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Developing and validation of Local Mastitis Test Reagent (LMTR) for diagnosis of subclinical mastitis in the farm animals

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Abstract

Mastitis is inflammation of the mammary gland and is considered one of the most common diseases that causing economic losses due to decreased milk production, high treatment costs, animal death, and earlier culling. Mastitis can affect one or more of the udder quarters and can be divided into different categories, clinical mastitis (CM) that reveals symptoms and subclinical mastitis (SCM). Unlike the clinical form, in subclinical form, there is neither visual detection of abnormalities in milk nor in the mammary gland. So, routine diagnostic screening tests for early detection of mastitis are necessary to treat it and avoid economic losses.

Mastitis screening tests are used the commercial kits that cost huge money. This research study intends to develop a low-price Local Mastitis Test Reagent (LMTR) for the detection of subclinical mastitis (SCM) and to examine its efficacy, accuracy, and validity at the field level. Moreover, to compare the results and the usefulness of the locally produce reagent with the commercial California mastitis tests and the Draminski mastitis test to correctly detect subclinical mastitis in dairy cows. The following substances, sodium carbonate (1%), sodium lauryl ethyl sulfate (0.7%), and Bromocreol purple (0.01%), were used for the preparation of LMTR. Thirty animals (74 Quarters) milk samples that comprised of 7 cows (7X 4 Quarters = 28 Quarters), 12 goats (12X 2 Quarters = 24 Quarters), and 11 ewes (11X 2 Quarters = 22 Quarters), were used to confirm the newly developed (LMTR) to validate its efficacy as an individual test kit in detecting SCM based on somatic cell count (SCC). The efficacy of the newly developed LMTR was compared with the California Mastitis Test (CMT) kit and Electrical conductivity test using DRAMINSKI Mastitis Detector. The results of this study reveal that subclinical mastitis test reagent, namely, (LMTR) was successful developed in this study. In this study, the percentages included 40 %, 37%, and 23% for cow, goat, and sheep, respectively. The results of CMT and LMRT for seven cows comprise 28 quarters, including RA, RH, LA, LH, which revealed agreement in the reactions and reading of the results. Additionally, 46 milk samples collected from 12 goats and 11 ewes were also showed the ability of LMRT to detect subclinical mastitis in goats and sheep in comparison to commercial CMT. The results of the current study, using the Draminski mastitis test / screening instrument for the examination of milk samples were revealed an obvious variations in all examined samples. Moreover, obvious variations in the correlation between the results of the Standard commercial California mastitis test (CMT), Local Mastitis Test Reagent (LMTR), and electroconductivity tests in the diagnosis of subclinical mastitis in cow, ewes, and goats were also seen.

In conclusion, the results of this study approved that the newly cheap-price mastitis test reagent LMTR was prepared successfully and revealed a good reliability for diagnosis of SCM in compare to California mastitis test and Electrical conductivity test using DRAMINSKI Mastitis Detector. The researchers recommend to use LMTR for the diagnosis of subclinical mastitis in the field as it is inexpensive and can simply prepare, moreover to do another future study including large numbers of animals in order to accurately validate the local product LMTR.

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Keywords: Local Mastitis test Reagent, Mastitis, California mastitis test, subclinical mastitis.

Introduction

Mastitis is an inflammation of the mammary gland. It is a response to the injury caused by bacteria in return to normal function. In dairy farms, many cases of mastitis are caused by microorganisms that invade the udder and then multiply and produce toxins that are dangerous to the mammary gland (Jones, 2009; Schroeder, 2012). As a result of inflammation in the udder, Schroeder, (2012) explained that it contributed to reductions in milk production and milk quality. The reduction of quality can be seen from levels of lactose, fat, minerals, and potassium. The changing of the milk's composition and quality can cause economic loss. The changing of milk quality is caused by bacteria that multiply in the milk, as milk is a good medium for bacterial growth.

Based on research conducted by Taponen *et al.,* (2009), the types of microorganisms that cause inflammation in the udder are *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Corynebacterium bovis*, *Staphylococcus aureus*, *Escherichia coli*, *Arcanobacterium pyogenes*. Mastitis is considered one of the most common diseases affecting economic losses due to reduced milk production, increased labor costs, increased treatment costs, animal death, and premature culling. Mastitis most commonly occurs in one of two forms - a clinical or subclinical infection.

Detection of clinical mastitis is easy, because of the visible changes in the affected mammary gland and its secretion. At the same time, diagnosis of subclinical is problematic since cow shows no physical symptoms. The milk can appears normal during subclinical mastitis, but more common can notice the increased somatic cell count and concentration of certain ions, Na^+, K^+, and Cl^-. In subclinical mastitis, clinical symptoms are not visible around the udder, such as swelling, temperature rise, redness, and pain when touched. Still, there is also a change in the milk composition (Hidayat *et al.,* 2002) that needs to be detected to prevent and treat mastitis. Subclinical mastitis acts as a continuous source of infection for other herd mates and decreases milk quality and quantity, causing huge economic loss.

In Iraq mastitis particularly, subclinical mastitis leads to huge economic losses due to the cost of treatment and culling of the infected animals. Moreover, economic losses, subclinical mastitis also have the risk for the transmission of zoonotic diseases like brucellosis, leptospirosis, tuberculosis, and streptococcal sore throat to humans. The early detection of mastitis is very important for dairy cows to diminish the economic losses related to drop in yield, increased treatment costs, and discarded milk (Bhutto *et al.,* 2012). Diverse screening approaches are used to diagnose subclinical mastitis during lactation, based on physical and chemical changes of milk (Sharma *et al.,* 2010). However, differences are found between these tests concerning accuracy (sensitivity and specificity) and cost (Fosgate *et al.,* 2013). According to the International Dairy Federation (IDF) recommendations, the detection of mastitis is based on the somatic cell count and microbiological status of the udder quarter. Though, somatic cell count increased in the first week post-calving and may remain high up to the first month of lactation (Atakan, 2008) and again increased towards the end of lactation as a normal physiological condition (Sharma and Pandey, 2011). The definitive diagnosis of mastitis needs the isolation of pathogenic bacteria, but this is an expensive method that requires time and cost. Further that, this method does not provide a measure of the degree of inflammation associated with the infection. The CMT test (California mastitis test) is one method to detect mastitis in low levels of abnormality (subclinical). This test is very easy to apply and effective at identifying mastitis (Surjowardojo *et al.,* 2008). The California mastitis test, first described and used by Schalm and Noorlander in (1957). It is a simple, quick, inexpensive, and rapid test that accurately predicts the somatic cell count in milk (Bhutto *et al.,* 2012). The California mastitis test is dependent upon the quantity of cellular nuclear protein present in the milk samples. The number of somatic cells in milk increases as the inflammatory process develops in udder tissue. The electrical conductivity/resistance of milk has been used as an indicator of mastitis for four decades, and it has a positive correlation with somatic cell count. Electrical conductivity is determined by the concentration of anions and cations in milk. As a result of the damage to the udder tissue during mastitis, the concentration of lactose and potassium decrease, and the concentration of sodium and chloride increase. Hand-held meters, such as the Draminski mastitis test, have been also endorsed as a screening instrument for subclinical mastitis in different countries (Fosgate *et al.,* 2013). However, data on the diagnostic value of this method is conflicting. Some authors point to a good correlation between electrical conductivity and bacteriological tests (Nielen *et al.,* 1992), while others consider this method inadequately sensitive (Pyörälä, 2003). Mastitis screening tests using commercially available foreign kits cost huge money. A review of the literature regarding using diagnostic screening tests for mastitis in Iraq revealed scarce publication. Consequently, the development and validation of a low-priced subclinical mastitis screening reagent are important for saving foreign money and also support the development of the farms of dairy cows in Iraq. Consequently, this study intends to develop a cheap-price local reagent for the detection of subclinical mastitis (SCM) and to examine its efficacy, accuracy, and validity at the field level. Moreover, to compare the results and the usefulness of the locally produce test reagent with the results of California mastitis tests and the Draminski mastitis test to accurately detect subclinical mastitis in dairy cows.

Materials and methods

1. Ethical statement

This study was approved by research ethical committee / College of veterinary medicine/ Al Muthanna University. Moreover, the milk samples from the animals were collected by veterinarians according to the international standard considering animal welfare and ethics.

2. Development of Local Mastitis Test Reagent (LMTR)

To develop and prepare LMTR, the following materials have used

A. sodium carbonate (1%)
B. sodium lauryl ethyl sulphate (0.7%)
C. Bromo cresol purple (0.01%) .

D. A series of trials were done to prepare LMTR by mixing the above substance and compared with commercial available foreign kits (commercial California Mastitis Test) reagent that imported form USA and cost about 125$.

3. Electrical conductivity test using DRAMINSKI Mastitis Detector

Electrical conductivity test is determined for milk samples using portable electrical conductivity meter (milk checker or digital mastitis detector) and is expressed in the unit of milk seimens/cm. For purpose of this study, DRAMIŃSKI ELECTRONIC MASTITIS DETECTOR was borrowed from Al Muthanna veterinary teaching hospital. The DRAMIŃSKI ELECTRONIC MASTITIS DETECTOR device (Figure. 1) was developed by Manufacturer DRAMIŃSKI / Poland (www.draminski.com) to detect mastitis in animals.

Figure. 1: Shows the DRAMIŃSKI ELECTRONIC MASTITIS DETECTOR

According to manufacturer, device consists of :

A. a measurement cup with electrodes
B. electronic unit with LCD panel
C. handle containing a standard 9 volt battery
D. a switch.

The instrument is made of polypropylene, resistant to atmospheric conditions, most chemicals, shock, breakage, and not simply wet by milk. It is sealed and waterproof, for easy washing and cleaning.

A minimum of 15 ml of milk (indicated by the line inside the cup) is required, and the sample must be first foremilk for the most accurate readings to be taken. Later milk samples will give slightly different readings as this milk comes from other parts of the quarter, which are often uninfected. Before use, examiner should be sure that the electrodes in the cup are clean. Contamination of electrodes with skin-oil from handling or examination while on display in stores and/or dried milk fat can result in incorrect (high) readings and methylated spirits is required to wipe the electrodes with on a clean cloth or tissue. The DRAMIŃSKI Mastitis Detector is a highly sensitive electronic instrument designed to measure very small changes in milk electrical resistance accurately. The instrument has been designed for use in the often hostile environment of the milking area and is sufficiently robust, shockproof, and waterproof to resist most normal working conditions. When switched on with no milk in the cup, first the LCD will display two dashes (a), indicating that the battery is connected and the instrument operating, but no measurement is being taken. we need to press the switch once again. The numbers “1 0” will be displayed (b).

a)
| - - |

b)
| 1 0 |

Procedures of measurement and reading the results:
1. Press the ON/OFF switch. After pressing the button two dashes are displayed (a).
2. Place the cup under the teat A. Squirt the milk directly into the cup to fill it up (minimum 1 cm from the cup edge).
3. After about 1 second press the switch to turn the detector on – the result will be displayed (c)

c)
| 370 |

4. Pour the milk out and shake off the milk remaining into the empty bucket.
5. Repeat these actions for the tits B, C and D to take measurements for all 4 quarters.
6. After examining the udder it is necessary to clean the measurement cup in the following way: immerse the cup in the water moving and turning the handle so that all milk remaining have been removed.

Interpretation of the results

The DRAMIŃSKI Mastitis Detector has a wide scale of readings on the LCD providing the opportunity to interpret the results.
There is no fixed point or number where mastitis is definitely present, or not present. Rather, there are increasing or decreasing degrees of infection as resistance changes.

1. Readings above 300 units: The milk sample is of high quality and is healthy. The incidence of subclinical mastitis is very low.
2. Readings between 300 and 250 units: A progressively increasing incidence of subclinical infection as readings decrease.
3. Readings below 250 units: This is an indication of a rapid increase in the severity of infection as subclinical mastitis progresses to clinical states. This is typified by somatic cells present rising from less than 1 million up to many millions.

4. **Samples collection**

In this study, 30 animals comprise seven cows, 12 goats, and 11 ewes (Figure. 2 A&B). These were used to collect milk samples; these animals were presented to Al Muthanna veterinary teaching hospital for various purposes such as pregnancy diagnosis or sick animals for another reason. The udder and teats were washed with warm water and air-dried for disinfection, whereas the udder, the tip were wiped off by 70% alcohol. The first few chills of milk were discarded, and milk samples were collected in a sterile test tube to avoid any type of contamination.

All samples were subjected to diagnosis of subclinical mastitis using the following tests:

A. Electrical conductivity test using DRAMINSKI Mastitis Detector was done according to previous described procedures.
B. Commercial California mastitis test (CMT)

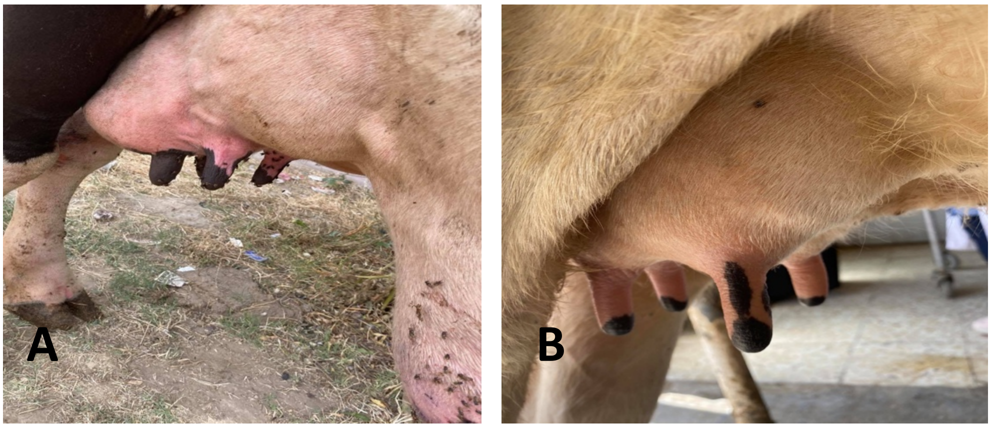

Figure. 2 A& B: Shows the udder of the cows

All milk samples collected were subjected to standard Commercial California mastitis test. The test was carried out according to the method described by *Schalm and Noorlander (1957)*, at cowside by mixing gently an equal volume of milk with reagent (2 mL) in a paddle (Figure. 3). Milk color changes or formation of a viscular gel are readable within 1-2 minutes. The results of CMT were scored according to the manufacturer's instructions and based on the reactions, the results were graded as negative (-), trace (T), weak positive (+), distinct positive (++), strong positive reaction (+++) and very strong reaction (++++) .

Figure. 3: Shows the standard CMT

C. **Local Mastitis Test Reagent (LMTR)**

All milk samples collected were subjected to LMTR to justify its efficacy and to validate it as an individual test reagent in compare to CMT in detecting subclinical mastitis based on somatic cell count (SCC). The results of LMTR were scored also like the scoring of CMT depending on the changes of the color and gel formation that also classified into as (-), (T), (+), (++) , (+++) and (++++) for negative, trace, weak positive, distinct positive and strong positive reaction and very strong reaction respectively (Figure.4).

Figure. 4: Shows the standard LMTR

Results

The total number of examined animals included in this study was 30 (74 Quarters) that comprised of 7 cow (7X 4 Quarters = 28 Quarters) , 12 goats (12X 2 Quarters = 24 Quarters) and 11 ewes (11X 2 Quarters = 22 Quarters). All these animals were used to collect milk samples (Table. 3). The percentages of animal species included in this study were 40 %, 37% and 23% for cow, goat and sheep respectively (Figure. 5).

Table. 3: Shows all the results of CMT, LMRT and Electric conductivity test for udder quarters of animals

No.	Animal species	CMT		LMTR		Electric conductivity test	
1	Cow	RA	+	RA	+	RA	320
		RH	±	RH	±	RH	380
		LA	++	LA	+	LA	340
		LH	+	LH	+	LH	440
2	Cow	RA	-	RA	-	RA	560
		RH	-	RH	-	RH	470

		LA	-	LA	-	LA	840
		LH	-	LH	±	LH	710
3	Cow	RA	+++	RA	+++	RA	290
		RH	+++	RH	+++	RH	260
		LA	+++	LA	+++	LA	290
		LH	++++	LH	++++	LH	340
4	Cow	RA	++++	RA	++++	RA	430
		RH	++	RH	++	RH	520
		LA	+++	LA	+++	LA	490
		LH	++	LH	++	LH	500
5	Cow	RA	-	RA	-	RA	480
		RH	-	RH	-	RH	870
		LA	-	LA	+	LA	857
		LH	-	LH	-	LH	560
6	Cow	RA	++	RA	++	RA	340
		RH	+++	RH	+++	RH	290
		LA	++	LA	++	LA	400
		LH	+++	LH	+++	LH	370
7	Cow	RA	+++	RA	++++	RA	250
		RH	+++	RH	+++	RH	290
		LA	++++	LA	++++	LA	340
		LH	+++	LH	+++	LH	310
8	goat	R	±	R	±	R	500
		L	-	L	-	L	760
9	goat	R	+++	R	++++	R	320
		L	±	L	±	L	440
10	goat	R	+++	R	+++	R	360
		L	+	L	+	L	400
11	goat	R	+++	R	+++	R	360
		L	+	L	+	L	370
12	goat	R	-	R	-	R	460
		L	-	L	-	L	320
13	goat	R	+	R	+	R	310
		L	+	L	+	L	290
14	goat	R	++++	R	++++	R	200
		L	++++	L	++++	L	190
15	goat	R	+++	R	+++	R	380
		L	+	L	+	L	370
16	goat	R	++++	R	++++	R	180
		L	++	L	+++	L	210
17	goat	R	±	R	±	R	335
		L	-	L	-	L	640
18	goat	R	-	R	-	R	900
		L	-	L	-	L	810
19	goat	R	++++	R	++++	R	257
		L	++++	L	++++	L	185
20	ewe	R	-	R	-	R	920

		L	-	L	-	L	650
21	ewe	R	+++	R	+++	R	290
		L	+++	L	+++	L	260
22	ewe	R	++++	R	++++	R	200
		L	+	L	+	L	460
23	ewe	R	++	R	+++	R	360
		L	+	L	+	L	370
24	ewe	R	-	R	-	R	900
		L	-	L	+	L	810
25	ewe	R	-	R	-	R	920
		L	-	L	-	L	810
26	ewe	R	+	R	±	R	680
		L	+	L	+	L	730
27	ewe	R	++	R	++	R	750
		L	±	L	+	L	640
28	ewe	R	++	R	+++	R	375
		L	±	L	±	L	387
29	ewe	R	-	R	-	R	857
		L	-	L	±	L	560
30	ewe	R	+	R	+	R	310
		L	++	L	+++	L	210

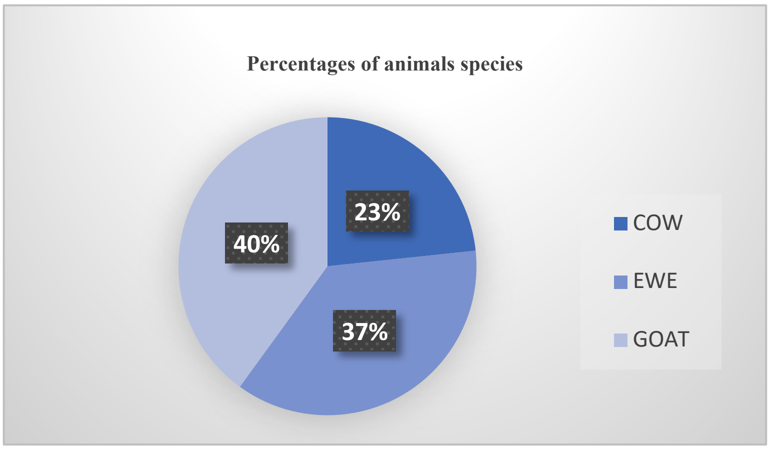

Figure. 5: Shows the distribution of the species of the animals used in this study

The Standard commercial California mastitis test (CMT) and Local Mastitis Test Reagent (LMTR) were revealed various results ranges between (-) negative for non-infected quarter to (++++) for very strong positive reaction. The Standard CMT and LMTR reactions and its percentages for cows, goat and ewes udder quarters are as following:

A. Cows

1. Right anterior

The results of CMT were 2 (7.14 %) , 0(0%), 1(3.57 %), 1(3.57 %), 2(7.14 %) and 1(3.57 %) for negative, suspected, positive 1, positive 2, positive 3, and positive 4 respectively. While the results of LMTR were 2(7.14 %), 0(0 %), 1(3.57 %),1(3.57 %),1(3.57 %), and 2 (7.14 %) for negative, suspected, positive 1, positive 2, positive 3, and positive 4 respectively (Table. 4; Figure. 6).

Table. 4: Shows the results of CMT and LMTR for Right anterior quarter udder (RA) / Cow

Quarter (RA)	CMT	LMTR
Negative	2	2
Suspected ±	0	0
Positive 1	1	1
Positive 2	1	1
Positive 3	2	1
Positive 4	1	2

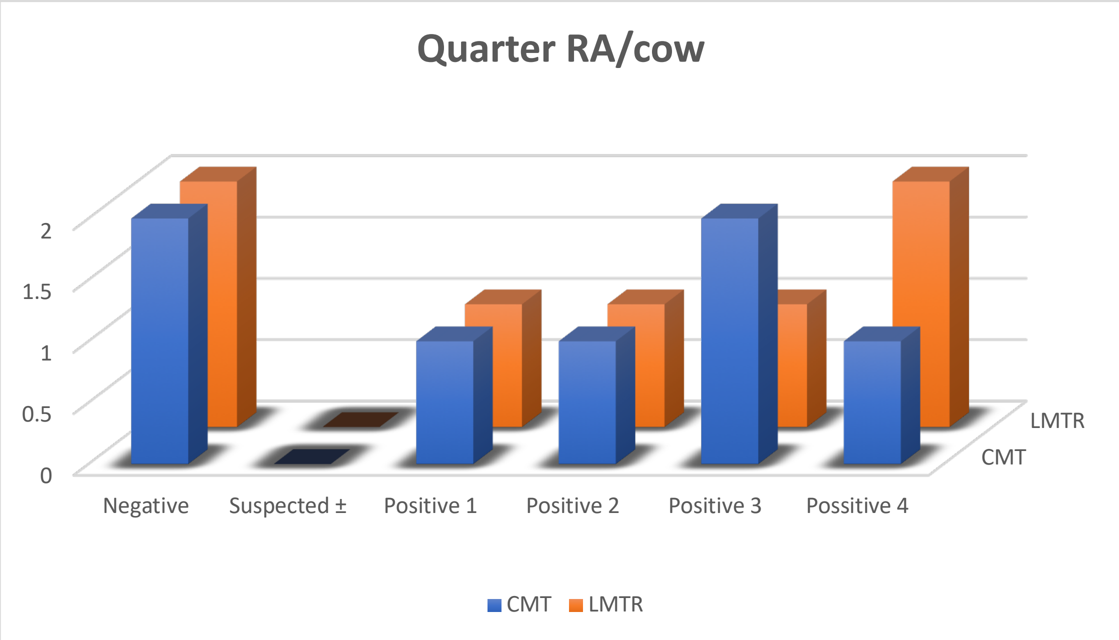

Figure. 6: Shows the results of CMT and LMTR for Right anterior quarter udder (RA) / Cow

2. Right Hind

The results of CMT were 2 (7.14 %) , 1(3.57 %), 0(0%), 1(3.57 %) 3 (10.17 %) and 0 (0 %) for negative, suspected, positive 1, positive 2, positive 3, and positive 4 respectively. While the results of LMTR were 2 (7.14 %) , 1(3.57 %), 0(0%), 1(3.57 %) 3 (10.17 %) and 0(0 %) for negative, suspected, positive 1, positive 2, positive 3, and positive 4 respectively (Table. 5; Figure. 7).

Table. 5: Shows the results of CMT and LMTR for Right Hind quarter udder (RH) / Cow

Quarter (RH)	CMT	LMTR
Negative	2	2
Suspected ±	1	1

Positive 1	0	0
Positive 2	1	1
Positive 3	3	3
Positive 4	0	0

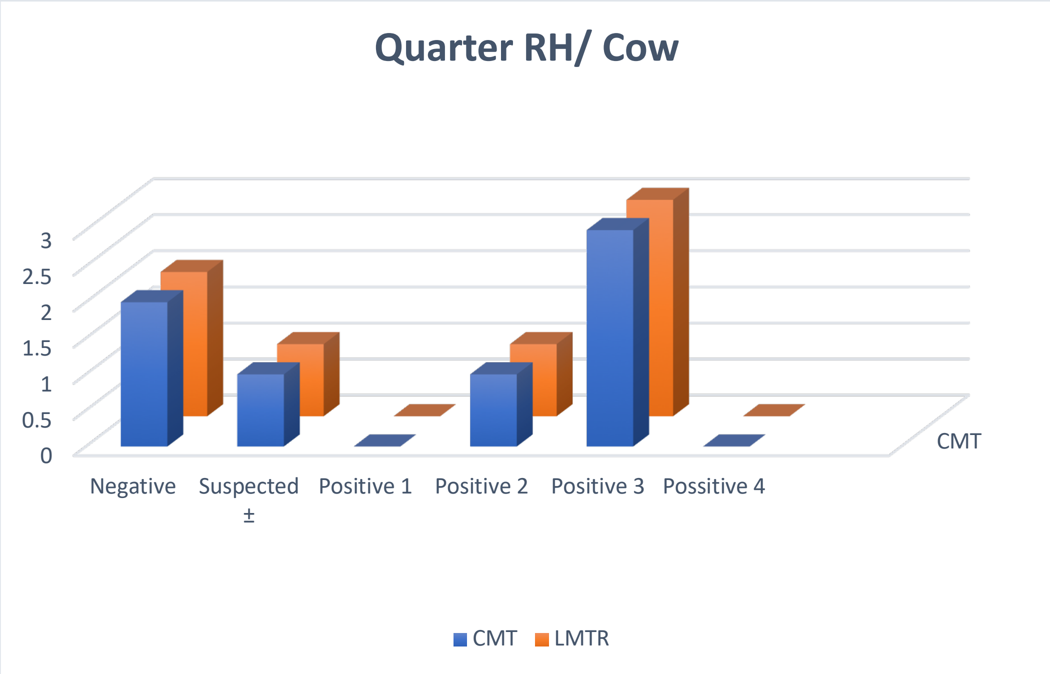

Figure. 7: Shows the results of CMT and LMTR for Right hind quarter udder/ Cow

3. Left anterior

The results of CMT were 1(3.57 %), 0 (0%), 2 (7.14 %) , 1(3.57 %), , 2(7.14 %) and 1(3.57 %) for negative, suspected, positive 1, positive 2, positive 3, and positive 4 respectively. While the results of LMTR were 1(3.57 %), 0(0%), 2 (7.14 %), 1(3.57 %), 2 (7.14 %), and 1(3.57 %) for negative, suspected, positive 1, positive 2, positive 3, and positive 4 respectively (Table. 6; Figure. 8).

Table. 6: Shows the results of CMT and LMTR for Left anterior quarter udder (LA) / Cow

Quarter LA	**CMT**	**LMTR**
Negative	1	1
Suspected ±	0	0
Positive 1	2	2
Positive 2	1	1
Positive 3	2	2
Positive 4	1	1

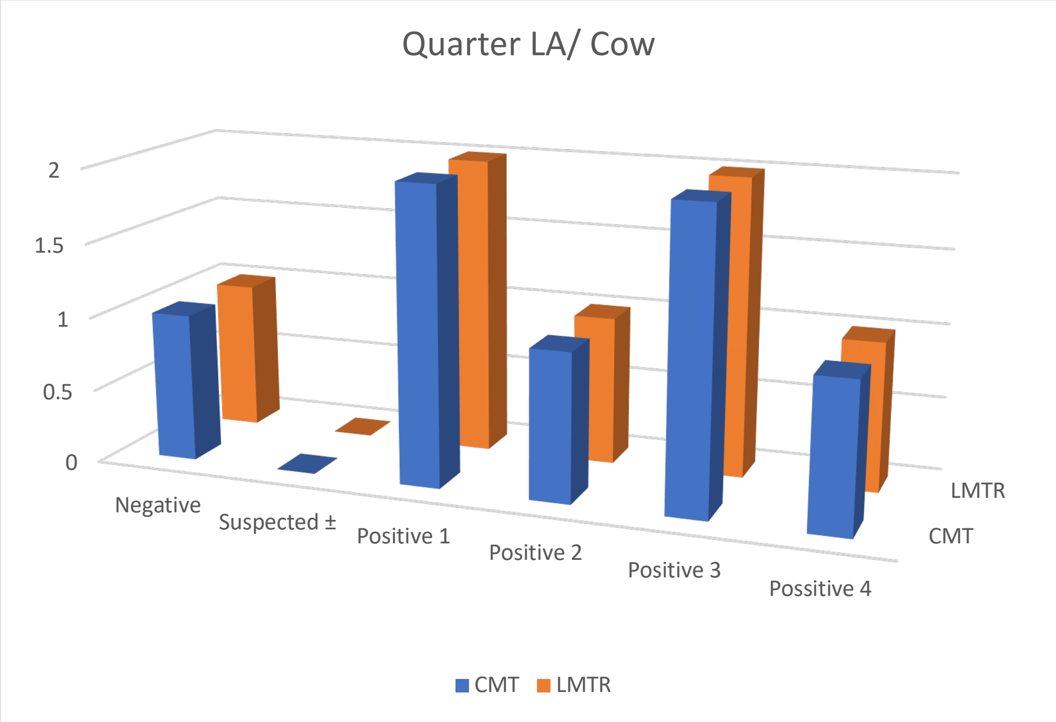

Figure. 8: Shows the results of CMT and LMTR for Left anterior quarter udder (LA) / Cow

4. Left Hind

The results of CMT were 2 (7.14 %) , 0(0%), 1(3.57 %), 1(3.57 %), 2 (7.14 %) and 1(3.57 %)for negative, suspected, positive 1, positive 2, positive 3, and positive 4 respectively. While the results of LMTR were 1(3.57 %), 1(3.57 %), 1 (3.57 %), 1(3.57 %), 2 (7.14 %), and 1(3.57 %) for negative, suspected, positive 1, positive 2, positive 3, and positive 4 respectively (Table. 7; Figure. 9).

Table. 7: Shows the results of CMT and LMTR for Left Hind quarter udder (LH) / Cow

Quarter LH	**CMT**	**LMTR**
Negative	2	1
Suspected ±	0	1
Positive 1	1	1
Positive 2	1	1
Positive 3	2	2
Positive 4	1	1

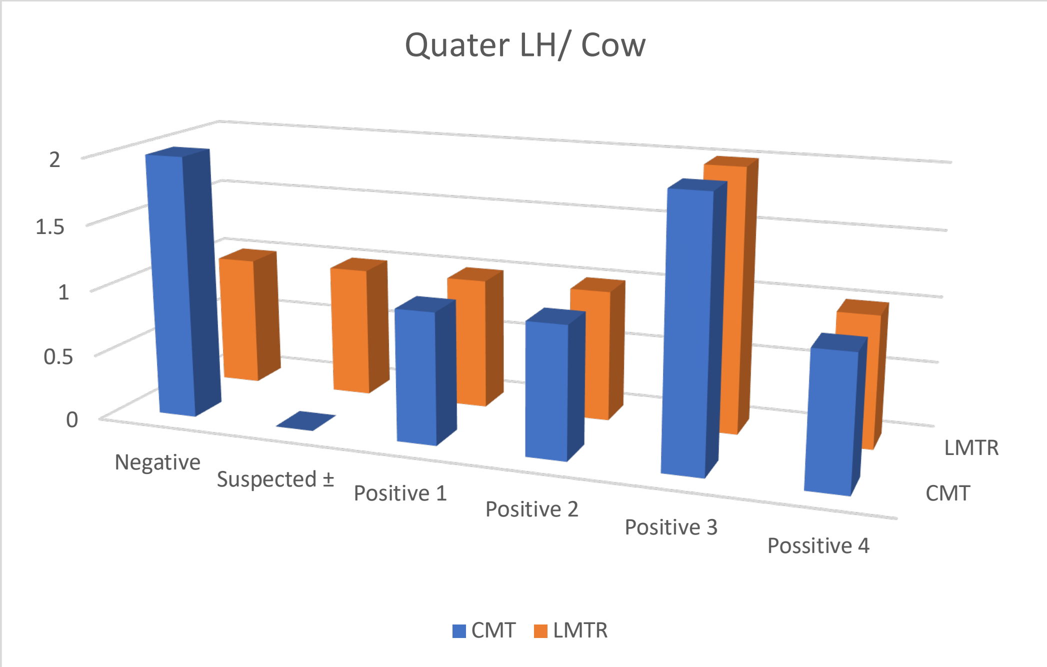

Figure.9: Shows the results of CMT and LMTR for Left Hind quarter udder (LH) / Cow

B. Goat

1. Right quarter

The results of CMT were 2 (8.33 %) , 2 (8.33 %), 1(4.16 %), 0(0%), 4(16.66 %) and 3(12.5 %) for negative, suspected, positive 1, positive 2, positive 3, and positive 4 respectively. While the results of LMTR were 2 (8.33 %), 3(12.5 %) , 1(4.16 %), 0(0 %), 2 (8.33 %), and 4(16.66 %) for negative, suspected, positive 1, positive 2, positive 3, and positive 4 respectively (Table. 8; Figure. 10).

Table. 8: Shows the results of CMT and LMTR for Right quarter udder (R) / Goat

Right quarter udder (R)	**CMT/Goat**	**LMTR/Goat**
Negative	2	2
Suspected ±	2	3
Positive 1	1	1
Positive 2	0	0
Positive 3	4	2
Positive 4	3	4

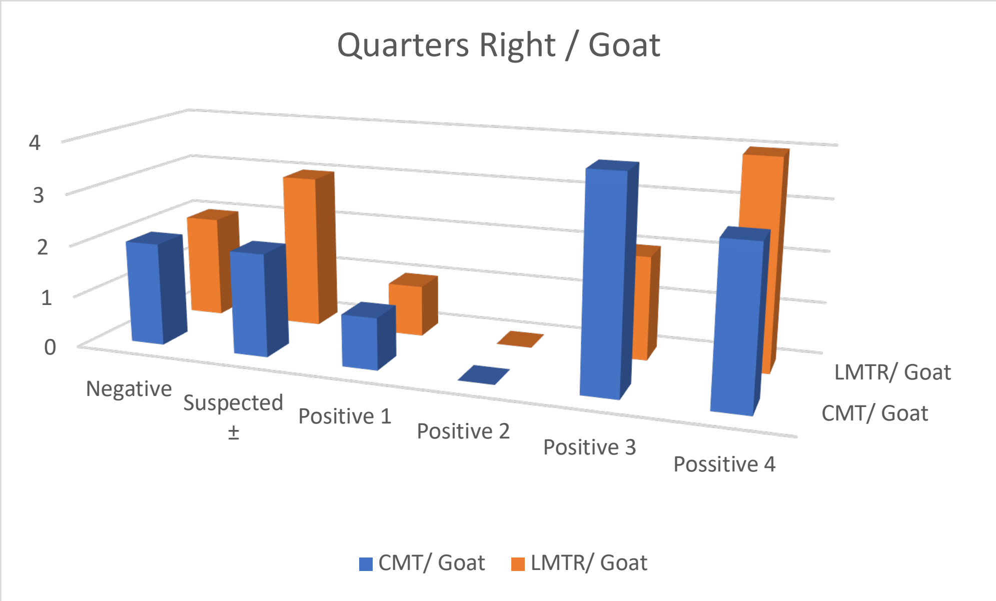

Figure.10: Shows the results of CMT and LMTR for Right quarter udder (R) / Goat

2. Left quarter

The results of CMT were 2 (7.14 %) , 0(0%), 1(3.57 %), 1(3.57 %), 2(7.14 %) and 1(3.57 %) for negative, suspected, positive 1, positive 2, positive 3, and positive 4 respectively. While the results of LMTR were 2(7.14 %), 0(0 %), 1(3.57 %),1(3.57 %),1(3.57 %), and 2(7.14 %) negative, suspected, positive 1, positive 2, positive 3, and positive 4 respectively (Table. 9; Figure. 11).

Table. 9: Shows the results of CMT and LMTR for Left quarter udder (L) / Goat

Quarter L	**CMT/ Goat**	**LMTR/ Goat**
Negative	4	4
Suspected ±	1	1
Positive 1	4	4
Positive 2	1	0
Positive 3	1	1
Positive 4	2	2

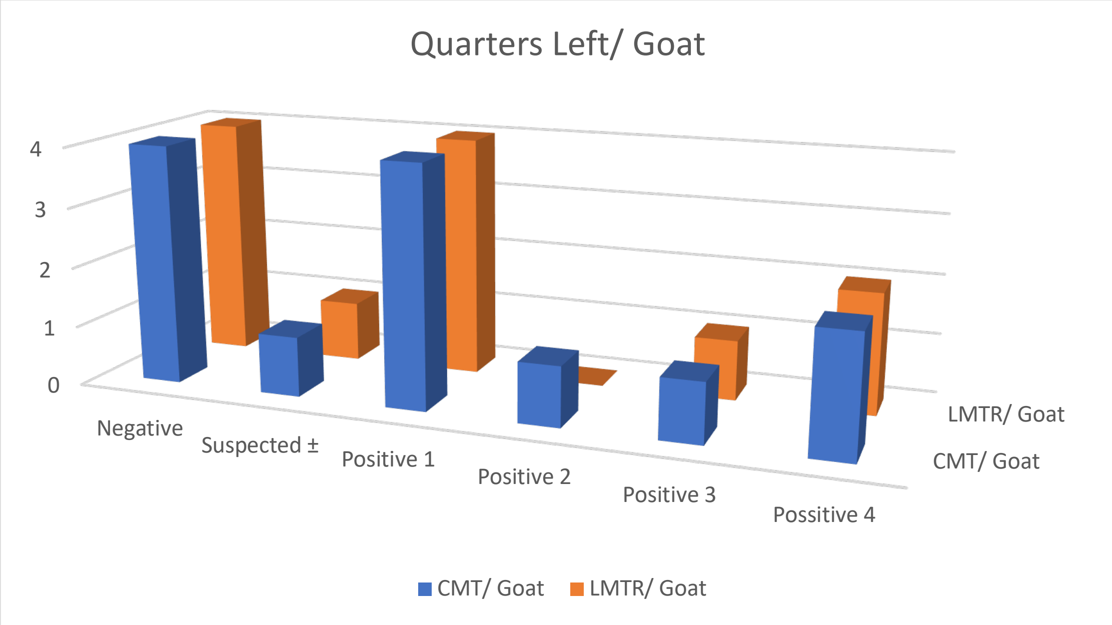

Figure.11: Shows the results of CMT and LMTR for Left quarter udder (L) / Goat

C. Ewes

1. Right quarter

The results of CMT were 4(18.18%), 0(0%), 2(9.09%), 3(13.63%),1(4.54 %) and 1(4.54 %)for negative, suspected, positive 1, positive 2, positive 3, and positive 4 respectively. While the results of LMTR were 4(18.18%), 1(4.54 %), 1(4.54 %),1(4.54 %), 3(13.63%)and 1(4.54 %) negative, suspected, positive 1, positive 2, positive 3, and positive 4 respectively (Table. 10; Figure. 12).

Table. 10: Shows the results of CMT and LMTR for Right quarter udder (R) / Ewe

Quarter R	CMT/Ewe	LMTR/Ewe
Negative	4	4
Suspected ±	0	1
Positive 1	2	1
Positive 2	3	1
Positive 3	1	3
Positive 4	1	1

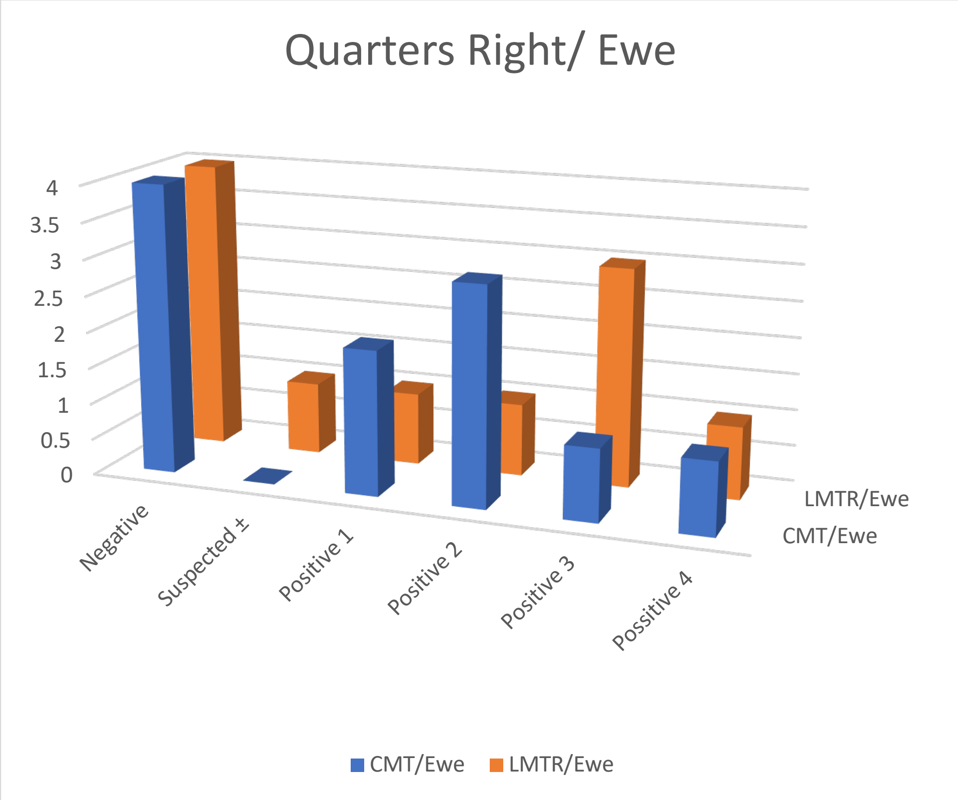

Figure. 12: Shows the results of CMT and LMTR for Right quarter udder (R) / Ewe

2. Left quarter

The results of CMT were 4 (18.18%), 2(9.09%), 3(13.63%), 1(4.54 %), 1(4.54 %) and 0(0%) for negative, suspected, positive 1, positive 2, positive 3, and positive 4 respectively. While the results of LMTR were 2(9.09%), 0(0 %), 5 (22.72%), 0(0 %),1(4.54 %) and 0(0 %) for negative, suspected, positive 1, positive 2, positive 3, and positive 4 respectively (Table. 11; Figure. 13).

Table. 11: Shows the results of CMT and LMTR for Left quarter udder (L) / Ewe

Quarter L	CMT/Ewe	LMTR/Ewe
Negative	4	2
Suspected ±	2	0
Positive 1	3	5
Positive 2	1	0
Positive 3	1	1
Positive 4	0	0

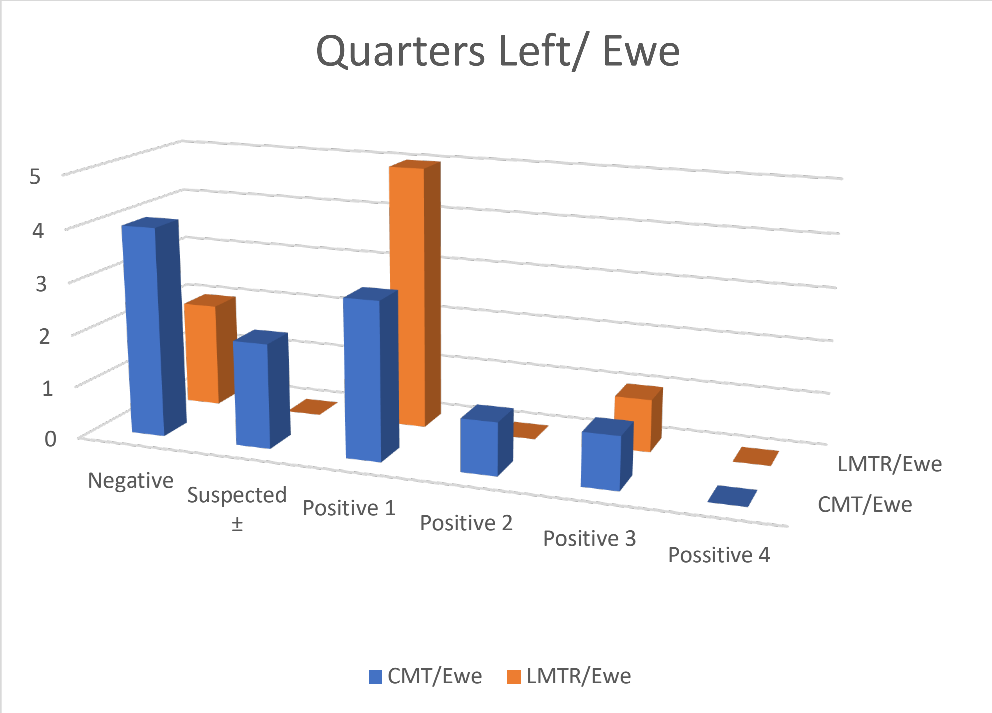

Figure. 13: Shows the results of CMT and LMTR for Left quarter udder (L) / Ewe

The results of the electroconductivity tests showed variation between the milk of udder quarters of different species of the animals. The electroconductivity were as following (the unit of milk seimens/cm):

1. Cow (Table. 12; Figure. 14).

A. Right anterior (RA) quarters: 320, 560, 290, 340, 480, 340, and 250 for C1, C2, C3, C4, C5, C6 and C7 respectively, and including the lowest value 250 in C7 and the highest value 560 in C2.
B. Right hind (RH) quarters: 380, 470,260,520, 870,290 and 290 for C1, C2, C3, C4, C5, C6 and C7 respectively, and including the lowest value 290 in C6 and C7 and the highest value 870 in C5.
C. Left anterior (LA) quarters: 340, 840, 290, 490, 857, 400 and 340 for C1, C2, C3, C4, C5, C6 and C7 respectively, and including the lowest value 290 in C3 and the highest value 857 in C5.
D. Left Hind (LH) quarters: 440, 710, 340, 500, 560, 370 and 310 for C1, C2, C3, C4, C5, C6 and C7 respectively, and including the lowest value 310 in C7 and the highest value 710 in C2.

Table. 12: Shows the results of electroconductivity tests in the RA, RH, LA and LH quarters in the cows (the unit of milk seimens/cm)

No	**RA**	**RH**	**LA**	**LH**
C1	320	380	340	440
C2	560	470	840	710
C3	290	260	290	340
C4	340	520	490	500
C5	480	870	857	560
C6	340	290	400	370
C7	250	290	340	310

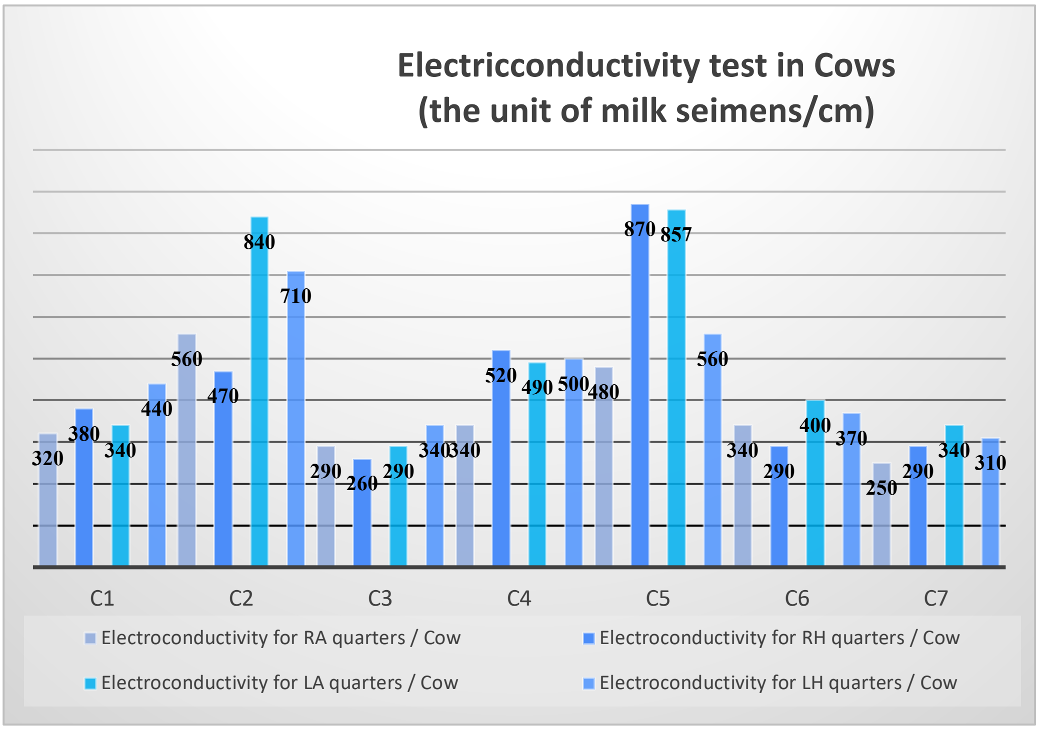

Figure. 14: Shows the results of electroconductivity tests in the RA, RH, LA and LH quarters in the cows (the unit of milk seimens/cm).

2. Goat (Table. 13; Figure. 15).

   A. Right quarters: 500, 320, 360, 360, 460, 310, 200, 380, 180, 335, 900 and 257 for G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, and G12 respectively, and including the lowest value 180 in G9 and the highest value 900 in G11.
   B. Left quarters: 760, 440, 400, 370, 320, 290, 190, 370, 210, 640, 810 and 185 for G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, and G12 respectively, and including the lowest value 185 in G12 and the highest value 810 in G11.

Table. 13: Shows the results of electroconductivity tests in the Right and left quarters in the goat (the unit of milk seimens/cm)

No of Goats	**Electric conductivity test/ Right Quarter/ Goat**	**Electric conductivity test / Left Quarter/ Goat**
G1	500	760
G2	320	440
G3	360	400
G4	360	370
G5	460	320
G6	310	290
G7	200	190
G8	380	370
G9	180	210
G10	335	640
G11	900	810
G12	257	185

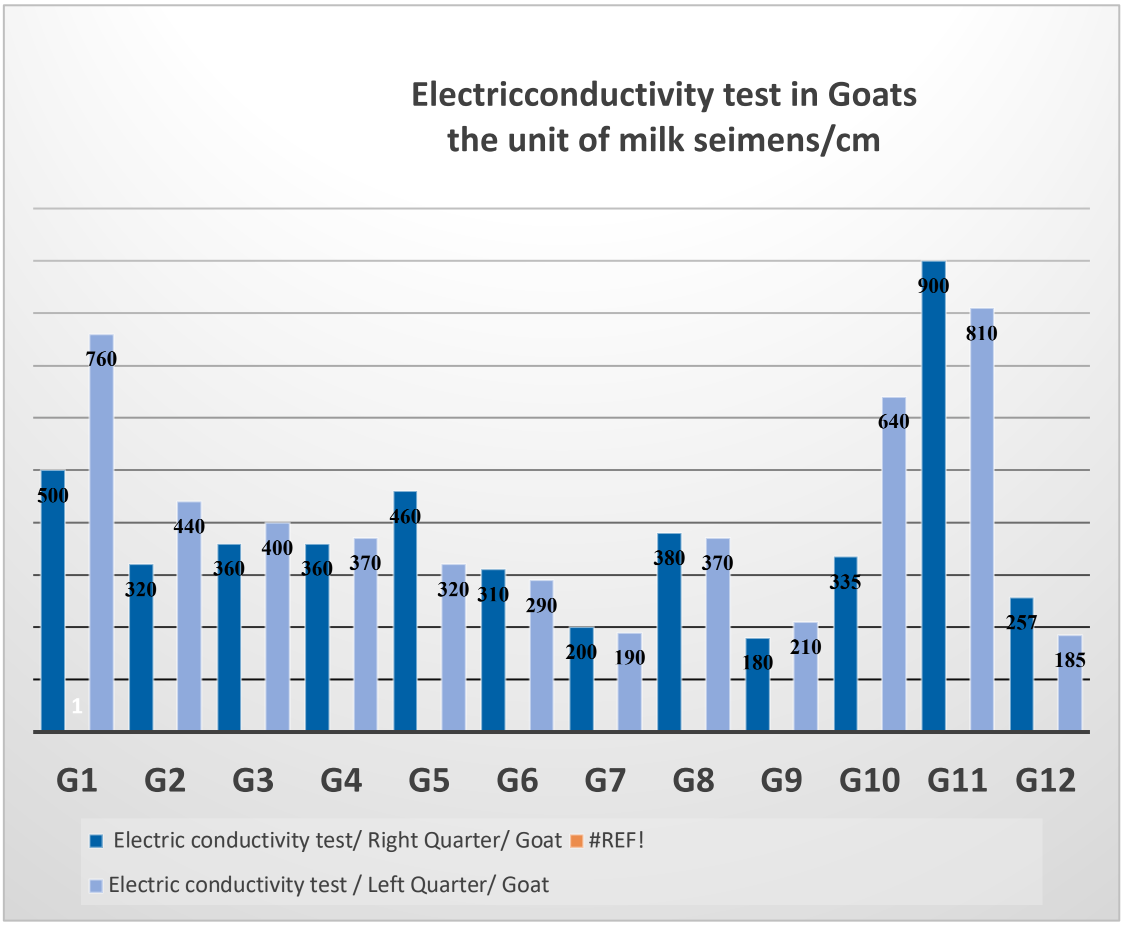

Figure. 14: Shows the results of electroconductivity tests in the Right and left quarters in the goat

3. Ewes (Table. 14; Figure. 16).

   A. Right quarter: 920, 290, 200, 360, 900, 920, 680, 750, 375, 857 and 310 for E1, E2, E3, E4, E5, E6, E7, E8, E9, E10, and E11 respectively, and including the lowest value 200 in E3 and the highest value 920 in E1 and E5.
   B. Left t quarter: 650, 260, 460, 370, 810, 810, 730, 640, 387, 560 and 210 for E1, E2, E3, E4, E5, E6, E7, E8, E9, E10, and E11 respectively, and including the lowest value 260 in E2 and the highest value 920 in E5.

Table. 14: Shows the results of electroconductivity tests in the Right and left quarters in the ewes (the unit of milk seimens/cm)

No of Ewes	**Electric conductivity test Right Quarter/ Ewes**	**Electric conductivity test / Left Quarter/ Ewes**
E1	920	650
E2	290	260
E3	200	460
E4	360	370
E5	900	810
E6	920	810
E7	680	730
E8	750	640
E9	375	387
E10	857	560
E11	310	210

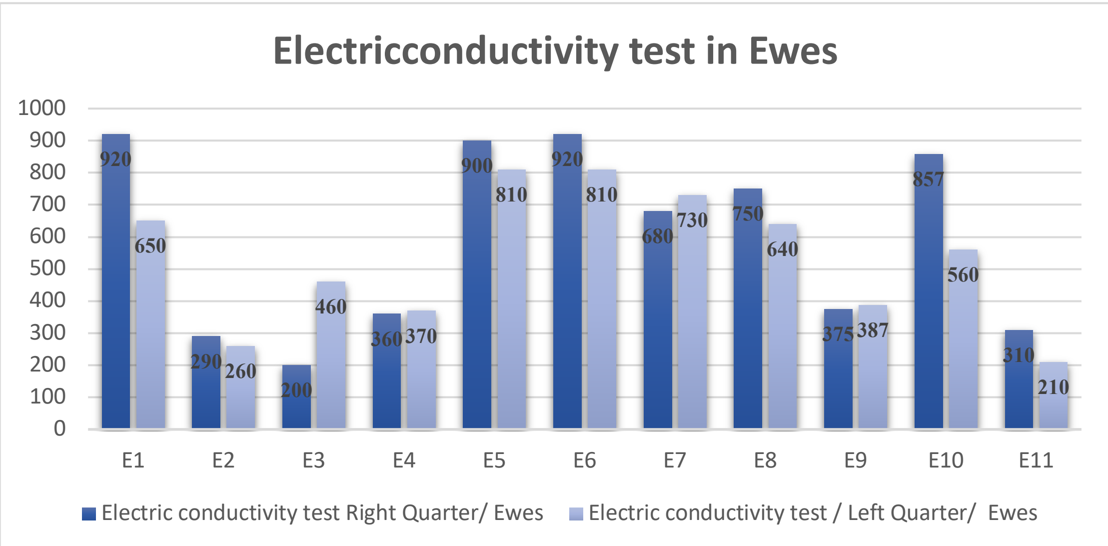

Figure. 16: Shows the results of electroconductivity tests in the Right and left quarters in the ewes

According to DRAMIŃSKI manufacturer instruction, the interpretation of the results revealed
no fixed point or number where mastitis is definitely present, or not present. Rather, there are increasing or decreasing degrees of infection as resistance changes. And there are 3 interpretation degrees:

1. Readings above 300 units: The milk sample is of high quality and is healthy. The incidence of subclinical mastitis is very low.
2. Readings between 300 and 250 units: A progressively increasing incidence of subclinical infection as readings decrease.
3. Readings below 250 units: This is an indication of a rapid increase in the severity of infection as subclinical mastitis progresses to clinical states. This is typified by somatic cells present rising from less than 1 million up to many millions.

And according to this interpretation degrees, the results of study showed that the examined milk samples from the cows revealed a variation results with the results of electroconductivity test and as follow:

1. Cows(Table. 15; Figure. 17).

   A. There were 6 and 6 positive results for CMT and LMTR that revealed under 300 units readings ranged from 250 to 290 units.
   B. There were 9 and 8 negative results for CMT and LMTR that revealed above 300 units reading ranged from 380 to 870 units.
   C. There were 13 and 14 positive results for CMT and LMTR that revealed above 300 units reading ranged from 310 to 520 units.

Table. 15: Shows the correlation between the results of the Standard commercial California mastitis test (CMT), Local Mastitis Test Reagent (LMTR) and electroconductivity tests in diagnosis of subclinical mastitis in cows.

No	**Animal Species**	**Quarter**	**CMT**	**LMTR**	**Electroconductivity test**
1	Cow	RA	1	1	320
		RH	0	0	380
		LA	2	1	340
		LH	1	1	440
2	Cow	RA	0	0	560
		RH	0	0	470
		LA	0	0	840
		LH	0	0	710
3	Cow	RA	3	3	290
		RH	3	3	260
		LA	3	3	290
		LH	4	4	340

4	Cow	RA	4	4	430
		RH	2	2	520
		LA	3	3	490
		LH	2	2	500
5	Cow	RA	0	0	480
		RH	0	0	870
		LA	0	1	857
		LH	0	0	560
6	Cow	RA	2	2	340
		RH	3	3	290
		LA	2	2	400
		LH	3	3	370
7	Cow	RA	3	4	250
		RH	3	3	290
		LA	4	4	340
		LH	3	3	310

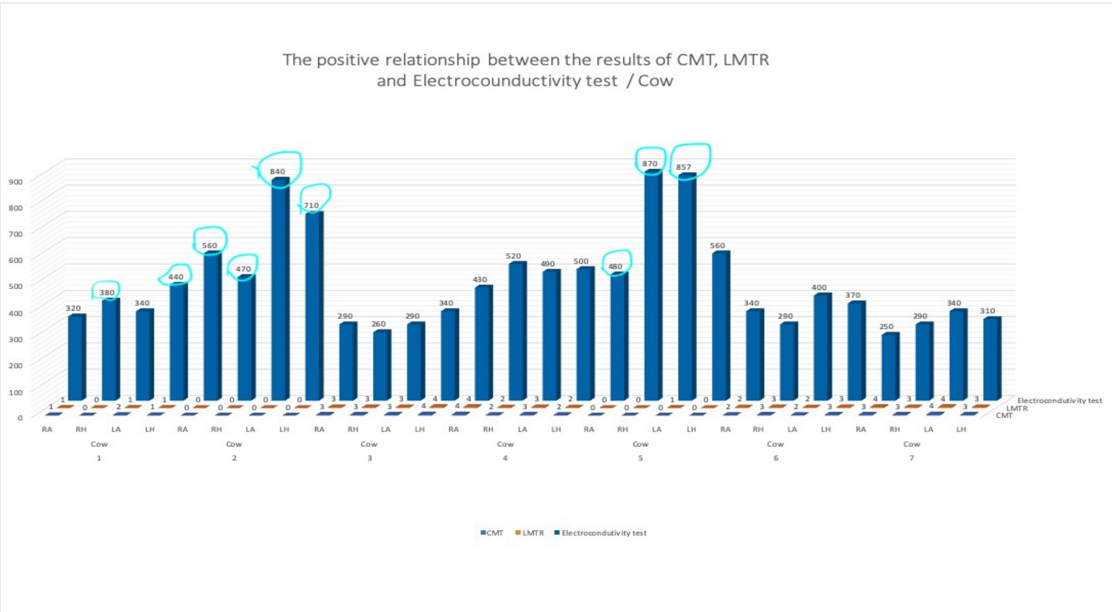

Figure. 17: Shows the correlation between the results of the Standard commercial California mastitis test (CMT), Local Mastitis Test Reagent (LMTR) and electroconductivity tests in diagnosis of subclinical mastitis in cows.

2. Goats(Table. 16; Figure. 18).

   A. There were 7 and 7 positive results for CMT and LMTR that revealed under 300 units readings ranged from 180 to 290 units.

B. There were 9 and 9 negative results for CMT and LMTR that revealed above 300 units reading ranged from 320 to 870 units.
C. There were 8 and 8 positive results for CMT and LMTR that revealed above 300 units reading ranged from 310 to 400 units.

Table. 16: Shows the correlation between the results of the Standard commercial California mastitis test (CMT), Local Mastitis Test Reagent (LMTR) and electroconductivity tests in diagnosis of subclinical mastitis in goats.

No	**Animal Species**	**Quarter**	**CMT**	**LMTR**	**Electrocondutivity test**
1	goat 1	R	0	0	500
		L	0	0	760
2	goat 2	R	3	4	320
		L	0	0	440
3	goat 3	R	3	3	360
		L	1	1	400
4	goat 4	R	3	3	360
		L	1	1	370
5	goat 5	R	0	0	460
		L	0	0	320
6	goat 6	R	1	1	310
		L	1	1	290
7	goat 7	R	4	4	200
		L	4	4	190
8	goat 8	R	3	3	380
		L	1	1	370
9	goat 9	R	4	4	180
		L	2	3	210
10	goat 10	R	0	0	335
		L	0	0	640
11	goat 11	R	0	0	900
		L	0	0	810
12	goat 12	R	4	4	257
		L	4	4	185

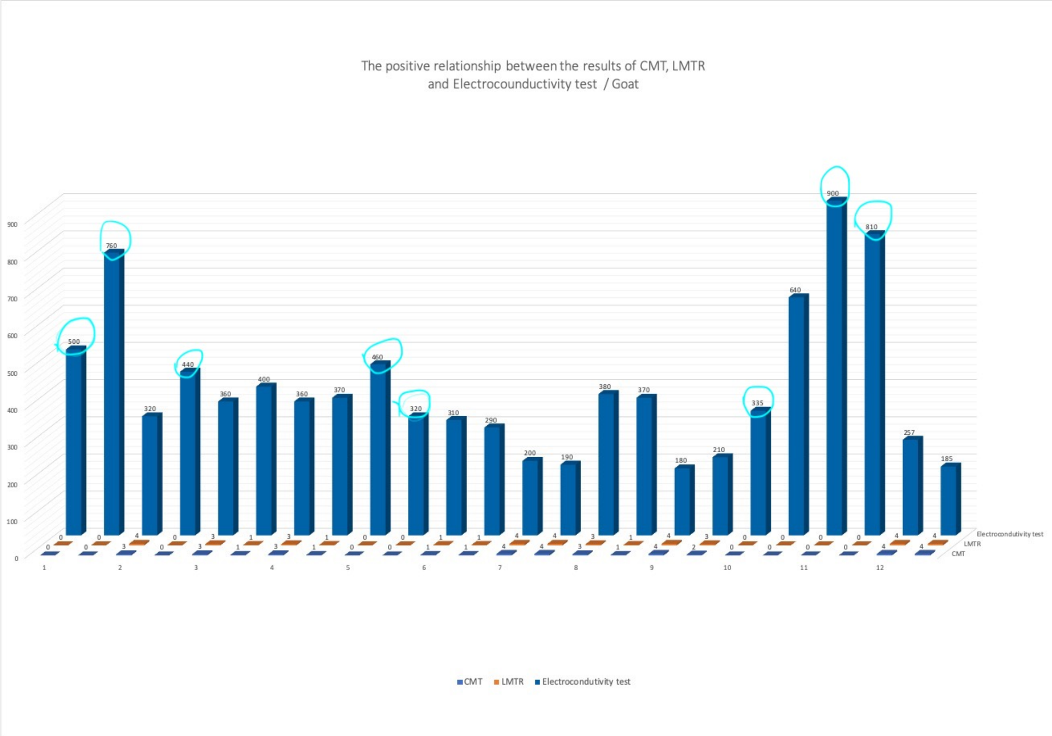

Figure. 18: Shows the correlation between the results of the Standard commercial California mastitis test (CMT), Local Mastitis Test Reagent (LMTR) and electroconductivity tests in diagnosis of subclinical mastitis in goats.

3. Ewes(Table. 17; Figure. 19).

   A. There were 4 and 4 positive results for CMT and LMTR that revealed under 300 units readings ranged from 200 to 290 units.
   B. There were 10 and 9 negative results for CMT and LMTR that revealed above 300 units reading ranged from 920 to 387 units.
   C. There were 8 and 9 positive results for CMT and LMTR that revealed above 300 units reading ranged from 310 to 750 units.

Table. 17: Shows the correlation between the results of the Standard commercial California mastitis test (CMT), Local Mastitis Test Reagent (LMTR) and electroconductivity tests in diagnosis of subclinical mastitis in ewes.

No	Animal Species	Quarter	CMT	LMTR	Electroconductivity test
1	ewe 1	R	0	0	920
		L	0	0	650
2	ewe 2	R	3	3	290
		L	3	3	260
3	ewe 3	R	4	4	200
		L	1	1	460
4	ewe 4	R	2	3	360
		L	1	1	370
5	ewe 5	R	0	0	900

		L	0	1	810
6	ewe 6	R	0	0	920
		L	0	0	810
7	ewe 7	R	1	0	680
		L	1	1	730
8	ewe 8	R	2	2	750
		L	0	1	640
9	ewe 9	R	2	3	375
		L	0	0	387
10	ewe 10	R	0	0	857
		L	0	0	560
11	ewe 11	R	1	1	310
		L	2	3	210

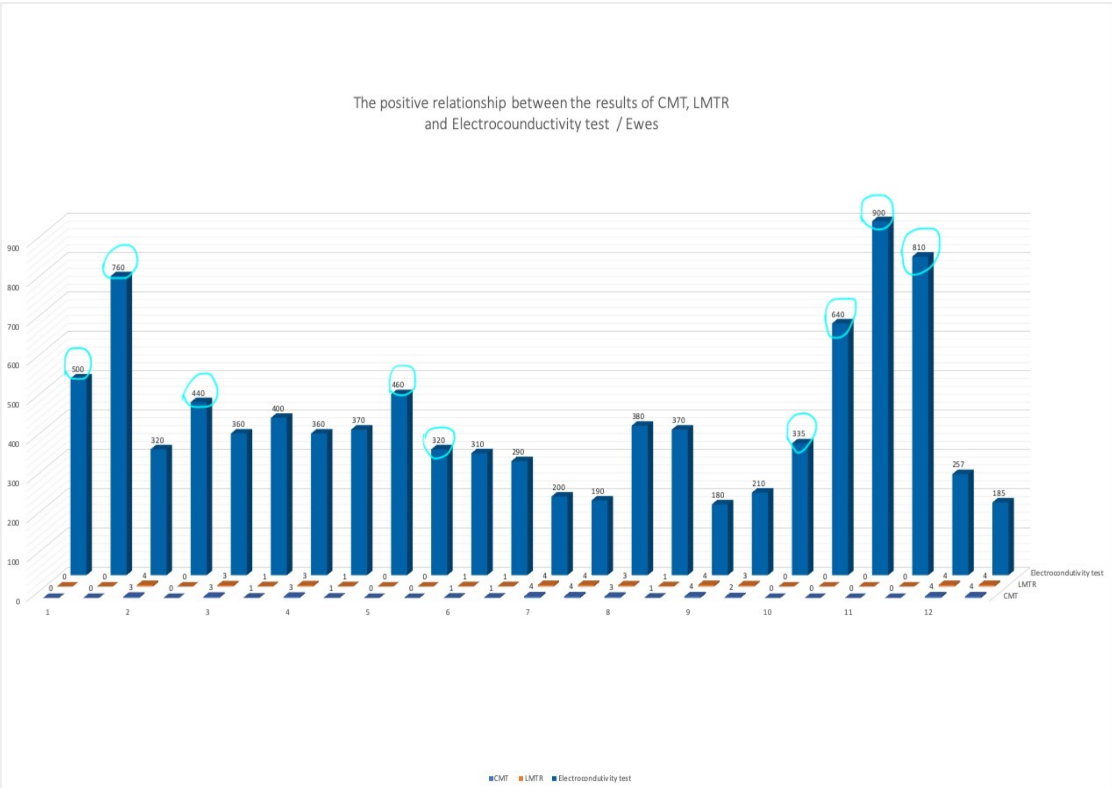

Figure. 19: Shows the correlation between the results of the Standard commercial California mastitis test (CMT), Local Mastitis Test Reagent (LMTR) and electroconductivity tests in diagnosis of subclinical mastitis in ewes.

Discussion

Mastitis is one of the important and difficult problems to understand since it is a multifactorial disease. There are different species of microorganisms responsible for causing infection. However, these microorganisms need to enter the mammary glands and establish themselves to the point that caused an infection and involved many factors, such as hygiene, milking machines, housing, environment, feed, and genetics. Moreover, there are variations in the importance of each factor about specific microorganisms in the development of mastitis (Elhaig and Selim, 2015; Selvaraju *et* *al.,* 2013; Kumar *et al.,* 2010; Barker *et al.,* 1998; Holdaway *et al.,* 1996). Therefore, early diagnosis of subclinical mastitis and timely treatment or culling of the infected animals will reduce the cost of treatment, management, and control procedures, as mastitis is contagious and can spread quickly for other animals.

Different diagnostic tests are used to investigate mastitis and changes in milk samples from the dairy animals. Various of these tests were applied by researchers, such as bacterial counts and somatic cell count. However, CMT, flow cytometry for counting lymphocytes, monocytes, and granulocytes, and chloride test called electroconductivity test are considered as important tests for diagnosis of mastitis. Moreover, these tests are regularly used diagnostically to investigate milk quality problems. (Leach *et al.,* 2008; Reddy *et al.,* 2014; Fernando *et al.,* 1985)

California mastitis test (CMT) is an important field screening test that uses for the diagnosis of subclinical mastitis in Iraq. It is a commercial kit and costs huge money. Therefore, in this study, a cheap Local Mastitis Test Reagent (LMTR) was developed and validated as a field test for diagnosis of subclinical mastitis compared to commercial CMT and electroconductivity test to save foreign currencies and support and develop the dairy farmers. In this study, there were variations in the results of the used tests between different udder quarters; however, the results of CMT and LMTR scores of examined milk samples in all animal species show compatibility. This means that the locally produced mastitis test reagent LMTR is valid and can detect subclinical mastitis compared to the commercial California Mastitis Test (CMT).

The results of CMT and LMRT for seven cows comprise 28 quarters including RA, RH, LA, LH were appeared in (Table. 4; Figure. 6), (Table. 5; Figure. 7), (Table. 6; Figure. 8) and (Table. 7; Figure. 9) respectively and revealed agreement in the reactions and reading of the results. Additionally, 46 milk samples collected from 12 goats comprise 24 quarters, and 11 ewes comprise 22 quarters were also showed compatible between the results of CMT and locally produce mastitis test reagent. These results are approved the ability of LMRT to detect subclinical mastitis in cows, goats and sheep compared to commercial CMT and LMRT. The results of this study are compatible with previous studies that used CMT as a field test in the diagnosis of subclinical mastitis in animals (Amit *et al.,* 2017; Leach *et al.,* 2008; Reddy *et al.,* 2014; Fernando *et al.,* 1985).

The principles of CMT are based on the action of the detergent reagent sodium lauryl ethyl sulfate that disrupts the cell membrane of somatic cells, DNA present in subclinical mastitis milk and considered as an indication of somatic cell count. The test is a very useful and well-known test for detecting subclinical mastitis. Additionally, the test has great advantages, such as the evaluation of the level of infection in an individual udder's quarter and also provides well results rather than providing an overall udder result. Surjowardojo *et al.*, (2008) mentioned that the CMT test (California mastitis test) is one technique used to distinguish the subclinical mastitis, characterized by low levels of abnormality. Moreover, much research approved the simplicity and effectiveness of applying the CMT test at identifying mastitis (Surjowardojo *et al.,* 2008). California mastitis test was used for the first time by Schalm and Noorlander, (1957) and the test is considered as the rapid test quantitively and accurate test in predict the somatic cell count in milk samples (Bhutto *et al.,* 2012). The somatic cells number are increased in milk as the inflammatory process develops in udder tissue. In this study, the principles of action of the locally produced mastitis test reagent LMTR are similar to the principles of commercial CMT, and these results are agreed with previously reported studies (Amit *et al.,* 2017; Annamaria *et al.,* 2017; Kabir *et al.,* 2019).

Another test used in the detection of subclinical mastitis is the electrical conductivity test of milk that use also as an indicator for the count of somatic cells present in milk samples. Moreover, this test has been used for four decades and expresses a positive correlation with the somatic count. The electrical conductivity is determined by milk anions and cations concentration. During mastitis or subclinical mastitis, damage that occurs to the udder tissue leads to a decrease in the concentration of lactose and potassium and an increase in the concentration of sodium and chloride. Different dairy countries are certified the hand-held meters, such as the Draminski mastitis test, as a screening instrument for subclinical mastitis (Fosgate *et al.,* 2013).

In this study, the Draminski mastitis test device was used as a second test to validate and evaluate the locally produced mastitis test reagent LMTR in diagnosing subclinical mastitis. The electrical conductivity is observed in milk samples, substances found in the solution that can ionize and conduct an electrical current. According to previous studies, there is a proportionally rising conductivity only if the concentration of sodium chloride rises. In this study, 74 milk samples from 7 cow (7X 4 Quarters = 28 Quarters) , 12 goats (12X 2 Quarters = 24 Quarters) and 11 ewes (11X 2 Quarters = 22 Quarters) were examined by Draminski mastitis test screening instrument. Variations were obvious in all examined samples as shown in (Table. 12; Figure. 14), (Table. 13; Figure. 15) and (Table. 14; Figure. 16) for Cow, Goat, and Ewes, respectively.

This study showed an obvious variations in the relationship between the results of the Standard commercial California mastitis test (CMT), Local Mastitis Test Reagent (LMTR), and electroconductivity tests in the diagnosis of subclinical mastitis. In cows, the relationship between these tests showed that 6 and 6 positive results for CMT and LMTR respectively were revealed the readings under 300 (the unit of milk seimens/cm) ranging from 250 to 290 (the unit of milk seimens/cm). These results showed a good relationship between the diagnosis of subclinical mastitis using the CMT and LMTR scores. Also, there were 9, and 8 negative results for CMT and LMTR were above 300 (the unit of milk seimens/cm) reading ranged from 380 to 870 (the unit of milk seimens/cm), which is also showed a good relationship between the diagnostic tests. Moreover, these results differentiated the healthy milk samples and omitted subclinical mastitis for the examined cows. However, some results revealed no agreement between the reading of the diagnostic tests, which showed 13 and 14 positive results for CMT and LMTR simultaneously revealed above 300 (the unit of milk seimens/cm) reading ranged from 310 to 520 (the unit of milk seimens/cm) with electroconductivity. According to manufacturer instruction, of the electroconductivity test, the readings above 300 (the unit of milk seimens/cm) related to high quality and healthy milk samples with a very low incidence of subclinical mastitis, but these results were contrary to the CMT and LMTR, which revealed positive reactions for subclinical mastitis.

The same scenario has also appeared in goats and ewes, the relationship between these tests showed that there were 7 and 7 positive results for CMT and LMTR that revealed under 300 (the unit of milk seimens/cm) readings ranged from 180 to 290 (the unit of milk seimens/cm); while in ewes, there were 4 and 4 positive results for CMT and LMTR that revealed under 300 (the unit of milk seimens/cm) readings ranged from 200 to 290 (the unit of milk seimens/cm). These results showed a good relationship between the diagnosis of subclinical mastitis using the CMT and LMTR scores. Moreover, in goat, There were 9 and 9 negative results for CMT and LMTR that revealed above 300 (the unit of milk seimens/cm) reading ranged from 320 to 870 (the unit of milk seimens/cm), while in ewes, there were 10 and 9 negative results for CMT and LMTR that revealed above 300 (the unit of milk seimens/cm) reading ranged from 920 to 387 (the unit of milk seimens/cm). These results showed a good connection between the diagnostic tests and differentiated the health milk samples, and omitted subclinical mastitis for the examined goats and ewes. Conversely, some results revealed no compatibility between the reading of the diagnostic tests. In goats, there were 8 and 8 positive results for CMT and LMTR that revealed above 300 (the unit of milk seimens/cm) reading ranged from 310 to 400 (the unit of milk seimens/cm). While for ewes, There were 8 and 9 positive results for CMT and LMTR that revealed above 300 (the unit of milk seimens/cm) reading ranged from 310 to 750 (the unit of milk seimens/cm). These results, as in cows, revealed a contrary between the CMT and LMTR reactions and the electroconductivity tests in the diagnosis of subclinical mastitis. These results are compatible with studies reported by other research; some researchers pointed to a good correlation between electroconductivity test and bacteriological examination (Nielen et al., 1992), whereas others reflect this method to be inadequately sensitive (Pyörälä, 2003). The results of the current study also are in agreement with the observations reported by Annamaria *et al.*, (2015).

Moreover, Langer *et al.,* (2014) and Ghahar, (2007) noticed a lower percentage of true positive milk samples by the Draminski mastitis test. However, Langer *et al.,* (2014) reported that recognizing subclinical mastitis with a hand-held electrical conductivity meter was very low. Another researcher indicated that milk electrical conductivity is determined by the type and concentration of ions, the interactive influence of the ions, and components contributing to milk viscosity (protein, fat, lactose) (Henningsson *et al.,* 2005). Additionally, Kitchen, (1981) explained that during subclinical mastitis, the concentration of sodium and chloride ions increases that leads to an increase in the electrical conductivity in milk. But The results of the current study are in agreement with the previous observation of Norberg *et al.,* (2004), who pointed that cows with subclinical mastitis may not always show an increased electrical conductivity of milk from the infected quarter. Still, the variations in electrical conductivity of milk from infected udder quarters may be larger than the variation in electrical conductivity of milk from healthy quarters. At the same time, Sheldrake *el al.,* (1983) showed that higher values of electrical conductivity of milk in infected quarters could be seen only in that quarter. While Morsi *et al*., (2000) approved that milk chlorine percentage alone cannot judge the presence of mastitis as it usually gives high results in colostrums or at the late stage of lactation. However, Biggadike *et al.,* (2000) approved that many factors influence the measurement of milk electrical conductivity such as breed, lactation stage, age of cow, oestrus, milk temperature, pH, and fat concentration in milk.

In conclusion, the results of this study approved that the newly cheap-price mastitis test reagent LMTR was prepared successfully and revealed a good reliability for diagnosis of SCM in compare to California mastitis test and Electrical conductivity test using DRAMINSKI Mastitis Detector. The researchers recommend to use LMTR for the diagnosis of subclinical mastitis in the field as it is inexpensive and can simply prepare, moreover to do another future study including large numbers of animals in order to accurately validate the local product LMTR.

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