

Investigation of subclinical mastitis cases in GCSAR Damascus goats from Humeimeh research station

M. Roukbi^{1*}, A. N. Omar², Z. Salam² and K. Dibeh²

¹Damascus Goat Research Station, General Commission for Scientific Agricultural Research, Humeimeh, Damascus, Syria.

²Animal Livestock Directorate, Livestock Research Directorate, General Commission for Scientific Agricultural Research, Damascus, Syria.

Accepted 3 October, 2014

ABSTRACT

The study was conducted to evaluate the performance of California mastitis test, electrical conductivity measurement and lactose content as markers for subclinical-intramammary infection (IMI) in goats. It was performed at Humeimeh research station for Damascus goats, General commission for scientific agricultural research (GCSAR) from February to march 2011 and done on dairy goats aged 2 to 6 years. Milk samples were taken from 134 dairy goats during morning milking according to habitual procedure and screened for evidence of subclinical mastitis by California Mastitis Test (CMT), electrical conductivity measurement (EC) and lactose content (LC) of milk. Positive (+1 to +3) CMT and/or high electrical conductivity milk samples were subjected to bacteriological examination to distinguish between healthy (absence of mastitis agents) and infected (presence of contagious or environmental mastitis agents). The overall prevalence of subclinical mastitis was found to be 24.6%. Percentage of positive (+1 to +3), suspected and negative CMT reactions were 76.85, 8.95 and 14.2%, respectively. The values of electrical conductivity for negative, suspected and positive (+1, +2 and +3) CMT milk samples were 3.93 ± 0.64 , 4.47 ± 0.61 , 4.68 ± 0.72 , 4.81 ± 0.76 and 6.56 ± 0.85 ms/cm, respectively. T-test has been shown statistical differences in California mastitis test reactions and electrical conductivity readings, and not statistically for milk lactose content, between positive and negative results for bacterial growth. Intramammary infection was found in 40.9% milk samples caused by *Staphylococcus* (75%), alone *E. coli* (16.6%) or associated with streptococci (8.3%). Correlation coefficient between CMT reactions with electrical conductivity readings was $R = 0.494$. Higher coefficient correlation for electrical conductivity measurement and Kappa agreement index ($R = 0.491$ and 0.42 respectively) with positive bacterial growth milk samples than for CMT ($R = 0.35$ and 0.12 respectively) make the electrical conductivity measurement more compatible with the results of bacteriological analysis than CMT despite a slight increase of CMT sensitivity, and reduced number of real negative cases.

Keywords: Damascus goats, subclinical mastitis, California CMT test, electrical conductivity, lactose content of milk.

*Corresponding author. E-mail: roukbi2009@gmail.com.

INTRODUCTION

Mastitis in small ruminants has a major effect in reducing both yield and quality of milk leading to strong economic losses (Stuhr and Aurlich, 2010). Moreover, mastitis is one the most frequently cited reason for culling (Tangorra et al., 2010). Many nations have taken attention to quality of the milk in small ruminants, especially after using the genetic improvement programs (Gabina and Barillet,

1991) and after intensifying of production systems for these animals (Haenlein, 1993). Attention is not longer based on the essential compounds of milk, but on the other indices providing a clear picture of the quality of the produced milk and the health of the udder such as the number of somatic cells and the level of electrical conductivity and others. The causes of mastitis are many

and varied; it is usually caused by injury but also due to incorrect feeding, unsanitary, and poor living conditions. Damage is caused to the udder by improper milking practices. Genetic and physiological factors (such as race, age, estrus, season of milking, milk yield or stage of lactation), increase mastitis susceptibility. Others as health standards and requirements increase somatic cells and electrical conductivity in the milk of goats under non-infectious conditions (Stuhr and Aurlich, 2010).

It seems that the most common methods (Somatic cell count, California Mastitis Test and electrical conductivity) for the control of udder health and udder infections are of limited value for goats [5]. Contrary to strong correlation between indirect diagnostic tests: CMT results and somatic cells count, and the results of the bacterial milk culture in ewes there is a problem in the interpretation of cell count and the results of California mastitis test of in goats. California Mastitis Test (CMT) is a common indirect method of measuring Somatic cell count (SCC) in cows, but some authors claim that CMT is an unreliable method for diagnosing intramammary infection (IMI) in goats (Schaeren and Maurer, 2006; Bergonier et al., 2003). Other studies, however, report that CMT may be useful for detection of healthy udders (Karzis et al., 2007; Petzer et al., 2008). The electrical conductivity is considered an rapid and non-directly indicator of the health of the udder in goats (Romero et al., 2010). It refers to the changes in permeability due to injury of the udder, associated with rising concentrations of ions and cations, as sodium Na^+ And chloride Cl^- secreted in the milk of infected udder halves (Krömer, 2007). Because of changes in the permeability of the udder tissues and its fluctuations of readings, electrical conductivity is not usually used in goat's milk. Electrical conductivity levels (Norberg, 2004) are affected by a variety of factors, the most important is the temperature of the milk samples (Alonso and Mimor, 2006), clinical subclinical mastitis (Hamann and Zeconi, 1998; Norberg, 2004), microbial contamination of milk (Daumoras and Knys, 2006), the acidity of the milk (Lanzanova et al., 1993; Mucchetti et al., 1994), animal race (Park, 1991), milking season (Dinsmor et al., 1998; Regin et al., 2002), the somatic cells content of the milk (Mucchetti et al., 1994; Fahr et al., 2001; Bansal et al., 2007). The value of electrical conductivity of milk changes during stage of milk production within the same lactation season (Sheldrake et al., 1983). The type of the devices used to measure of electrical conductivity also plays very important role in the results (Maatje et al., 1983; Onyango et al., 1988; Crame, 1987).

There are reported values of electrical conductivity got an average values of electrical conductivity 6.6 ± 0.5 ms/cm (Schuppel and Schwoppe, 1999) close to values 6.33 ± 0.75 ms/cm obtained by Boulaaba (2009). According to Tangorra et al. (2010) there is no absolute threshold reliable permitting to distinguish between the inflamed and non-inflamed udder glands. Also Ryffel et al. (2007) could not put comparable limits values in goat

milk. The fact that the direct count of somatic cells in milk samples helped to diagnose of subclinical mastitis and therefore the percentage of infection in milk samples taken from herds has been increased from 20-50% to 70.2% (Boscós et al., 1996). The levels of lactose content in milk decrease after the udder becoming infected and partially permeable for lactose formed in the udder from glucose and galactose (Stuhr and Aurlich, 2010). Noticing also that Jendretzke (2009) indicated that the lactose level in milk is depending of some factors like estrus, feeding, lactation number and age. It ranges between 3.8 and 4.6% and falls at the end of milking season.

Milk samples can be considered infected if they accompanied by a positive $\geq +1$ CMT reaction, white cell count ≥ 0.63 million cells / ml and the level of lactose $\leq 4.33\%$, or chloride content $\geq 0.109\%$ (Upadhyaya and Rao, 1993).

In this study, indirect diagnose subclinical mastitis methods are used: measurement of somatic cell count (SCC) by California Mastitis Test (CMT), electrical conductivity (EC) measurements lactose content LC analysis in milk is evaluated. The purpose of the present study was to compare the performance of CMT, EC and LC as possible markers for subclinical-intramammary infection (IMI) in goats, to assess the prevalence of subclinical-intramammary infection in Humeimeh Research Station for Damascus goats and moreover to see how well indirect measurements of SCC (CMT and EC) correspond to growth of pathogens in the udder.

MATERIALS AND METHODS

The study was conducted at Humeimeh Research Station for Damascus goats/General Commission for Agricultural Scientific Research (GCSAR). Animals that were used for the study were born in the station but their origin was from Damascus region. Animals are usually herded twice a day (mornings before getting out and after being herded afternoon). The does were milked twice per day (during morning and evening hours). The lactating does were given barley grain, wheat bran and cotton seed cake and concentrate during milking period. The study was conducted from February to march 2011. A total of 134 foremilk samples (about 10 ml) were collected from dairy goat aged 2 to 6 years once during morning milking from one half or two halves according to habitual procedure and screened for evidence of subclinical mastitis by California Mastitis Test (CMT), electrical conductivity measurement and lactose content of milk. Positive (+1 to +3) CMT and/or high electrical conductivity milk samples were subjected to bacteriological examination to distinguish between healthy (absence of mastitis agents) and infected (presence of contagious or environmental mastitis agents). Milk samples for the bacterial culture of (+1 to +3) CMT and/or highly electrical conductivity reading were taken after cleaning, washing and disinfecting the udder with alcohol 70.0% in sterile plastic bottles excluding the first fore-milk stream.

The following indirect methods for the detection of subclinical mastitis have been used:

Electrical conductivity measurements

This was conducted using Suntex SC-17 which measures electrical

Table 1. Grading CMT reactions or defining CMT scores.

CMT Reaction	Symbol	Interaction	Cell count/ml
unchanged	0	Negative	68,000
Precipitate a mild viscous form disappears with continuous movement	T	Effect	268.000
distinct precipitated 'mucus' Disappears with continuous movement	+1	Weak positive	800,000
directly thickness of the mixture with the emergence of a gel	+2	Clear	2,560,000
Forming gelatinous with convex surface	+3	Strong	≥ 10.000.000

Shearer JK, and Harris B., Jr. Mastitis in Dairy Goats. In: Schalm, OW, Carroll, EJ, and Jain, NC: Bovine Mastitis. Lea and Febiger, Philadelphia, PA,: Interpretation of California Mastitis Test scores on goat milk.

conductivity or current flow resistance of milk in ohms(mho) or Siemens (S) per meter (ms/m = 10² mmho/cm), and that between the negative and positive sensitive poles for measuring the temperature of samples automatically.

California CMT

The main advantages with CMT are that it is quick, cheap and simple and that it is an "animal-side". This test was performed as described (Schalm et al., 1971; Ikram, 1997) to classify the milk samples in intact and infected udders before performing bacterial growth culture (Tangorra et al., 2010). The principle of the test is the reactive reagent (BOVI.VET CMT-Test from KRUUSE Danish Company) which is a solvent reacts with the DNA of somatic cell nuclei after the dissolution of their outer wall and the nucleus cell wall with the formation of filamentous mass which is proportional with the somatic cells count. The presence of neutrophils, which are directly related to glandular irritation leads to increase of somatic cell count (Sheldrake et al., 1983). The presence of positive interactions does not necessarily indicate the presence of mastitis. The bacterial toxins that reach the bloodstream leads also to massive destruction of white blood cells without being associated with a positive result in CMT (Wagner et al., 2007).

To interpret the results of CMT test (Shearer and Harris, 1992) (Table 1):

a) Trace or weak result of CMT (viscous to mucus disappears with continuous movement) indicate suspicion of the udder inflammation (≤268 × 10³ cell/ml), which means that the udder has just recovered from a previous infection or that there is an evidence of inflammation of the udder. In both cases the test must repeated to verify the presence of inflammation or make another diagnostic test.

b) Clearly positive results without any doubt (directly thickness of the mixture with the emergence of a gel): indicate evaluative inflammatory response associated with advanced high somatic cells count (≥800 × 10³ cell/ml). This may be due to mechanical injury of the mammary gland or more likely caused by germ infection. Causes led to inflammation of the udder (still electricity or problems related to machine milking must be defined and corrected immediately).

Lactose content of milk

This was measured once in positive and negative in positive and negative milk samples for microbial growth by Melchoscans S 50 From Foss Company.

Bacterial analysis

CMT positive (+1 to+3) and/or high electrical conductivity milk samples, which numbered 66 samples were cultured once at the

Table 2. Goat's status according to the results of bacterial growth as a diagnostic test.

CMT	bacterial growth	Status
≥ +1 reaction	Positive result	Real positive
≥ +1 reaction	Negative result	False positive
negative reaction	Positive result	False negative
negative reaction	Negative result	real negative

Laboratory of the Animal Health Department, Aleppo Directorate of Agriculture on McConkie culture and blooded agar plates which were incubated at 37°C for 16 to 24 h, and re-evaluated at 48 h on milk samples, the bacteria were identified by means of typical colony morphology according to the standard modalities of Barth (2009).

If moderate to high growth of a major udder pathogen was found the sample would be diagnosed as positive for bacterial analysis.

Prevalence of the subclinical, accuracy, sensitivity and specificity values, positive and negative predictive and positive likelihood ratio for the CMT and EC

Prevalence of the subclinical, accuracy, sensitivity and specificity values positive and negative predictive and Positive likelihood ratio for the CMT and EC were calculated (McGee, 2002; Reid et al., 1998) after goats have been classified into groups according to CMT reactions and electrical conductivity readings (threshold of electrical conductivity is ≥ 4.75 ms/cm) depending on the result of bacterial growth as a diagnostic test (Table 2).

Prevalence

The prevalence is the proportion of true (a) positive (positive for either diagnostic test or bacterial culture), and false(c) negative (positive diagnostic test but negative bacterial culture) in the population or:

$$\frac{a+c}{a+b+c+d} \times 100$$

Accuracy

The proportion of true positive (a) and true negative (d) in the population or:

$$\frac{a+d}{a+b+c+d} \times 100$$

Sensitivity

The proportion of true positive (a) in true positive (a) and false negative(c) or:

$$\frac{a}{a+c} \times 100$$

Specificity

The proportion of true negative in true negative and false positive or:

$$\frac{d}{b+d} \times 100$$

Positive predictive value (PPV)

The proportion of true positive in true positive and false positive or:

$$\frac{a}{a+b} \times 100$$

Negative predictive value (NPV)

The proportion of true negative in true negative and false negative or:

$$\frac{d}{c+d} \times 100$$

Positive likelihood ratio (LR⁺)

$$= \frac{\text{Sensitivity}}{(1 - \text{Sensitivity})}$$

Negative likelihood ratio (LR)

$$\frac{(1 - \text{Sensitivity})}{\text{Specificity}} \text{ or } \frac{\frac{c}{a+c}}{\frac{d}{b+d}}$$

Pre-test probability

The proportion of true positive and false negative in the total number of cases:

$$\frac{a+c}{a+b+c+d} \times 100$$

Pre-test odds

$$\frac{\text{prevalence}}{(1 - \text{prevalence})} \text{ or } \frac{\text{pre-test probability}}{(1 - \text{pre-test probability})}$$

Post-test probability

$$\frac{\text{post-test odds}}{(\text{post-test odds} + 1)}$$

Post-test odds

Pre-test odds × Likelihood ratio

Positive posttest probability

$$\frac{a}{a+b}$$

Negative posttest probability

$$\frac{c}{c+d}$$

Statistical analysis

T-test was carried out using Genstat 7.2 (Genstat Seventh Edition, 2004) to study the differences in CMT reactions and electrical conductivity readings between positive and negative bacterial growth results. T-test for independent for CMT group reactions was also performed to test the differences between the means of electrical conductivity readings using the program (StatPac, 2011).

Agreement measure (Cohen kappa index)

The performance of the modern technologies or test' evaluation need to be interpreted whether the differences between different tests due to the methods used or for the opinions public commentators. The Cohen kappa agreement index is preferred statistically because it measures the agreement between the results of diagnostic methods, and interprets what's real or accounts for chance (Bland and Altman, 1986; Kundel and Polansky, 2003) (Table 3).

Cohen's kappa coefficient was used to measure the agreement between in hand: CMT, electrical conductivity EC measurements with the results of the bacteriological analysis in the other hand (<0 no agreement, 0 to 0.2 slight agreement, 0.2 to 0.4 fair agreement, 0.4 to 0.6 moderate agreement, 0.6 to 0.8 substantial agreement, and 0.8 to 1 almost perfect agreement).

RESULTS**Statement of assorted goats basing on the results of California mastitis test and electrical conductivity**

Goats were assorted according to the results of CMT and electrical conductivity measurements basing on the result of bacterial growth as a diagnostic test and so it was obtained four groups symbolized A to D. As shown in Table 4, an increase of the truth negative cases for the

Table 3. Data entering model to measure the Cohen kappa index for the agreement.

	Diagnostic test positive		Total
	Yes	No	
Negative diagnosis test	Yes	Positive bacterial growth, positive result of diagnostic test	Negative bacterial growth but positive result of diagnostic test
	No	Positive bacterial growth but negative result of diagnostic test	Negative bacterial growth, negative result diagnostic test
Total			

Table 4. Issue of goat's assortment basing on the results of CMT and EC.

Symbol	Statement	California test	Electrical conductivity
A	Positive for the disease with a positive diagnostic test or true positive	30	28
B	Negative for the disease with positive diagnostic test or false positive	71	32
C	Positive for the disease with negative diagnostic test or false-negative	3	5
D	negative for the disease, as well as diagnostic test or true negative	30	69
Total			134

Table 5. Values of electrical conductivity (ms/cm) for CMT reactions.

Symbol	0	T	1	2	3
SD + Mean	3.93 ± 0.64	4.47 ± 0.61	4.68 ± 0.72	4.81 ± 0.76	6.56 ± 0.85
SE	0.15	0.16	0.092	0.13	0.35

measure of electrical conductivity is faced by an increase of false positive cases for CMT.

Prevalence of udder subclinical mastitis in GCSAR' Damascus goat research stations

The prevalence of subclinical mastitis in GCSAR' Damascus goat research station (for CMT and EC) which is the proportion of true positive and false in the population according to (Byrt et al., 1993 to Feinstein and Cicchetti, 1990) was amounted to 24.6%.

CMT results

The percentage of positive reactions (+1 to +3) and suspected and negative CMT was 76.85, 8.95 and 14.2%, respectively.

Results of measuring the electrical conductivity according to CMT reactions

The values of electrical conductivity for negative, suspected and positive (+1, +2 and +3) CMT milk samples were 3.93 ± 0.64, 4.47 ± 0.61, 4.68 ± 0.72, 4.81 ± 0.76 and 6.56 ± 0.85 ms/cm, respectively. The t-test showed highly significant differences ($P \leq 0.002$) in CMT

reactions and in electrical conductivity readings between positive and negative bacterial growth milk samples. T-test for independent groups showed using (Genstat Seventh Edition, 2004) also significant differences ($P \leq 0.05$) between means of electrical conductivity values (Table 5) for CMT inter-group reactions between negative and +1 CMT ($P \leq 0.05$), between negative and +2 and +3 CMT ($P \leq 0.01$), between suspected and +3 CMT ($P \leq 0.002$), and between +1 and +3 CMT ($P \leq 0.002$), while no differences were observed between means and standard deviations for electrical conductivity readings between suspected and +3 CMT inter-groups and for inter-group CMT and between suspected and +1 and +2 CMT inter-groups, and also between +2 and +1 CMT inter-groups.

Correlation coefficients between CMT reactions with electrical conductivity readings, and between both electrical conductivity readings and CMT reactions with positive bacterial growth in milk samples were ($R = 0.494$, $R = 0.491$ and $R = 0.35$ respectively). T-test has been shown statistical differences in California mastitis test reactions and electrical conductivity reading between positive and negative results for bacterial growth.

Lactose content of milk

Low levels of lactose content were recorded in positive milk samples for bacterial growth compared to negative

Table 6. Statistically performance of CMT and EC.

Type of diagnostic test	CMT	EC
Accuracy %	44.8	72.4
Sensitivity %	90.9	84.8
Specificity %	29.7	68.3
Positive predictive value PPV %	29.7	46.7
Negative predictive value NPV %	30.9	93.2
Positive likelihood ratio LR +	1.29	2.68
Negative likelihood ratio LR-	0.31	0.22
Pre-test probability;	0.246	
Post- Probability	0.297 or 3/10	0.467 or 5/10
Pre-test odd s	0.327	
Post-test odds	0.42	0.88
Cohen's kappa index	0.1217	0.4168
Pearson correlation factor with the result of bacterial analysis	0.35	0.49

Table 7. Cohen's kappa index for CMT and EC according to Sharma et al. (2010).

Measurement of the electrical conductivity					California test				
		Positive		Total			Positive		Total
		Yes	No				Yes	No	
Negative	Yes	28	32	60	Negative	Yes	30	71	101
	No	5	69	74		No	3	30	33
Total		33	101	134	Total		33	100	134

milk samples for bacterial growth ($4.1 \pm 0.18\%$ vs. $4.15 \pm 0.19\%$).

Results of the bacteriological analysis in suspected and positive milk samples

Intramammary infection, defined as growth of udder pathogens, was found in 40.9 % milk samples caused by contagious pathogens represented by *Staphylococcus staphylococci* (75%). In addition to environmental pathogens represented by alone *E. coli* (16.6%) or associated with streptococci (8.3%) all sensitive to the following antibiotics: Cephalosporin, Cephalixin, Ciprofloxacin, Ofloxacin, Enrofloxacin, Doxycycline Sellin - neomycin, Amikacin (cannot be administrated to pregnant does).

Statistical measures of the performance of CMT and EC

Table 6 shows high accuracy and specificity of the measure of the electrical conductivity despite of high CMT sensitivity, Noting also that an increase of truth negative cases for electrical conductivity was faced by similar increase of false positive cases of CMT (Table 6).

However, Cohen's kappa index which was obtained for

the measuring electrical conductivity is closer to 1 than for CMT (slight) and therefore it was more compatible (Sharma et al., 2010) (Table 7).

DISCUSSION

The prevalence of subclinical mastitis in GCSAR' Damascus Goat Research Station Humeimeh was amounted to 24.6%. The overall prevalence of subclinical mastitis in goats at Tigray Regional State, North Ethiopia was found to be 28.14% (Gebrewahid et al., 2012).

The percentage of positive reactions (+1 to +3) and suspected and negative CMT were 76.85, 8.95 and 14.2%, respectively. The percentage of positive reactions was very close to the results obtained (76.7%) in dairy goats in the province of Morogoro in Tanzania by Mbilu (2007), but higher than what obtained (Upadhyaya and Rao, 1993) for (+1 to +3) CMT (67.7%) and therefore lower for suspected (trace) and negative CMT reactions (32.8%). Noticing that there are significant differences between CMT readings in milk samples as well as between udder halves whose somatic cell count is $\geq 0.750 \times 10^3$ cells/ml, as well as between infected and uninfected udders as indicated (Petzer et al., 2008).

The values of electrical conductivity for negative, suspected and positive (+1, +2 and +3) CMT milk samples were 3.93 ± 0.64 , 4.47 ± 0.61 , 4.68 ± 0.72 , 4.81

± 0.76 and 6.56 ± 0.85 ms/cm, respectively.

The electrical conductivity readings for (+3) CMT was (6.56 ± 0.85 ms/cm) closed to the average level obtained by Deiad et al. (2010) for normal milk (6.33 ms/cm) for the yield milk of 1564.2 g. Fluctuations in electrical conductivity measurements for CMT ratings may be due to the type of device used for measurements and temperature of the samples (Alonso and Mimor, 2006; Maatje et al., 1983; Onyango et al., 1988; Crame, 1987). This is supported by the fact that it would be necessary of time-series analysis of historical data of the electrical conductivity among the latest highly recorded 20th readings during the beginning and middle of the end lactation season and the health status of the two udder halves and the comparison between the values of different halves through lactation season to obtain satisfactory results (Tangorra et al., 2010).

T-test for independent groups using (Genstat Seventh Edition, 2004) showed also significant differences ($P \leq 0.05$) between means of electrical conductivity values for CMT inter-group reactions between negative and +1 CMT ($P \leq 0.05$), between negative and +2 and +3 CMT ($P \leq 0.01$), between suspected and +3 CMT ($P \leq 0.002$), and between +1 and +3 CMT ($P \leq 0.002$), while no differences were observed between means and standard deviations for electrical conductivity readings between suspected and +3 CMT inter- groups and for inter- group CMT and between suspected and +1 and +2 CMT inter-groups, and also between +2 and +1 CMT inter- groups.

As Kifaro et al. (2009) no significant influence on lactose content in positive milk samples for bacterial growth was observed ($4.1 \pm 0.18\%$ vs. $4.15 \pm 0.19\%$), contrary to Leitner et al. (2004) who reported significantly lower concentration of lactose in the infected glands (4.17 ± 0.13) in comparison to uninfected one (4.7 ± 0.1). The measurement in this study was done once and could not be repeated and therefore there were no statistical differences between positive or negative milk samples for bacterial growth.

Intramammary infection was found in 38.9% milk samples. There was found lower Intramammary infection IMI than in other studies (Persson and Olofsson, 2011), because only 18% of all udder halves had IMI, where the proportion of udder halves with subclinical IMI in goats ranged from 23 to 70% (Leitner et al., 2004; Persson and Olofsson, 2011; McDougall and Prosser, 2010). The lower proportion of Persson and Olofsson (2011) study might be the result of good udder health in Sweden. *Staphylococcus* (75%) isolated in this present study correspond to Schaeren and Maurer (2006) and Leitner et al. (2004) who indicated that *Staphylococcus aureus* is the most prevalent pathogens of the mammary gland in goats (60 to 80%) in goats. In addition to isolated *Staphylococcus* there were environmental pathogens represented by alone *E. coli* (16.6%) or in association with streptococci (8.3%). Noticing that Gebrewahid et al. (2012) had isolated from CMT positive samples:

Staphylococcus aureus (27.7%), *Esherchia coli* (17.0%) and *Streptococcus* (10.63%) in goats at Tigray Regional State, North Ethiopia.

Correlation coefficients between CMT reactions with electrical conductivity readings, and between both electrical conductivity readings and CMT reactions with positive bacterial growth in milk samples were ($R = 0.494$, $R = 0.491$ and $R = 0.35$ respectively). T-test has been shown statistical differences in California mastitis test reactions and electrical conductivity readings between positive and negative results for bacterial growth. According to Schaeren and Maurer (2006) in 20 to 30% of the cases the SCC is $\geq 750,000$ cells/ml. Also, the relation between California Mastitis Test (CMT) reactions and udder infections is not very close. Moreover, over 20% of mammary halves infected with CN (coagulase-negative) *Staphylococcus* showed negative CMT reactions. On the other hand, 25% of samples from mammary halves without a proven infection reacted positively (Schaeren and Maurer, 2006). This confirms the idea what pointed it to (Koop et al., 2011) that the sensitivity of the bacterial analysis is very low compared to the count of somatic cells SCC. The prevalence of mastitis by *S. aureus* in dairy goats must be higher than what obtained by bacterial analysis.

The low number of false positive, relative high accuracy and positive PPV and negative NPV predictive values, relative high positive likelihood ratio LR+ and relative high agreement of Cohen' kappa index, and Pearson correlation between measuring electrical conductivity with bacterial culture (32 vs. 71 for CMT, 72.4%, 46.7%, 93.2%, 0.4168, 2.68 and 0.49 respectively) make this test more compatible with the results of bacteriological analysis than CMT (Sharma et al., 2010; Martin et al., 1987) despite the high number of real negative of electrical conductivity in comparison to CMT (69 vs.30).

Conclusions

1. Both California test and electrical conductivity give nearly the same number of true positive and false-negative but different number of false positive and true negative.
2. The electrical conductivity measurement is more compatible with the results of bacteriological analysis than CMT.
3. It can be concluded that the CMT does not reflect really the health status of the udder; it is more influenced than EC by different factors cited by the authors like increased days in milking, parity and reduced milk production.
4. The fact that the positivity CMT does not mean necessarily the infection of the udder. This is supported by the fact that most of the milk samples were bacteriologically negative.
5. Lactose content in positive or negative milk samples

for bacterial growth was unchanged (4.1 ± 0.18 and $4.15 \pm 0.19\%$, respectively). It needs probably repeated measurements.

ACKNOWLEDGEMENTS

I would like to thank the Government of Syria through the Ministry of Agriculture, the director of the General Commission for Scientific Agricultural Research (GCSAR) for sponsoring this work. My sincere gratitude goes to the Laboratory of the Animal Health Department, Aleppo Directorate of Agriculture for their assistance in the laboratory work. Lastly but not least, I acknowledge all those who in one way or the other assisted in the completion of this work.

REFERENCES

- Alonso VLM, Mimor MA, 2006.** Design and construction of a system for measuring the concentration of water and milk. Robotics and Automotive Mechanism Electronics Conference, 2:47-51.
- Bansal BK, Hamann J, Lind O, Singh ST, Dhaliwal PS, 2007.** Somatic cell count and biochemical components of milk related to udder health in buffaloes. *Ital J Anim Sci*, 6(2):1035-1038.
- Barth K, 2009.** Eutergesundheitsüberwachung bei Milchständen und Milchzeigen- Welch Methoden sind geeignet? *Landbauforsch. SH*, 332:89-95.
- Bergonier D, de Cremoux R, Rupp R, Lagriffoul G, Berthelot X, 2003.** Mastitis of dairy small ruminants. *Vet Res*, 34(5):689-716.
- Bland JM, Altman DG, 1986.** Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*, 1:307-310.
- Boscós C, Stefanakis A, Alexopoulos C, Samartzi F, 1996.** Prevalence of subclinical mastitis and influence of breed, parity, stage of lactation and mammary bacteriological status on Coulter Counter Counts and California Mastitis Test in the milk of Saanen and autochthonous Greek goats. *Small Rumin Res*, 21:139-147.
- Boulaaba A, 2009.** Untersuchung zur Eutergesundheit von Ziegen sowie durchflusszytometrische Differenzierung carpiner Milchzellen. Gießen: DVG-Service, 285p.
- Byrt T, Bishop J, Carlin JB, 1993.** Bias, prevalence and kappa. *J Clin Epidemiol*, 46:423-429.
- Cohen's kappa index. On-line calculation. www.olmosantonio.com.
- Crame S, 1987.** Automatic computerized herd management: Heat detection mastitis and health monitoring. Page 18 in proc. 3rd symp.
- Daumoras J, Knys A, 2006.** Research into correlation of milk electrical conductivity and bacterial contamination. *Elect Eng*, 6(70):95-98.
- Deiad O, Kaskous S, Skouti J, 2010.** Relationship between electrical conductivity and other milk production parameters of Shami goat in Al-quneitra area. *J Anim Poultry Prod Mansoura Univ*, 1(9):415-420.
- Dinsmor RP, Goodell GM, Chard P, 1998.** Electrical conductivity of milk in cows with subclinical mastitis. *J Anim Sci*, 76, Supp 1.1.
- Fahr R, Suess R, Schulz J, 2001.** Einflussfaktoren auf die somatische zellzahl bei Schaf and Ziege. *Tierz.Dummerstorf*, 44:288-298.
- Feinstein AR, Cicchetti DV, 1990.** High agreement but low kappa: I. The problems of two paradoxes. *J Clin Epidemiol*, 43:543-549.
- Gabina D, Barillet F, 1991.** Actual tendencies for sheep selection within the European Community. *Inf Tec Econ Agrar*, 87:227-235.
- Gebrewahid TT, Abera BH, Menghistu HT, 2012.** Prevalence and etiology of subclinical mastitis in small ruminants of Tigray Regional state, North Ethiopia. *Vet.Word*, 5(2):103-109.
- Genstat Seventh Edition (Release 7.2), 2004.** Laws Agricultural Trust (Rothamsted Experimental Station).
- Haenlein G, 1993.** Producing quality goat milk. *Int J Anim Sci*, 8:79-85.
- Hamann J, Zeconi A, 1998.** Evaluation of electrical conductivity of milk as a mastitis indicator. *Bulletin IDF N 334/1998*.
- Ikram M, 1997.** Diagnostic microbiology. In: W Paul and VMD Pratt (editors), *Laboratory Procedures for Veterinary Technicians*. RR Donnelley and Sons Company. St. Louis, Missouri. pp 159-160.
- Jendretzke K, 2009.** Untersuchung zuur Laktosegehalt, somatischer Zellzahl und bakteriologischer Beshaffenheit von Ziegenmilch aus hessischer Bestanden Giessen: WB Laufersweiler, 120 p.
- Karzis J, Donkin EF, Petzer IM, 2007.** The influence of intramammary antibiotic treatment, presence of bacteria, stage of lactation and parity in dairy goats as measured by the California Milk Cell Test and somatic cell counts. *Onderstepoort J Vet Res*, 74(2):161-167.
- Kifaro GC, Moshi NG, Minga UM, 2009.** Effect of sub-clinical mastitis on milk yield and composition of dairy goats in Tanzania *AJFAND*, 9(1):623-634.
- Koop G, van Werven T, Toft N, Nielen M, 2011.** Estimating test characteristics of somatic cell count to detect *Staphylococcus aureus*-infected dairy goats using latent class analysis. *J Dairy Sci*, 94(6): 2902-2911.
- Krömer V, 2007.** Kurzes Lehrbuch Milchkunde and Milchhygiene. Stuttgart: Parey, 232.
- Kundel HL, Polansky M, 2003.** Measurement of observer agreement. *Radiology*, 228:303-308.
- Lanzanova M, Mucchetti G, Neviani E, 1993.** Analysis of conductance changes as growth index of lactic acid bacteria in milk. *J. Dairy Sci*, 76:20-25.
- Leitner G, Merin U, Silankove N, 2004.** Changes in milk composition as affected by subclinical mastitis in goats; *J.Dairy Sci*, 87:1719-1726.
- Maatje K, Rossing W, Gassen J, Plugers G, 1983.** Automation of electrical conductivity measurements during milking. page 89 in proc. 3rd Symp. Automation in Dairying, Wageningen, Neth.
- Martin SW, Meek AH, Willeberg P, 1987.** *Veterinary Epidemiology: Principles and Methods*. 1st ed. Ames, Iowa: Iowa State Univ Pr: 62-73.
- Mbilu TJNK, 2007.** Status of mastitis in lactating goats at Sokoine University of agriculture and neighbouring smallholder farms in Morogoro Municipality, Tanzania *Livestock Research for Rural Development*, 19 (3).
- McDougall S, Prosser C, 2010.** Prevalence and incidence of intramammary infections of lactating dairy goats. In 5th IDF Mastitis Conference. Christchurch NZ: *Vet Learn*, 235-240.
- McGee S, 2002.** Simplifying likelihood ratios. *J Gen Intern Med*, 17(8):647-650.
- Mucchetti G, Gatti M, Neviani E, 1994.** Electrical conductivity changes in milk caused by acidification: determining factors. *J Dairy Sci*, 77(4):940-944.
- Norberg E, 2004.** Electrical conductivity of milk: Ability to predict mastitis Status. *J Dairy Sci*, 87:1099-1107.
- Onyango C, Marchant A, Kake R, Stambridge D, 1988.** A low maintenance conductivity sensor for detecting mastitis. *J Agric Eng Res*, 40:215-217.
- Park YW, 1991.** Electrical conductivity, bacteria counts, percent fat and protein in goat milk. *Small Rumin Res*, 5(4):367-375.
- Persson Y, Olofsson I, 2011.** Direct and indirect measurement of somatic cell count as indicator of intramammary infection in dairy goats. *Acta Vet Scand.*; 53(1):15
- Petzer IM, Donkin EF, Du Preez E, Karzis J, van der Schans TJ, Watermeyer JC, van Reenen R, 2008.** Value of tests for evaluating udder health in dairy goats: somatic cell counts, California Milk Cell Test and electrical conductivity. *Onderstepoort J Vet Res*, 75(4):279-287.
- Regin F, Eckhard S, Wolfgang J, Joa CK, 2002.** Systematic effects on activity, milk yield, milk flow rate and electrical conductivity. *Arch Tierz Dummerstorf*, 45(3):213-222.
- Reid MC, Lane DA, Feinstein AR, 1998.** Academic calculations versus clinical judgments: practicing physicians' use of quantitative measures of test accuracy. *Am J Med*, 104(4):374-80.
- Romero G, Sendra E, Muelas R, Ramon DSJ, 2010.** Effect of electrical conductivity of goat's milk on characteristics of fresh cheese. *Milchwissenschaft*, 65(1):56-59.
- Ryffel S, Maurer J, Shaeren W, 2007.** Comparison of udder health and cell count pattern in Swiss goats and milking Ewes. 5th International Symposium on The Challenge to Sheep and Goats Milk Sectors,

- Algher in Italy, April 18-20th.
- Schaeren W, Maurer J, 2006.** Prevalence of subclinical udder infections and individual somatic cell counts in three dairy goat herds during a full lactation] *Schweiz Arch Tierheilkd*, 148(12):641-648.
- Schalm OW, Carroll EJ, Jain NC, 1971.** *Bovine Mastitis*. Lea and Febiger: Philadelphia, Pennsylvania, USA. pp 117-120.
- Schuppel H, Schwoppe, 1999.** Zum Gehalt somatischer Zellen zur mikrobiologischen Beschaffenheit der Milch von Ziegen mit klinisch unauffälligem Euterbefund. *Milchwissensch*, 54:13-15
- Sharma N, Pandey V, Sudhan NA, 2010.** Comparison of some indirect screening tests for detection of subclinical mastitis in dairy cows. *Bulgarian J Vet Med*, 13(2):98-103.
- Shearer JK, Harris B, Jr. 1992.** *Mastitis in Dairy Goats*. Inn. Schalm, OW, Carroll, EJ, and Jain, NC: *Bovine Mastitis*. Lea and Febiger, Philadelphia, PA: Interpretation of California Mastitis Test scores on goat milk;. series of the Animal Science Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Original publication date November 1992. Reviewed June 2003. Visit the EDIS Web Site at <http://edis.ifas.ufl.edu>.
- Sheldrake M, Hoar T, Gregor T, 1983.** Lactation stage, parity and infection affection somatic cell, electrical conductivity, and serum albumin in milk. *J Dairy Res*, 66:31-41
- StatPac, 2011.** admin@statpac.com.
- Stuhr T, Aurlich K, 2010.** Intramammary infections in dairy goats: knowledge and indicators for detection of subclinical mastitis. *Landbauforschung-vTI Agric Forestry Res*, 4(60):267-280.
- Tangorra F, Zaninellim M, Agazzi A, Savioni G, 2010.** Milk electrical conductivity and mastitis in dairy goats: results from a pilot study. *Small Rumin Res*, 90:109-113.
- Upadhyaya TN, Rao AT, 1993.** Diagnosis and threshold values of subclinical mastitis in goats, *Small ruminant research*, 12(2):201-210.
- Wagner SA, Joner DE, Apley MD, 2007.** Effect of endotoxin mastitis on epithelial cell numbers in the milk of dairy cows. *Am J Vet Res*, 70:796-799.
-
- Citation:** Roukbi M, Omar AN, Salam Z, Dibeh K, 2015. Investigation of subclinical mastitis cases in GCSAR Damascus goats from Humeimeh research station. *Net J Agric Sci*, 3(1): 5-13.
-