Detection of clinical and subclinical mastitis in dairy cows of Diyala Province, Iraq

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المستخلص

يعتبر التهاب الضرع من احدى الامراض المهمة التي تسبب خسائر اقتصادية في حقول ابقار الحليب. أجريت الدراسة الحالية للتحري عن التهاب الضرع السريري اعتماداً على مستوى ارباع الضرع والعلامات السريرية والتهاب الضرع تحت السريري اعتماداً على الاختبارات المباشرة وغير المباشرة لعينات الحليب المأخوذة من الابقار في مناطق مختلفة من محافظة ديالى, من تشرين الثاني 2013 ولغاية اذار 2014. ان العدد الكلي للعينات بلغ 100 عينة اخذت من 25 بقرة اجريت لها الاختبارات الفيزيائية والكيميائية اضافة الى ذلك فحص حساب الخلايا الجسمانية والزرع الجرثومي . ثمانية (32%) من اصل 25 بقرة ظهرت عليها علامات سريرية وتغيرات في قوام الحليب من حيث وجود التخثر, اللون التبني والاحمرار وعدم تناظر ارباع الضرع , بينما (68%) من الحالات عانت من التهاب الضرع تحت السريري. بينت النتائج فروق معنوية (P<0.05) لأختبارات الأريل (88%), وايت ساید (85%), الكلورايد (100%) , ال pH (80%) و حساب الخلايا الجسمانية (97%).

بلغت عدد العزلات البكتيرية المعزولة من الابقار المصابة بالتهاب الضرع السريري وتحت السريري حوالي 40 عزلة بالجراثيم الموجبة لصبغة كرام و19 عزلة سالبة لصبغة كرام وكانت (80%) البكتريا العنقودية الذهبية , (20%) البكتريا العنقودية السالبة لاختبار التجلط , (26.31%) البكتريا القيحية , (15.79%) لكل من الباستوريلا, البكتريا القولونية , الكليبسيلا والسالمونيلا و(10.52%) لبكتريا البروتيز.

بينت نتائج الدراسة نسبة انتشار واسع للالتهاب الضرع تحت السريري لأبقار الحليب المتواجدة في حقول اقضية محافظة ديالى.

الكلمات المفتاحية : الكشف ,التهاب الضرع السريري وتحت السريري , أبقار الحليب، محافظة ديالى .

Abstract

Mastitis continues to be one of the economically most important diseases in dairy farming. The present study conducted to investigate the clinical mastitis at cow and quarter level based on clinical signs and direct / indirect tests for subclinical mastitis in dairy cows in different area of Diyala Province, from November 2013 to April 2014. The total number of samples is 100 from 25 dairy cows. These samples is tested to physical and chemical examination, as well as Somatic cell count (SCC) and bacteriological examination. Eight (32%) of twenty five cows suffered abnormalities in consistency of milk in which clot, straw color, redness and non-symmetrical quarter of udder.Whenever, 68% of cows passing subclinical mastitis. The result show significant difference at ($p<0.05$) represented by AMT (88%), WST (85%), chloride test (100%), increased pH (80%) and (97%) SCC.

Total number of bacteria species isolated from infected cow by clinical and sub clinical mastitis were 40 isolate caused by gram positive &19 caused by gram negative as follows, *Staphylococcus aureus* (80.0%) Coagulase negative *Stapylococci* (20.0%*); Pseudomonas areaginosa* (26.31%); *Pasteurella Spp.*(15.79%) , *Escherichia coli* (15.79 %); *Klebsiella Spp.*(15.79%) ;*Salmonella Spp*(15.79%) and *Proteus Spp.*(10.52%).

The study show high prevalence of subclinical mastitis among dairy cattle raised in small private farms at Diyala Province districts.

Key words: Detection, Clinical and subclinical mastitis, Dairy cows, Diyala Province.

Introduction

Mastitis is an inflammation of mammary gland parenchyma which is characterized by a range of physical and chemical changes of the milk and pathological changes in the udder tissues (1). There are two main classes of mastitis. The first is clinical mastitis, which manifests signs observed from the animal a comprise swelling, heat and pain in the udder or the milk which represent by presence of clots in milk, milk discoloration and high numbers of leukocytes in affected milk. The other is subclinical mastitis, which produces no visible signs from the udder except when using diagnostic tools. Since the quality and quantity of the milk is influenced by mastitis, it is considered to be one of the most important causes of economic losses in the dairy industry worldwide (2). Somatic cells are part of the natural defense mechanism and include lymphocytes,macrophages, polymorphonuclear cells and some epithelial cells(3). Somatic cell count (SCC) can be measured quantitatively by California mastitis test (CMT). It is a simple, easy and low cost screening test for subclinical mastitis at dairy farms (4).

There are many factors may affect SCC such as age, lactation period, parity, season, stress, management, day-to-day variation, and mainly the intramammary infection (IMI) status. The ability to correctly interpret somatic cell counts depends on an understanding of the factors which may affect the number of somatic cells (5,6 & 7).

Mastitis is usually caused by bacterial pathogens which can be classified into two groups. The contagious pathogens which include *Streptococcus agalactiae*, *Staphylococcus aureus* and *Mycoplasma bovis*as well as environmental pathogens which include *Streptococcus* species (*Streptococcus uberis*and *Streptococcus dysgalactiae*). And environmental coliforms (Gram negative bacteria *Escherichia coli*, *Klebsiella*spp., *Citrobacte*r spp., *Enterobacter*spp., *Enterobacterfaecalis*and *Enterobacterfaecium*., and other gram negative bacteria such as *Serratia*, *Pseudomonas* and *Proteus* (1).

Materials and methods

Area of study

This study carried out in five region of Diyala province which extends to the northeast of Baghdad.The total number of milk samples collected from 25 cows about 100 samples in different of crossbred dairy cows (10 cows Friesian and 15 native breed), age groups (2.5 to 7 years), parities (1 to 4) and stage of lactation (early and late) were included in the study groups.

Collection of samples:

Milk samples (n=100) were collected after proper disinfection of teat surface with 70% ethanol. 10 mL of milk from each four quarters were collected aseptically inseparate sterile polyethylene screw-capped, (8). The milk samples were placed in an ice box and carried to laboratory of clinical pathology of internal and preventive medicine department of Diyala University, where they were kept at 4 to10C^0 in a refrigerator for further laboratory investigation.

Diagnostic tests

The milk samples from apparently healthy cows were subjected to following diagnostic tests:

Indirect Examination: Arial mastitis test (AMT), Whiteside test (WST), Chloride test (CT) and the Bovi-mastitis test (Card test) for detection of milk pH

AMT or CMT: It was prepared similar to the Surf field mastitis test (SFMT) according to (1) with slight modification. 3% solution was prepared by addition of three grams of commonly used detergent powder (Arial) in 100 ml of warm distilled water; Bromocresol purple at the ratio of 1:10,000 was added to the solution. A four-well plastic paddle is used, one well-being for each quarter of the cow to be tested. The reaction was then visually scored depending upon the amount of gel formation as follows:

Negative = no reaction Trace = appearance of streaks can be made visible during rotation of the plate.

1+ = distinct thickening during rotation, but no gel

2+ = slight formation of gel which follows the rotating plate very slowly

3+ = solid formation of gel that adheres to the base of the plate

The WST was carried out according to (9) by mixing 2 drops of 4 % sodium hydroxide with 5 drops of milk on a dark glass plate, stirring for 20 seconds and show the degree of coagulation .

The Chloride test was conducted according to (9). The milk containing abnormally high percentage of chloride (> 0.14%), yellow color appeared, indicating a positive sample. Persistent red color (due to formation of silver chromate) indicated a negative sample.

The pH of milk samples were measured using the Bovi Vet. Indicator paper (Fig.1)

Figure (1): Card test.

(VMED SUPPLY, INC). A couple of drops of milk on the *test* paper recording change in the spot color. Change in colour was correlated with pH according to the manufacturer instructions and as follows; pH 6.6-6.7 pale-green, pH 6.8 moderate green, pH 7.1 green, pH 7.4 dark blue-green.

- **Direct examination** Somatic cell count (SCC).

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Procedure:

a-Thoroughly mix freshly collected milk sample. Spread 0.01 ml (10µL) of milk over an area of (1 square cm^2) on a clean slide. Spread milk evenly. Dry the slide on a flat horizontal surface, don't heat the slide. Stain with Newman- Lambert stain (Methylene blue 12gm, ethyl alcohol 95% 54ml, tetrachloroethane 40ml and glacial acetic acid 6ml). Advantage of this stain will * Remove fat. * Fix. *Stain bacteria and leukocytes.

b-Dip air dried smear in the stain for 15 seconds to 1 minute depending on stain quality.

c-Dry in air.

d-Wash with water.

e-Dry in air.

f-Examine under oil immersion field for the presence of leukocytes and bacteria.

g-Count leukocytes in 20-30 microscopical fields. Fig. (2).

Number of leukocytes/ ml of milk=
No. of cells counted x microscopical factor (4x 10^5). (8).
No. of fields examined

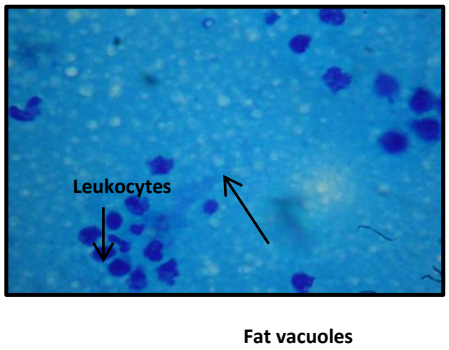

Figure (2):Milk stain –high somatic cells (leukocyte) and fat vacuole (X 100)

h-Bacteriology :

Bacterial culture was performed according to (10). Milk samples from both clinical and subclinical cases were streaked on blood and MacConkey agar plates under 24 h after sampling, then incubated under aerobic conditions at 37°C and analyzed at 24 and48 h. The culture was considered negative if no growth occurred after 48 h of incubation. Bacteria on culture-positive plates were identified according to their size, shape, color, hemolytic characteristics, Gram's stain and catalase production of colonies. For confirmation, different biochemical tests were used following sub culturing and isolation of distinct colonies (10).

Results

All dairy cows examined clinically in which if the redness, pain, enlargement, symmetrical or non-symmetrical udder and abnormalities in milk sample present or absent. Eight (32%) of twenty five cows suffered abnormalities in consistency of milk in which clot, straw color, redness and non-symmetrical quarter of udder. Whenever, 68% of cows revealed subclinical mastitis, which produces no visible signs from the udder except when using diagnostic tools.

The result of indirect and direct examination show significant difference at ($p<0.05$) as in table (1).

Table (1): Number and percentage of milk samples giving positive reaction using the four tests

Total No of milk samples	**+ AMT**	**+ WST**	**+ Chloride test**	**+ Increased pH**	**+ SCC**
100	88* (88%)	85* (85%)	100* (100%)	80 (80%)	97* (97%)
X^2=30.889 *p value* = 0.00000323 P< 0.05 S.D*					
X^2= Chi-square, S.D = Significant difference*					

AMT: Arial mastitis test. WST: Whiteside test.

The present study show the age, breed, parities and stage of lactation of cows effected on SCC values as in table (2).

Table (2): The case history, age, breed of cows and (DSCC) of milk sample.

Case number		**Age**	**Breed**	**Case history**	**Direct somatic cells (SCC) count values (Mean ±SD)**
1	4 quarter	3.5 years	Friesian	Pregnant in the eighth month	6,815,000± 3334. 921*
2	4 quarter	3.5years	local	Calving more than one month and normal lactation	2,045,000±875, 652*
3	4 quarter	7years	local	first calving and all signs are normal	1,440,000±400, 666
4	4 quarter	3 years	cross	Calving more than month ,skin lesion ,	1,625,000±754, 895
5	4 quarter	2.5 years	Friesian	Normal signs	1,223,500±1,120,462
6	4 quarter	3years	Friesian	Normal signs 2 parity	825,300±622,218
7	4 quarter	7 years	local	It's have a new parturient and normal lactation	990,000±258,457
8	4 quarter	2.5years	local	There is no signs of mastitis and symmetrical udder	975,000±114,746

9	4 quarter	3.5years	local	There is no signs of mastitis symmetrical udder	1,365,000±156,098
10	4 quarter	2 years	local	The milking and udder are normal and also the milk consistency is normal	760,000±105,830
11	4 quarter	2.5years	local	In the first stages of gestation and non-symmetrical quarter of udder	3,235,000±2,156,687
12	4 quarter	2.5years	local	Normal signs and symmetrical quarter udder	846,667±374,344
13	4 quarter	2.5years	Friesian	Normal signs 3parity hind quarter non-symmetrical	3,230,000±676,166
14	4 quarter	2.5years	Friesian	Normal signs and 2 parity	1,720,000±1,393,604
15	4 quarter	3 years	Friesian	Normal quarters of udder	980,000±192,527
16	4 quarter	4years	cross	Abnormal milk, clot, straw color and hind quarter non-symmetrical	4,005,000±2,860,507
17	4 quarter	3years	cross	Normal signs	1,235,000±425,010
18	4 quarter	2.5years	local	Abnormal milk consistency with blood, non-symmetrical quarter	4,870,000±885,513
19	4 quarter	2.5years	local	Abnormalities in consistency and presence of blood in milk	4,265,000±3,773,959
20	4 quarter	4years	local	Normal	2,095,000±874,586
21	4 quarter	5-6 years	Friesian	Abnormal consistency , blood with milk. 3parities	3789500±1838,687

22	4 quarter	7 years	Friesian	Abnormal milk, blood milk. 3parities	2940750±663253.785
23	4 quarter	4 years	Friesian	Normal, 2 parities	680750±122265.7624
24	4 quarter	6 years	local	Abnormal milk , blood milk 3parities	1277000±63608.175
25	4 quarter	4-5 years	local	Non symmetrical udder, abnormal milk	1033750±107500

Bacteriology

Milk samples from both clinical and subclinical quarters were collected for bacteriological culture. Total isolated were 40 isolate caused by gram positive &19 caused by gram negative as in table (3).

Table (3): Show the number and percentage of isolated bacteria.

Bacteria species	**No. isolated bacteria**	**(%)**
Staphylococcus. aureus	32	80.0%
Staphylococcus coagulase negative (CNS)	8	20.0%
pseudomonas Spp.	5	26.31%
Pasteurella Spp.	3	15.79%
E.coli,	3	15.79%
Klebsiella Spp.	3	15.79%
Salmonella Spp.	3	15.79%
Proteus Spp.	2	10.52%

Discussion

Clinically, cases of clinical mastitis recorded in present study agreement with other result of (11).Subclinical mastitis is highly prevalent in cattle raised at various districts of Diyala Province.

Table (1), show close correlation between number of positive reactions with AMT and WST; both tests are known to be used as indirect methods for counting leukocytes in samples of milk in the field and in the laboratory. This result was agreement with that obtained by (12), they detected similar sensitivity of Surf mastitis test (SMT), WST and chloride test.

According to the chloride test; all samples showed increase the chloride ion. It seems that there is variation in the normal level of chloride ion recorded in normal milk

samples. Elango*et al.*,(2010) (13) reported that the normal range of chloride content of healthy animal's milk was 0.08 - 0.14%. While, (14) considered that the chloride content of normal milk sample was 0.91%. The result for the chloride test agreed with that obtained by (15); they considered the chloride test invaluable to diagnose subclinical mastitis in buffaloes.

The result of compared various diagnostic tests for detection of subclinical mastitis was indicated 97% sensitivity for SCC and 88% for AMT, 85% WST reaction were agreement with result of (16,17).

In the present study showed that SCC was the most reliable test and closest to the bacteriological results. The direct microscopical examination of somatic cell count was more accurate test for diagnosis of subclinical mastitis in dairy cows. SCC was the most accurate test for the diagnosis of subclinical mastitis followed by the modified California mastitis test (MCMT) and the modified White side test (MWST), (18). Patel *et al.* (2000) (19) reported higher reliability of CMT (85.69%) followed by MWST (79.74%).

Dependent on analysis data of SCC values in table (2), the study show SCC was effected by age and breed and increased as parity increased. These finding agreement with other researches of (20,21,22) also reported higher SCC for later parities. De Haas, (2003) (20) indicated that there was a different defense mechanism against mammary infection at younger and older ages. SCC increases with progressing lactation (late lactation) regardless of whether the cow is infected or not (7). During early and late lactation the percentage of neutrophils tends to increase while the percentage of lymphocytes decreases (14). At parturition SCC are usually higher than one million per ml and decreases to 100,000 cells/ml in the 7 to 10 days post-partum.

The SCC increased above the normal range in case of inflammation or udder infection. Subclinical mastitis is a complex disease and the differences in results could be due to differences in management systems between farms, stage of lactation, parity, breed (23).

The high SCC values recorded in the present study of infected cows by subclinical mastitis may explained to differences in management systems between farms, stage of lactation, parity, breed , age and intramammary infection,. This finding agreement with other authors (22,23,24,25).

Results of culturing and isolation of the causative agents revealed that *S.aureus* were the most predominant bacteria as they were isolated from 32 (57.14%) in mammary glands infected with subclinical mastitis while , *Staphylococcus coagulase negative* (CNS)was isolated from 8 (14.28%) samples This result was agreed with the most studies on bovine mastitis where many investigators demonstrated that *S.aureus* were the most common pathogens isolated from bovine milk and the common bacteria isolated from the teat skin , the teat – end (3); (26,27) .The predominance of *Staphylococcus* spp.is also agreed with results of (28), They found that (36.8%) of bovine milk samples infected with *S.aureus* , while 10.5% were infected with *CNS*.

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Staphylococcus aureus was the most prevalent pathogens in the clinical mastitis (44.44%) while *Staphylococcus*

epidermidis was the most prevalent pathogens in the subclinical mastitis (53.84%)in Baghdad (27). Enterobacteriaceae were also isolated from several cases of bovine mastitis in this study like *E.coli* 3 (5.30%), *Klebsiella Spp.* 3(5.3%), *Salmonella Spp.* 3 (5.3%) and *Proteus Spp.* 1(1.78%), this result is agreed with results obtained from other studies in Iraq and in other countries (28,29,30).

Conclusions

In light of our findings it can be concluded that the prevalence of subclinical and clinical mastitis is high and both contagious and environmental pathogens are involved in Diyala Province. SCC was the most accurate test after cultural isolation, followed by AMT and WST.

We realized that the use of the locally prepared AMT, WST and the imported Bovi card test are suitable for early detection of subclinical mastitis in cattle in spite of slight differences in the scores recorded.

Staph. aureus in bovine mastitis were more predominant than other intramammary infecting and environmental pathogens .

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