تشخيص مرض التهاب الضرع غير السريرى
فى الأبقار العراقية

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تم فحص ٥٥٩ عينه لبن جمعت من أرباع الضرع لابقار حلوب للكشف عن مرض التهاب الضرع غير السريرى بالفحوص البكتريولوجية وكذلك بالاختبارات المعملية الريعة الاتية : تعيين التركيز الايدروجينى ، النسبة المئوية للكلورين ، انزيم الكاتاليز ، عد الخلايا الجسمية ، اختبار وايت ساید المحسن واختبار كاليفورنيا لالتهاب الضرع ٠

وقد أثبتت نتائج الفحوص البكتريولوجية وجود مرض التهاب الضرع غير السريرى فى ٢٥٫٤٠ ٪ من العينات ٠ وتم عزل الميكروب السبحى أجالاكتيا والميكروب العنقودى الذهبى من معظم العينات كما تم عزل الميكروب السبحى ديجالاكيتا ، سيد ومونس ارجينوزا ، الشيرشيان القولونية ، السبحى يوبرمس وكورينى بيوجينس بنسب مختلفة ٠

كما اعطت اختبارات وايت ساید المحسن ، كاليفورنيا لالتهاب الضرع ، تعيين الكلورين وعد الخلايا الجسمية أعلا نسبة مئوية من الدقة مع الفحوص البكتريولوجية ٠ واعتبر الباحثان فحوص عد الخلايا الجسمية ، وايت ساید المحسن وكاليفورنيا لالتهاب الضرع من الاختبارات التى يعول عليها التشخيص مرض التهاب الضرع غير السريرى أكثر من اختبارات التركيز الايدروجينى وأنزيم الكاتاليز وتعيين الكلورين لما تعطيه الفحوص الاخيرة من نسب مئوية عالية لنتائج سلبية زائفة ٠

أوصى الباحثان بالتدقيق على الممارسات التقنية والمقاييس الصحية المانعة لانتشار مرض التهاب الضرع ٠

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DIAGNOSIS OF SUBCLINICAL MASTITIS IN IRAQI DAIRY CATTLE

(With 11 Tables)

By

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SUMMARY

A total of 559 individual milk samples from clinically normal quarters of dairy cows, were examined bacteriologically as well as by using 6 screening tests: pH, chloride , catalase, DMSCC, MWT and CMT, for detection of subclinical mastitis.

The bacteriological findings reveal that 25.40% of examined samples proved to be mastitic.

Str. agalactia and Staph. aureus were the most prevalent infective agents, while Str. dysagalactia, Ps. Aeruginosa, E. coli, Str. uberis and C. pyogenes could be isolated in a descending manner.

The highest percent of accuracy with the bacteriological results was obtained by MWT (95.7%), CMT (93.9), chloride test (93.6%) and DMSCC (91.8%).

DMSCC, MWT and CMT can be considered more reliable tests for diagnosis of mastitis than pH, catalase and chloride test due to the high percentage of false negative results obtained with the later tests.

Preventive peractices and hygienic measures for mastitis control should be highly recommended.

INTRODUCTION

Healthy mamary glands are essential to the secretion of milk that is wholesome to drink and sufficient in quantity to be profitable to dairymen. The most important disease affecting

udder is mastitis. Since most mastitic cases occur in subclinical forms, hence the diseased animal continues, for a time, to be a dangerous source of infection.

Several investigators have reported that, bovine mastitis is of great economic importance to the dairy industry (DALLING, 1947; HOPSON, 1950; LEECH et al., 1960; DHANDA and SETHI, 1962; BUTOZAN et al., 1963; McCARTHY, 1965 and CAMPBELL and MARCHALL, 1975).

Regarding public health, mastitis is considered of quite vital importance due to its association with many zoonotic diseases in which milk acts as a vehicle of infection (LITTLE and PLASTRIDGE, 1946; DACK, 1956 and APHA, 1972).

Owing to the lack of information about the incidence of subclinical mastitis in Iraqi dairy cattle, this work was planned to fulfill this gap and to evaluate the common mastitis tests applied.

MATERIAL AND METHODS

For conducting this work, 559 individual fore-milk samples were aseptically drawn from clinically normal quarters of dairy cows belonging to three gorvernmental dairy farms in Iraqi.Collected samples were transferred to the laboratory to be examined using the following tests:

A. Bacteriological examination:-

1- Cultivation of milk sediment: The milk sediment obtained by centrifugation of 10 ml of the sample for 20 minutes at 3000 rpm., was seeded onto plates of nutrient agar, blood agar, Edward's medium and MacConkey's agar.

2- Examination of incubated milk: Loopfuls from the incubated samples were streaked on blood agar, Edward's medium and MacConkeys agar plates.

Inoculated plates were incubated at 37°C for 48 hours. Suspected colonies, appearing on different media, were examined microscopically before being isolated in pure culture for further identification according to BREED et al., (1957).

B. Rapid screening tests:

1- pH determination Using Pye potentiometer model 293, Pye unicam.

2- Catalase test: as described by CHALMERS (1962). The production of more than 1.5 ml of gas in the closed arm of the Einharimeter tube is regarded as positive.

3- Gel test: Modified Whiteside (MWT) and California Mastitis tests (CMT) were done according to the procedure described by American Public Health Association "APHA" (1972).

4- Dierct Microscopic Somatic Cell count (DMSCC): was carried out according to APHA (1972)using Breed's and Newman Lampert stain.

5- Chloride test: as described by LING (1963) based on the principale that the chloride content of milk increases beyond the critical value of 0.14 percent when mastitis is present.

Evaluation of the common mastitis test:

The reliability of the rapid screening tests was varified by comparing their results with the bacteriological findings.

For convenience, a percentage accuracy score was devised as:

$$\frac{\text{No.of true positive samples + No.of true negative samples}}{\text{Total No. of sample tested}} \times 100$$

RESULTS

The results obtained from the bacteriological examination of milk samples drawn from clinically normal quarters of dairy cows and the incidence of subclinical mastitis are recorded in tables 1 to 4.

The reliability of the rapid screening tests and the percentage agreement of its results as compared with the bacteriological findings are recorded in tables 5 to 11.

Table (1)

Incidence of mastitis in regards to the number of cows and quarter samples examined.

	Total number examined	Mastitic cases	
		No.	%
Cows	140	79	56.43
Quarter samples	559	142	25.40

Table (2)

Incidence of mastitic cows in relation to the number of quarters affected.

Number of quarters	Mastitic cases	
	No.	%
One quarter	38	48.10
Two quarters	24	30.38
Three quarters	12	15.19
Four quarters	5	6.33
Total	79	100.00

Table 3: Incidence and Frequency of mastitic quarters in relation to the bacteriological results.

Quarter	Mastitic Cases	
	No.	%
Left - Hind quarter	40	28 . 17
Left - Fore quarter	40	28 . 17
Right- Hind quarter	34	23 . 94
Right- Fore quarter	28	19 . 72

Table 4: Frequency distribution of isolated organisms from examined milk samples.

Isolated organisms	Frequency	
	No.	%
Str. agalactia	45	8 . 05
Str. agalactia + E. colI	17	3 . 04
Str. dysgalactia	14	2 . 50
Str. dysgalactia + E. col.	2	0 . 36
Str. uberis	2	0 . 36
Staph. aureus	30	5 . 37
Staph. aureus + E. coli	19	3 . 40
E. coli	4	0 . 71
Pseudomonas aeruginosa	7	1 . 25
C. Pyogenes	2	0 . 36
Total	142	25 . 40

Table 5: Comparative statement showing the percentage agreement of diagnostic tests taking Bacteriological results as a standard.

Test	Number of samples examined	Bacteriological results No. of samples +ve	Bacteriological results No. of samples -ve	Test reaction True +ve	Test reaction True -ve	Test reaction False +ve	Test reaction False -ve	percent accur. True +ve	percent False +ve	inacouracy False -ve
PH	559	142	417	90	23	394	52	86.5	4.1	9.3
Catalase Test	559	142	417	92	--	417	50	91.0	---	9.9
Chloride Test	559	142	417	116	10	407	26	93.6	1.8	4.6
DMSCC	559	142	417	142	16	371	--	91.8	8.2	---
MWT	559	142	417	138	20	397	4	95.7	3.6	0.7
CMT	559	142	417	142	34	383	--	93.9	6.1	---

Table 6: Correlation between positive pH and the bacteriological results

Range	No. of Samples	Bacteriological results		Agreement %
		- Ve	+ Ve	
-- 6.6	221	204	17	
-- 6.7	225	190	35	
-- 6.8	59	23	36	61.02
-- 6.9	20	---	20	100.00
-- 7.0	12	---	12	100.00
-- 7.1	16	---	16	100.00
-- 7.2	6	---	6	100.00
Total	559	417	142	

Table 7: Correlation between positive catalase test and the bacteriological results

Range	No. of Samples	Bacteriological results		Agreement %
		- Ve	+ Ve	
0-- / 0.5	132	132	---	
0.5-- / 1.0	272	244	28	
1.0-- / 1.5	63	44	22	
1.5-- / 2.0	44	--	44	100.00
2.0-- / 2.5	13	--	13	100.00
2.5-- / 3.0	17	--	17	100.00
3.0-- / 3.5	10	--	10	100.00
3.5-- 4.0	8	--	8	100.00
Total	559	417	142	

Table 8: Correlation between positive chloride test and the bacteriological results.

Range	No. of Samples	Bacteriological results		Agreement %
		- Ve	+ Ve	
-- 0.09	40	40	---	
-- 0.10	82	81	1	
-- 0.11	106	104	2	
-- 0.12	134	126	8	
-- 0.13	71	56	15	
-- 0.14	58	7	51	87.93
-- 0.15	46	3	43	93.48
-- 0.16	22	---	22	100.00
Total	559	417	142	

Table 9: Correlation between the Direct Microscopic Somatic Cell Count (DMSCC) and the bacteriological results.

Range	No. of Samples	Bacteriological results		Agreement %
		- Ve	+ Ve	
10^4/ 10^5	92	92	----	
10^5/ $5X10^5$	279	279	----	
$5X10^5$/ 10^6	96	42	54	56.25
10^6	92	4	88	95.65
Total	559	417	142	

Table 10: Correlation between positive Modified Whiteside Test (M W T) and the bacteriological results

Score	No. of Samples	Bacteriological results		Agreement %
		- Ve	+ Ve	
--	401	397	4	
+	92	20	72	78.26
++	53	---	53	100.00
+++	13	---	13	100.00
Total	559	417	142	

Table 11: Correlation between positive California Mastitis Test (C M T) and the bacteriological results

Score	No. of Samples	Bacteriological results		Agreement %
		- Ve	+ Ve	
--	383	383	---	
+	107	34	73	68.22
++	48	--	48	100.00
+++	21	--	21	100.00
Total	559	417	142	

DISCUSSION

Results achieved reveal that, 56.43% of the dairy cows were suffering from subclinical mastitis (Table 1). Most of mastitic cows showed one or two quarters infected (48.10% and 30.38% respectively). While the four quarters were infected in five cases (Table 2).

These findings substantiate what have been reported by McCARTHY (1965);HARVEY and HILL (1967)and CAMPBELL and MARCHALL (1975).

It is worthmentioning that, the incidence percent of mastitic cases was more prevalent in the left side of the udder, while infection among the right-hind quarters was higher than that in fore quarters (Table 3).

The bacteriological findings indicates that, out of the 559 milk samples examined, 142 (25.40%) were found to be mastitic (Table 1). Nearly similar percentage was reported by NASR (1956); BUTOZAN et al.(1963); ZAKARYA (1969) and MARINSEK, (1976).

The results reported in table (4) show the frequency distribution of organisms implicated in mastitic cases, from which it is evident that Str. agalactia and Staph. aureus were the most prevalent organisms (11.09% and 8.77% respectively), while Str. dysgalactia, Ps. aeruginose, E. coli, Str. uberis and C. pyogenes could be isolated in varying percentages (Table 4).

The findings obtained nearly simulate those reported by FIELD (1944); LITTLE and PLASTRIDGE (1949); NASR (1956) ; RADOSTILES (1961); ZAKARYA (1969); JACQUET et al. (1975) and FARID et al., (1976).

Realizing that infected quarters show no obvious symptoms and secreted apparently normal milk for a long time, during which the causative organisms act as an investible potential source of spreading infection among the flock, thus rendering the problem more difficult to solve. Moreover, produced milk from such animals becomes mostly abnormal in its physical and chemical properties rendering the product of impaired utility or even unfit for consumption, thus causing haevy economic losses.

From the pbulic health point of view, members of isolates has been incriminated in food-poisoning outbreaks, while others were implicated in certain diseases (DACK, 1956; SLANETZ and BARLLOY, 1962; WALLACE et al., 1962; FRAZIER, 1967 and APHA, 1972).

The summarized results given in table (5) point out that, M W T, C M T, chloride test and D M S C C gave the highest percent of accuracy as compared with the bacteriological findings, while catalase test and pH determination gave a lower of scoreuracy. D M S C C and C M T gave the highest percent of false positive results, while pH determination, M W T and chloride test showed a less percentage. On the other hand, no false positive cases could be detected by catalase test. Consulting the results recorded in table (7), it is evident that all examined milk samples which produced 1.5 ml gas or more proved to be mastitic (100 % agreement). The highest percent of false nagative findings were produced by pH determination, catalese test,while nonfalse negative results were detected by D M S C C and C M T, and only 4 cases (0.7%) could be detected by M W T.

Correlation between the different screening tests and bacberiological findings was recorded in tables 6 to 11. The percent of agreement differed from one test to another but one can safely conclude that milk samples showing pH 6.8; produce 1.5 ml of gas; contain 0.14% chloride; one million somatic cells/ml; or producing scores (2 + or 3 + 2 by M W T or abnormality. While samples giving pH 6.7-6.8; chloride 0.13-0.14%; D M S C C 500,000 - 1000,000 and score (+) by M W T or C M T, should be considered supicious and further confirmatory tests have to be applied.

Although results of the different tests often show good correlation with bacteriological finings, yet no one test was completely satisfactory for the detection of mastitis.

In conclusion,the application of the screening tests leads to earlier detection of subclinically infected quarters and aid in the selection of dairy animals for either production or therapy.

As the cost of a sound mastitis control program is normally for 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.

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