

**The Direct Microscopical
Examination of Somatic Cell Count
to Detection of Subclinical Mastitis
in Bovine of Diyala Province.**

By

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Supervised

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَإِنَّ لَكُمْ فِي الْأَنْعَامِ لَعِبْرَةً ۖ نُسْقِيكُمْ مِمَّا فِي
بُطُونِهِ مِنْ بَيْنِ قَرْتٍ وَدَمٍ لَبَنًا خَالِصًا سَائِغًا
لِّلشَّارِبِينَ

صدق الله العظيم

سورة النحل آية ٦٦

Summary

The present study conducted to detection of subclinical mastitis by direct microscopic examination of somatic cells count (SCC) in bovine of different region of Diyala Province from November 2013 to April 2014.

The total number of milk samples collected from 20 cows examined clinically in different ages and breeds about 80 sample. The milk sample collected about 5 ml for each quarter in marked and sterile test tube with disinfected procedure at the night and refrigerated and then presented to laboratory of clinical pathology of preventive and internal medicine department of Diyala University.

The direct microscopic examination of milk staining with Newman lambert stain apparent somatic cells (leukocyte ,neutrophils, lymphocyte and monocyte), epithelial cell and gram positive (G^{+ve}) as Staphylococcus sp. (large and small cocoid) and Bacillus Sp. and negative (G^{-ve}) bacteria (rod or bacilli in shape) .The SCC in this study a ranged 90-98 % and epithelial cells 2-4% .

The present study show the age and breed of cows effected on SCC in which high SCC recorded in 2-3.5 years and Frisian cows, the SCC recorded in this study ranged 620,000 – 10,680,000 per/ μ L.

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 (Radostitset *al.* 2000). Significant milk changes that can be observed in bovine mastitis are the presence of clots in milk, milk discoloration and high numbers of leukocytes in affected milk. Furthermore, apparent clinical signs in bovine mastitis comprise swelling, heat and pain in the udder.
- 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., *Citrobacter* spp., *Enterobacter* spp., *Enterobacter faecalis* and *Enterobacter faecium*., and other gram negative bacteria such as *Serratia* , *Pseudomonas* and *Proteus* (Radostitset *al.* 2000).

Introduction

- Somatic cell count (SCC) is a useful predictor of intra mammary infection(IMI) that includes leucocytes (75%) i.e. neutrophils , macrophages, lymphocytes, erythrocytes and epithelial cells (25%). Leucocytes increase in response to bacterial infection, tissue injury and stress. Somatic cells are protective for the animal body and fight infectious organisms(Sharma *et al.*,2011).
- Somatic cells are always present in milk and they increase due to mammary gland infections. When udders are healthy the somatic cell count (SCC) in milk is between 50,000 and 100,000 cells/ml . If the SCC is greater than 200,000 cells/ml, it is assumed to be a threshold distinguishing a healthy udder from a diseased udder (Sharma *et al.*,2011).
- High SCC in milk reduces the quality of both milk and dairy products, and also affects milk shelf life and flavor, as well as cheese and butterfat yield (Sharma *et al.*,2011).

Aim of study:

This study conducted to detection of subclinical mastitis by direct microscopic examination of somatic cell count SCC in bovine of diyala province .

Literature of review

1-2 The Somatic cells

Somatic cells are mainly milk-secreting epithelial cells that have been shed from the lining of the gland and white blood cells (leukocytes) that have entered the mammary gland in response to injury or infection when udders are healthy the somatic cell count (SCC) in milk is between 50,000 and 100,000 cells/ml (Skrzypek , *et al.*, 2004). If the SCC is greater than 200,000 cells/ml, it is assumed to be a threshold distinguishing a healthy udder from a diseased udder (Harmon ,*et al.*,2001 and Skrzypek, *et al.*, 2004). High SCC in milk reduces the quality of both milk and dairy products, and also affects milk shelf life and flavor, as well as cheese and butterfat yield (Skrzypek, *et al.*, 2004 and Sharma *et al.*,2011).

The milk somatic cells include 75% leucocytes, i.e . neutrophils, macrophages, lymphocytes, erythrocytes, and 25% epithelial cells. Erythrocytes can be found at concentrations ranging from 0 to $1.51 \times 10^6 / \mu\text{l}$. The epithelial cells of the glands are normally shed and get renewed, however, during infection the numbers increase. The white blood cells serve as a defense mechanism to fight infection and assist in the repair of damaged tissue. During inflammation (mastitis) the major increase in SCC is due to the influx of neutrophils into the milk to fight infection and have been estimated at over 90% (Miller and Paape, 1985; Harmon, 1994) and the measurement of SCC in milk is known as a somatic cell count.

Literature of review

- **2-2 Function of the somatic cells**
- Mastitis is caused by bacterial invasion into the udder . The small numbers of somatic cells that are normally present in milk attempt to resolve this intra mammary infection immediately. The cellular presence in milk is one of the important protective mechanisms of the mammary gland and may be considered as a surveillance function in the uninfected gland. Both bacteria and leukocytes in the infected quarters release chemo-attractive products for leukocytes, especially neutrophils (Sharma *et al.*,2011).
- The neutrophil polymorphonuclear (PMN) leukocytes are the second line of defense against mammary gland infection. PMN's are phagocytic cells which engulf and kill bacteria. However, in bovines, the phagocytic ability of PMN of milk can consume milk fat globules and casein (Opdebeeck, 1982) leading to putrefaction of milk. An inflammatory response is usually initiated when bacteria enter the mammary gland through the teat canal and multiply in the milk. Although bacterial toxins, enzymes and cell-wall components have a direct effect on the function of the mammary epithelium, they it also stimulate the production of numerous mediators of inflammation, mainly neutrophils (Gallinet *al.*, 1992), due to edema, vasodilation and increased vascular permeability (Nonnccke and Harp, 1986).

Function of the somatic cells

- Blood monocytes become macrophages in the tissues and are the major cell type in milk during involution of the udder. During bacterial pathogenesis, macrophages serve to facilitate either innate or acquired immune responses. During lactation, the proportion of macrophages is highest (68%) in the early post-partum period and lowest (21%) in late lactation (Park *et al.*, 1992). Similar to neutrophils, the non-specific functions of macrophages are to phagocytize invading bacteria and destroy them with proteases and reactive oxygen species (ROS) (Boysoet *al.*, 2007).
- Lymphocytes are the only cells of the immune system that recognize a variety of antigenic structures through membrane receptors, which define their specificity, diversity and memory characters (Boysoet *al.*, 2007). T-lymphocytes and B-lymphocytes are two subsets of lymphocytes that differ in function and protein products and play specific immune functions (Harmon, 2001).
- The mammary epithelial cells may play a protective role in prevention of infection via ingestion and possible digestion of phagocytosed microbes. The mammary epithelial cells are able to produce a variety of inflammatory mediators such as cytokine, chemokines, host defense peptides and arachidonic acid metabolites (Harmon, 2001).

Literature of review

- **2-3 Factors affecting somatic cell count**
- Many factors may affect SCC such as age, lactation period, parity, season, stress , management, day-to-day variation, and mainly the 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(Sargeant *et al.*, 2001; Pyorala, 2003 and Berglund *et al.*, 2007).

2-3-1 Mammary gland infection level (Mastitis)

The most important factor affecting the somatic cell count of the milk from an individual quarter depends upon the infection status of the quarter (Sharma *et al.*, 2011). Sharma (2003) analyzed 2161 milk samples from lactating cows and demonstrated that $SCC \leq 100,000$ cells/ml could be considered as threshold or negative for the California mastitis test (CMT). The degree and nature of the cellular response are likely to be proportional to the severity of the infection.

2-3-2 Stage of lactation

SCC increases with progressing lactation (late lactation) regardless of whether the cow is infected or not (Berglund *et al.*, 2007). SCC elevation has been linked with an animal's innate immune response in preparation for calving and to enhance the mammary gland defense mechanism at this critical calving time (Sharma *et al.*, 2011). During early and late lactation the percentage of neutrophils tends to increase while the percentage of lymphocytes decreases (Sharma *et al.*, 2011). 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.

2-3-3 Age and Breed

Various researchers have reported that SCC increases with increasing age. This increase is primarily due to an increased prevalence of infection in older cows and is not due to any large increase due to age *per se*. SCC variation has been noted between breeds of dairy animals (Singh, 2002).

2-3-4 Parity, Season and Stress

Somatic cell counts are generally lowest during the winter and highest during the summer season (Khate and Yadav, 2010). During summer, the growth and number of environmental bacteria is increased in the bedding material of housed stock due to favorable temperature and humidity (Harmon, 1994). Free radicals are generally produced during stress due to milking techniques, environmental and infectious organisms (teat injury). These radicals are unstable and react quickly with other compounds in order to capture the electron to gain stability (Smith *et al.*, 1985).

2-3-5 Diurnal variation

In general, SCC that is lowest just before milking increases rapidly on stripping, and may persist for up to 4 hours after milking and then gradually declines. Studies have also shown that two consecutive milking from the same cow could fluctuate in SCC by 30%. Day to day variation in cell counts has also been investigated and revealed that SCC could fluctuate to more than 40% without any of the circumstances described above (Sharma *et al.*, 2011).

2-3-6 Milk transportation and Management

Methods of transportation and storage of milk samples have been demonstrated to affect SCC count (Gonzalo *et al.* (2003). There are many management factors that play a most important role in the development of contagious disease like mastitis in dairy animals. Amongst these, unhygienic conditions are more important in increasing the chances of intra mammary infection (IMI) and resulting in high SCC . Other management factors pertain to the type of flooring , feeding, teat dipping and milking techniques etc. Teat injuries and leakers commonly develop because of stall and plat form design raising the incidence of mastitis and causing higher SCC. Using a post-milking teat dip appears to predispose some very low SCC herds to more clinical mastitis-in particular mastitis caused by *E. coli*. Recently , hygienic milking has come into practice routinely to prevent the spread of *Staph. Aureus* infecting contagious mastitis (Sharma *et al.*,2011).

Materials and methods :

3-1 Instrument and requirement

Table(1). Instrument and requirement used in this study

- Newman lambert stain
- Slides
- Micropipettes 10-100 μ l and tip
- Ethanol alcohol 70%
- Sterile Cotton
- Test tube without anticoagulant
- Microscope

3-2Area of study:

All milk samples was collected in different region of Diyala province .

3-3 Sample collection :

The total number of milk samples collected from 20 cows about 80 sample in different age and breed .The milk sample collected about 5 ml for each quarter in marked and sterile test tube, with disinfected procedure at the night and refrigerated and then presented to laboratory of clinical pathology of preventive and internal medicine department of Diyala University.

Procedure:-

- Prepare four sterile test tubes, label them as; FR, FL, HR and HL.
- Wash the udder with soap and disinfect with a disinfectant.
- The teat orifice should be disinfected with iodine.
- When quarter milk samples are taken, two or three streams of milk should be discarded. In an aseptic way collect about five ml of milk from each quarter.
- If infection is present start with the infected quarters. When teat ends are dry, milk samples should be collected in the prelabelled sterile test tubes and closed with caps from near teats first and far teats last . put on ice water or refrigerated until delivered to the laboratory.
- Milk samples should not be frozen if the somatic cell counting is to be conducted as this destroys the somatic cells.(Buswell, 1995).

Procedure

Preparation of sterile tube and marked tube as
FR,FL,HR,HL



Wash udder with soap and disinfect with a
disinfectant



Procedure

An aseptic way collect about 5 ml of milk from each quarter



3-4 Laboratory examination

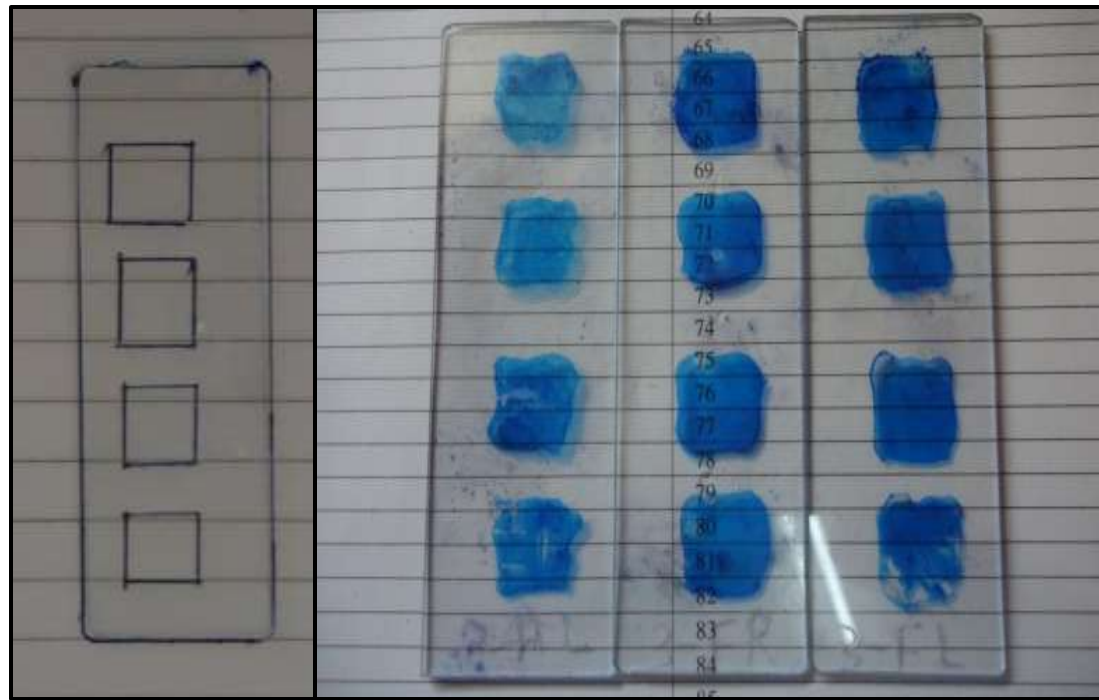
3-4-1 The direct examination of somatic cell count (DESCC).



Preparation of milk smear for detection of SCC

3-4 Laboratory examination

Slide of SCC staining with Newman Lambert stain



3-4 Laboratory examination

1. Thoroughly mix freshly collected milk sample.
2. Spread 0.01 ml (10 μ L) of milk over an area of (1 square cm²) on a clean slide. Spread milk evenly.
3. Dry the slide on a flat horizontal surface, don't heat the slide.
4. Stain with Newman- Lambert stain.

3-4-2 Composition

Methylene blue 12gm

Ethyl alcohol 95% 54ml

Tetrachloroethane 40ml

Glacial acetic acid 6ml .

This stain will; * Remove fat. * Fix. *Stain bacteria and leukocytes.

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

6. Dry in air.

7. Wash with water.

8. Dry in air.

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

10. Count leukocytes in 20-30 microscopical fields.

Number of leukocytes/ ml of milk=

No. of cells counted x microscopical factor (4×10^5). (Buswell, 1995).

No. of fields examined

Result

Clinical signs :

All udders of cows examined clinically in which if the redness, pain, enlargement, symmetrical, non symmetrical and abnormalities in milk sample present or absent. Some of cows suffered from non-symmetrical quarter of udder, redness and abnormal milk as in table (2).

Result

Laboratory examination

Direct microscopic examination of somatic cell count (DMSCC)

The direct microscopic examination of milk smear staining with Newman Lambert stain apparent SCC (leukocyte, neutrophils, lymphocyte and monocyte), epithelial cell and gram positive (G^{+ve}) as *Staphylococcus* sp. (large and small coccoid) and *Bacillus* Sp. and negative (G^{-ve}) bacteria (rod or bacilli in shape). The SCC in this study ranged 90-98% and epithelial cells 2-4%.

The present study shows the age and breed of cows affected on SCC in which high SCC recorded in 2-3.5 years and Frisian cows as in table (2). The SCC recorded in this study ranged 620,000 – 10,680,000 per/ μ L as in table (2).

Result

(A-B)milk stain high somatic cell (leukocyte) and fat vacuole (X100)

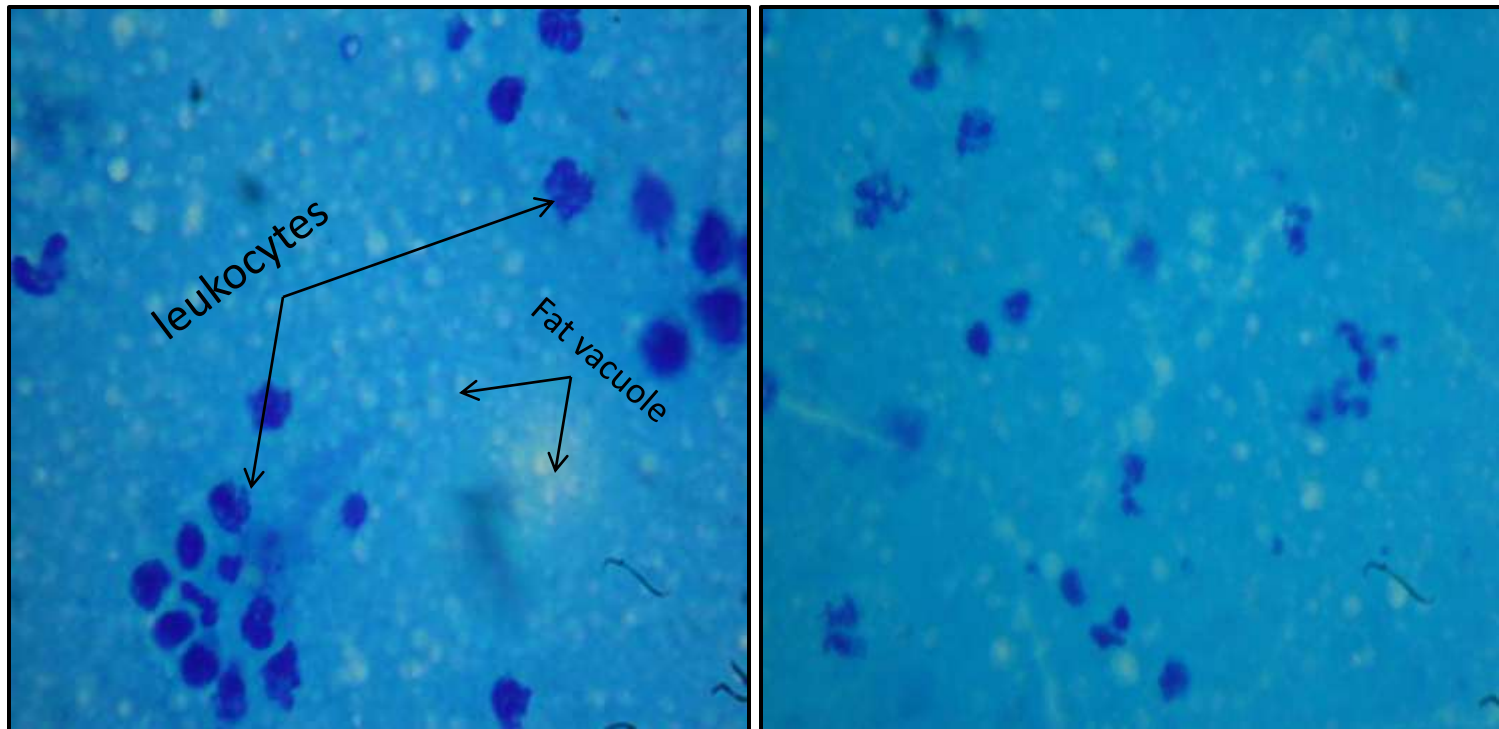


Table (2) The clinical signs, age, breed of cows and (DSCC) of milk sample .

Case number		Age	breed	Clinical signs	Direct somatic cell count (DSCC)
1	FL	3.5 years	Friesian	Pregnant in the eighth month	10,680,000
	FR				8,460,000
	HL				4,580,000
	HR				3,540,000
٢	FL	3.5 year	local	Calving more than one month and normal lactation	1,100,000
	FR				2,620,000
	HL				1,520,000
	HR				2,940,000
3	FR	2.5 year	local	first calving and all signs are normal	1,340,000
	FL				1,300,000
	HR				1,100,000
	HL				2,020,000
٤	HL	3 year	cross	parturated more than month no skin lesion	1,640,000
	FL				1,040,000
	FR				2,680,000
	HR				1,140,000

Table (2) The clinical signs, age, breed of cows and (DSCC) of milk sample

5	HL	2.5 year	Friesian cow	Normal signs	1,600,000
	FR				2,640,000
	FL				940,000
	HR				2,540,000
6	HL	2.5 year	Friesian	Normal signs 2 parity	1,140,000
	FR				1,220,000
	FL				940,000
	HR				1,200,000
7	FL	3 year	local	It's have a new parturient and normal lactation	760,000
	FR				1,360,000
	HL				940,000
	HR				900,000
8	FR	2 year	local	There is no signs of mastitis and symmetrical udder	1,120,000
	HL				980,000
	FL				960,000
	HR				840,000

Table (2) The clinical signs, age, breed of cows and (DSCC) of milk sample

9	HL	3.5 year	local	There is no signs of mastitis symmetrical udder	1,140,000
	FL				1,420,000
	FR				1,400,000
	HR				1,500,000
10	HR	2 year	local	The milking and udder are normal and also the milk consistency is normal	740,000
	FL				620,000
	HL				860,000
	FR				820,000
11	HR	2.5 year	local	In the first stages of gestation and non-symmetrical quarter of udder	5,240,000
	FR				4,960,000
	HL				1,340,000
	FL				1,400,000
12	HL	2 year	local	Normal signs and symmetrical quarter udder	720,000
	FR				Fat presence
	HR				1,120,000
	FL				1,000,000

Table (2) The clinical signs, age, breed of cows and (DSCC) of milk sample

13	HL	2.5 year	Friesian	Normal signs	2,520,000
	FR			3parity hind	2,820,000
	FL			quarter non-	3,980,000
	HR			symmetrical	3,600,000
14	FL	2.5 year	Friesian	Normal signs and	3,800,000
	FR			2 parity	900,000
	HL				960,000
	HR				1,220,000
15	HL	2.5 year	Friesian	Normal quarters	760,000
	HR			of udder	1,220,000
	FR				1,020,000
	FL				920,000
16	HL	4 year	CROSS	It's has 2 cases of	5,440,000
	FR			parturants and	1,960,000
	HR			suspected has	1,300,000
	FL			mastitis	7,320,000

Table (2) The clinical signs, age, breed of cows and (DSCC) of milk sample

17	FL	3 year	cross	Normal signs	1,860,000
	FR				1,140,000
	HR				1,000,000
	HL				940,000
18	HL	2.5 year	local	Abnormal milk consistency with blood, non- symmetrical quarter	3,980,000
	HR				4,560,000
	FL				4,860,000
	FR				6,080,000
19	FR	2.5 year	local	Abnormalities in consistency and presence of blood in milk	9,860,000
	FL				2,480,000
	HL				1,660,000
	HR				3,060,000
20	FL	4 year	local	Normal	1,760,000
	HR				1,820,000
	FR				3,380,000
	HL				1,420,000

Discussion

- In the present study it was apparent 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 of this study, present findings are in agreement with Sharma *et al.* (2008). They reported that 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).
- The result of this study in which variable value of SCC above 500,000 was agreed with (Almawet *al.*, 2008 and Sharma,etal.,2010) .The SCC increased above the normal range incase of inflammation or udder infection.
- The high SCC 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 intra mammary infection,. This finding agreement with other authors (Eyduran,*et al.*,2002, Sederevicius,*et al.*,2006 and Almawet *al.*, 2008).
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Conclusion

- According to results of study , we concluded the following
 1. The cows of area of study is infected by subclinical mastitis
 2. The SCC influencing with some factor as inramammary infection, age, parity and time of milking
 3. The SCC is accurate technique for detection of subclinical mastitis

Recommendation

- Further studies are needed to obtain more information about the mastitis and subclinical mastitis
 1. Epidemiological study of subclinical mastitis in animals of Diyala province.
 2. Study of some enzyme activity of milk in infected animal.

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الخلاصة

- أجريت الدراسة الحالية للكشف عن حالات التهاب الضرع تحت السريري بالفحص المجهرى المباشر للخلايا الجسمانية لعينات الحليب المأخوذة من أبقار مناطق مختلفة من محافظة ديالى من تشرين الثاني ٢٠١٣ ولغاية نيسان ٢٠١٤ .
- بلغت عدد العينات الكلية ثمانون عينة حليب من عشرون بقرة فحصت سريريا بمختلف الأعمار والسلالات. تم جمع عينات الحليب من الأبقار بمقدار ٥مل في أنابيب اختبار معقمة ومعلمة لكل ربع من أرباع الضرع و باستخدام طريقة التعقيم عند المساء وحفظت في الثلاجة وبعدها تم جلب العينات الى مختبر التشخيصات المخبرية فرع الطب الباطني في كلية الطب البيطري بجامعة ديالى.
- الفحص المجهرى المباشر لعينات الحليب المصبوغة بصبغة نيومان لمبرت بينت وجود الخلايا الجسمانية والتي تمثل (كريات الدم البيض ويضمونها الخلايا المتعادلة ، خلايا اللمفاوية، خلايا وحيدة النواة والخلايا الظهارية وبعض أنواع البكتريا الموجبة مثل البكتريا العنقودية والسالبة للصبغة ،حيث بلغت الخلايا البيض حوالي ٩٠-٩٨ % والخلايا الظهارية ٢-٤ % .
- أظهرت الدراسة الحالية بان العمر والسلالة لها تأثير على الخلايا الجسمانية حيث سجلت أعلى شدة إصابة بأبقار نوع الفريزيان وبأعمار ٣,٥ سنة ، حيث بلغت عد الخلايا الجسمانية من ٦٢٠,٠٠٠ إلى ١٠,٦٨٠,٠٠٠ لكل مايكرو ليتر من عينة الحليب.

الفحص المجهرى المباشر لعد الخلايا
الجسمانية للكشف عن التهاب الضرع تحت
السريري في أبقار محافظة ديالى

من قبل الطالب

احمد كامل عواد

المشرف

المدرس المساعد

طارق رفعت منت