygienic condition of the animal, milkers and equipments used for milking should be taken in consideration. Early detection of infected gland is the most important factor in the control of mastitis by applying screening tests supported by confirmatory bacteriological examination.

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Table (1) :   Correlation between Koestler No. and
                bacteriological results of examined quarter
                milk samples.

Koestler number	No. of samples	Bacteriological results		Agreement %	False Koestler No.	
		+ve	-ve		+ve%	-ve%
1 - 2	14	5	9	-	-	35.71
2 - 3	2	1	1	50.0	50.0	-
> 3	84	42	42	50.0	50.0	←
Total	100	48	52			

Table (2) :   Correlation between positive modified white-
side test (MWT) and bacteriological results.

Score	No.of samples	Bacteriological results		Agreement %	False MWT results	
		+ve	-ve		+ve%	- ve %
-ve	79	31	48	-	-	39.24
+ve	4	3	1	75.0	25.0	-
++ve	11	9	2	81.82	18.18	-
+++ve	6	5	1	83.33	16.67	-
Total	100	48	52			

-ve   =   Negative

+ve   =   Weak positive

++ve   =   Moderate positive

+++ve =   Strong positive.

Table (3) :   Correlation between somatic cell count (SCC)
and bacteriological results.

Cell count / ml milk	No.of samples	Bacteriological results		Agreement %	False SCC	
		+ve	-ve		+ve %	-ve %
<500.000	79	31	48	-	-	39.24
>500.000	21	17	4	80.95	19.05	-
Total	100	48	52			

Table (4) : Frequency distribution of isolated bacteria
from examined quarter milk samples.

Single infection			Double infection		
Bacteria isolated	No.	%	Bacteria isolated	No.	%
Str. agalactiae	5	10.42	Str.agalactiae+S.aureus	6	12.50
Str. dysgalactiae	2	4.17	Str.agalactiae+ E.coli	3	6.25
E.coli	7	14.58			
S.aureus	11	22:91			
S.epidermidis	12	25.0			
Cory. pyogenes	2	4.17			
Total	39	81.25		9	18.75