Rev. Salud Anim. Vol. 38 No. 2 (Mayo.-ago. 2016): 131-135
ISSN: 2224-4700

SHORT COMMUNICATION

Evaluation of an electrical conductivity portable device as an alternative for subclinical mastitis detection

Ana Beatriz da Costa Ribeiro[I], Joice Sifuentes dos Santos[I], Daniel Zanol[II], Letícia Neves Leme Lombarde[II], Samera Rafaela Bruzaroski[II], Agostinho Ludovico[I], Elsa Helena Walter de Santana[I]*

[I] Professor from Master in Science and Technology of Milk and Dairy Products. R. Marselha, 591, CEP 86041-140 Londrina, PR, Brazil. [II]Undergraduate Course in Veterinary Medicine, University North of Parana (UNOPAR), Av. Paris, 675, CEP 86041-120 Londrina, PR, Brazil.

ABSTRACT: The use of efficient methods for the early diagnosis of subclinical mastitis is of great importance in milk production. By using an electrical conductivity meter (EC), the efficiency of subclinical mastitis diagnosis was evaluated in dairy farms in northern Parana, Brazil, and the results were compared with those obtained by using the California Mastitis Test and the Somatic Cell Count (SCC). Samples from 941 quarters of 259 cows of the Holstein and Jersey breeds were analyzed in four dairy farms between June and July 2014. The EC method correctly evaluated 54.8% of the samples (264 samples with subclinical mastitis) and 250 without infection when compared with the diagnosis by CMT. Of the 445 samples diagnosed as positive by EC, 19.7% were classified by CMT with score 2 and 24.7% with score 3. Of the samples with negative results (496), most of them had scores 0 and 1 (50.4% and 21.4 %). The correlations between EC and SCC (r = 0.14) and EC and CMT (r = 0.17) were positive but low, which generated false positive and false negative results of subclinical mastitis in the herd.

Key words: California Mastitis Test, Somatic Cell Count, milk.

Evaluación del dispositivo portátil de la conductividad eléctrica como alternativa para la detección de mastitis subclínica

RESUMEN: El uso de métodos eficientes para el diagnóstico rápido de la mastitis subclínica es de gran importancia en la producción de leche. Mediante el empleo de un medidor de conductividad eléctrica (CE), se evaluó la eficacia del diagnóstico de mastitis subclínica en granjas de leche del norte de Paraná, en Brasil, y se compararon los resultados con la Prueba de California para Mastitis (CMT, *del inglés California Mastitis Test*) y con el conteo de células somáticas (CCS). Se analizaron 941 cuartos mamarios, obtenidos de 259 vacas de las razas Holstein y Jersey en cuatro granjas lecheras, entre junio y julio de 2014. La CE evaluó correctamente 54,8% de las muestras (264 muestras con mastitis subclínica) y 250 sin infección en comparación con el diagnóstico de CMT. De las 445 muestras diagnosticadas como positiva para EC, 19,7% se clasificaron por la CMT, con una puntuación de 2 y 24,7% con una puntuación de 3. Entre los resultados negativos (496 muestras), la mayoría de las muestras tuvieron una puntuación entre 0 y 1 (50,4% y 21,4%). Hubo una correlación positiva (p <0.001); sin embargo, resultó baja entre CE y CCS (r = 0.14) y CE y CMT (r = 0.17), por lo que generaron resultados falsos positivos y falsos negativos de la mastitis subclínica en el hato.

Palabras clave: Prueba de California, Conteo de Células Somáticas, leche.

***** Corresponding author: *Elsa Helena Walter de Santana.*
Correo electrónico: elsahws@hotmail.com

Mammary gland inflammation, usually in response to an invasive agent, is characterized by an increase in the milk somatic cell count (SCC). A milk SCC above 200,000 cells/ml indicates the presence of mastitis in cattle. However, counts greater than 100,000 cells/ml are already considered as indicative of subclinical mastitis (1). Brazilian legislation, as required by the Ministry of Agriculture, Livestock and Supply (MAPA), through the Normative Instruction No. 62 of 2011, established maximum SCC levels of 600,000 cells/ml for cool raw milk, until June 30.2014 and 500,000 cells/ml from July 1, 2014, to June 30, 2016 (2).

Subclinical mastitis must be detected at an early stage and by a reliable method to minimize clinical mastitis outbreaks, which can cause significant losses to the producers. With an increased SCC, alterations in milk components such as lactose, protein, and fat are observed. Changes in milk composition caused by mastitis allow performing indirect tests such as SCC, California Mastitis Test (CMT), and electrical conductivity (EC). The content of chloride and lactose can also be used to diagnose this disorder (3).

CMT is the most widespread form of subclinical mastitis diagnosis among producers and veterinarians in Brazil. EC measurement can be an alternative method replacing CMT because it is easy to use in the dairy farm routine. EC evaluates the ability of a solution to conduct an electric current between two electrodes and is given in milliSiemens per centimeter (mS/cm). EC increases are proportional to SCC increases. When the epithelium of the mammary gland is damaged as a result of mastitis, EC is modified due to changes in sodium, potassium, and chlorine balance (4).

The use of EC measurement as a form of diagnosis of subclinical mastitis in the routine of dairy farms can be a valuable tool for the producer because it enables early diagnosis of subclinical mastitis, facilitating the treatment and minimizing economic losses. Besides, EC can be an alternative to the use of CMT. Thus, the present work evaluated the subclinical mastitis diagnostic efficiency in dairy farms through the use of a portable electrical conductivity (EC) meter, and comparing the results obtained with those obtained by other methods currently available to the producer, like the California Mastitis Test (CMT) and the Somatic Cell Count (SCC).

Milk samples were collected from the daily milking routine of four dairy farms in northern Parana, Brazil, between June and July 2014. Those farms had mechanical milking system parlours and a herd of Holstein and Jersey breeds. Cows in early lactation (up to 10 days calved) and cows near to their dry period were excluded. The animals were prepared for milking routine according to each farm prescription. Milk samples were collected after discarding the first three strips of milk and disinfecting the teats with a 2% sodium hypochlorite solution. The following milk was used for analysis by CMT, the next for the EC measurement, and then, samples for SCC were collected. A total of 941 individual quarter samples were collected and analyzed by the three diagnostic methods.

The CMT was performed with the use of a plastic tray containing four chambers, where the milk collected was mixed with a violet base bromocresol reagent (Tadabras Industria e Comércio de Produtos Veterinarios Ltda). The milk and reagent mixture was slowly stirred for 10 seconds and the following results assigned: Negative (0), weak (1), distinct positive (2), and strongly positive (3).

For SCC analysis, the milk samples were placed in test tubes containing bromopol, and the somatic cells were determined by electronic counting using the flow cytometry technique in the Somacount 500® equipment (Bentley Intruments Inc.).

The EC measurement was performed with a commercial hand-held conductivity meter. The EC portable meter uses a combination of two values to indicate the result of subclinical mastitis: absolute electric conductivity (AEC) and differential electric conductivity (DEC). The AEC refers to the actual conductivity measurement of each teat, and the DEC is the difference of the absolute measure found and the lowest value of AEC of the cow, considered as 0 to calculation. The combination of these values is calculated by computer in the system known as inter quarter ratio (IQR), which indicates the presence or absence of subclinical mastitis (Table 1). As the non mastitic milk has similar electrical conductivity in all teats, the EC difference among all the teats of a cow may indicate mastitis in a particular one (5). The EC portable meter has a mechanism to compensate the possible differences in temperature of the samples and is calibrated with a 0.05 M KCl solution. The EC values and their interpretation can be seen in Table 1.

The data were analyzed by Statistical 12 software (Statsoft Inc.) through descriptive statistical analysis and non-parametric Kruskal-Wallis at 5% significance ($p<0.05$) to compare the results of AEC and DEC vs. CMT, and Spearman correlation test at 5% significance ($p<0.05$) between CMT vs. DEC and SCC vs. DEC.

By comparing data from CMT and EC, it could be stated that the EC values found for CMT 0 and 1 showed no statistical difference ($p>0.05$; Table 2). The same

TABLE 1. Interpretation of the values of absolute electric conductivity (AEC) and differential electric conductivity (DEC) in milk./ *Interpretación de los valores de conductividad eléctrica absoluta (CEA) y la conductividad eléctrica diferencial (CED) en la leche.*

AEC (mS/cm)	DEC (mS/cm)	Interpretation
< 6.2	< 0.5	Normal Milk
≥ 6.2	< 0.5	Abnormal Milk, indicating colostrum or end of the lactation
≥ 6.2	≥ 0.5	Subclinical mastitis
< 6.2	≥ 0.5	Subclinical mastitis

was observed between scores 2 and 3 (p>0.05). However, differences in the assessments were observed in comparing the CMT scores 0 and 1 with scores 2 and 3 (p<0.05) when absolute and differential values were considered. Milk with CMT 0 is considered normal, and scores 1, 2 and 3 are indicative of subclinical mastitis. It was observed that some samples tested with CMT score 0 presented DEC greater than or equal to 0.5 (187 samples), which indicated the presence of subclinical mastitis. This can be attributed to the fact that the EC detects subclinical mastitis earlier than the CMT method routinely used in dairy farms in the country (6).

Of the 172 quarters with CMT 1, 106 tested negative for mastitis by the EC. It is known that the electrical conductivity measures the presence of Na^+ and Cl^- ions in milk, which are released in higher amounts in the presence of an inflammatory process and tissue damage, depending on the damage extension of the affected quarter (7).

It is assumed that the milk before the removal of the first three strips and the residual milk (milk located in the mammary gland at the end of milking), are those containing the highest values of SCC (8). It was also confirmed that there might be variations in Na^+ concentration depending on the milk portion collected for the sample. Higher levels of Na^+ are present in milk before the withdrawal of three strips and in cisternal milk (milk before starting the milking process) and may decrease by up to 25% when measured in alveolar milk (during milking) (9).

The milk samples were collected soon after the withdrawal of the first three strips for all the tests (EC, CMT and SCC), assuming then that the portion used was alveolar milk. The cisternal milk, which contains higher SCC and higher EC can only be obtained when it is not done the pre-stimulation (removal of the initial strips) (8), which is used in Brazil to detect clinical mastitis through the strip cup test. As the manufacturer does not indicate in its instruction manual which is the

TABLE 2. Results of the California Mastitis Test (CMT), absolute electric conductivity (AEC), differential electric conductivity (DEC) and somatic cell count (SCC) of 941 individual quarter milk samples obtained from four dairy farms in northern Parana, Brazil, between June and July 2014./ *Resultados de la Prueba de California para Mastitis, conductividad eléctrica absoluta (CEA), conductividad eléctrica diferencial (CED) y Conteo de Células Somáticas (CCS) de muestras de leche de cuartos individuales obtenidos de cuatro granjas lecheras en el norte de Paraná, Brasil, entre junio y julio de 2014.*

CMT	n	AEC (mS/cm)	DEC (mS/cm)	SCC ($x10^3$ cells/ml)
0	438	4.91 ± 0.73^b (1.70 - 9.40)	0.52 ± 0.63^b (0.00 - 5.00)	263.19 ± 874.94^c (13 - 9500)
1	172	4.98 ± 0.64^b (3.00 - 6.70)	0.46 ± 0.59^b (0.00 - 3.30)	442.59 ± 1359.79^b (13 - 9500)
2	149	5.22 ± 0.92^a (2.20 - 9.10)	0.86 ± 0.89^a (0.00 - 4.10)	793.91 ± 1638.51^a (13 - 9893)
3	182	5.49 ± 1.42^a (1.70 - 10.40)	1.04 ± 1.13^a (0.00 - 4.70)	1419.31 ± 2301.49^a (13 - 9999)

Results are presented as Mean ± Standard Deviation (Minimum - Maximum).
Different superscript letters in the same column indicate statistical difference by the non parametric Kruskal-Wallis at 5% of significance (p<0.05).

exact time to use the portable EC measuring device, the same application protocol for CMT was used; that is, after discarding the first three strips. Therefore, there may be some influence of the collecting time on the measuring of the electrical conductivity. In a research, the residual milk after the milking process removing the milk left in the udder manually (postmilking strippings) was evaluated and showed an increase in the accuracy of EC when compared with the first milk drawn from the udder before proper milking (foremilk) in the same cow (10). Other authors (11), however, indicate that the best results are achieved when the milk is collected before milking.

There was a positive correlation (p<0.001) between DEC and SCC (r = 0.14) and DEC and CMT (r = 0.17). However, the correlation coefficients were low. A significant correlation between EC and log SCC was found; however, the correlation (r = 0.21) was higher than in the present study (10).

Samples with DEC at or above 0.5 mS/cm (Table 1), an index determined by the manufacturer, were considered positive for subclinical mastitis. Of the 941 quarter samples investigated, 445 samples (47.2%) were diagnosed as positive and 496 (52.8%) as negative by EC. Of the 445 samples diagnosed as positive by EC, 19.7% were classified with score 2 and 24.7% with score 3 by CMT. Of the negative results (496 samples), most samples had scores 0 and 1 (50.4% and 21.4%, respectively).

When the samples were diagnosed as positive for subclinical mastitis by EC (Table 3), 19.9% had a score of 0 on CMT and could be classified as false positive, while 25.3% of the samples were considered normal by EC and had scores 1, 2 and 3 on CMT, being considered as false negative. We can consider that 54.8% of the samples were evaluated correctly using the portable electrical conductivity meter. A similar frequency of false positives (16.5%) and higher of false negatives (47.7%) were observed in the first milk taken from the quarter before the actual milking (foremilk) (12). A correct identification of regular milk higher than in the present work has been indicated, around 90% (13). The apparatus used for EC evaluation was more efficient for detecting extreme change in conductivity values, or animals that were already presenting clinical mastitis and animals that were not showing any electrical conductivity alteration yet.

Considering milk with SCC lower than 100,000 cell/ml as normal milk, EC diagnosed 44.3% of the samples as subclinical mastitis and 16.8% as normal milk. When considering SCC higher than 100,001 cell/ml as subclinical mastitis, 20.6% were normal milk by EC, being considered as false negative, and 18.3% as samples from cows with subclinical mastitis (Table 4). Cows with SCC between 200,000 and 300,000 cell/ml must be identified to start their preventive or therapeutic treatment, like frequent milking and antibiotic therapy, to avoid new contaminations or other herd cow problems (14). Therefore, considering up to 100,000 cell/ml as a normal milk and SCC the pattern test, 35.1% of the samples were evaluated correctly using electrical conductivity.

TABLE 3. Diagnosis of subclinical mastitis by electrical conductivity and California Mastitis Test (CMT) of 941 individual quarter milk samples obtained from four dairy farms in northern Parana, Brazil between June and July 2014./ *Diagnóstico de mastitis subclínica por conductividad eléctrica y la Prueba de California para Mastitis de muestras de leche de 941cuartos individuales obtenidos de cuatro granjas lecheras en el norte de Paraná, Brasil, entre junio y julio de 2014.*

Diagnosis by electrical conduvity	California Mastitis Test (CMT)			
	0	1	2	3
Subclinical mastitis (%)	19.9	7.0	9.4	11.7
Normal Milk (%)	26.7	11.3	6.3	7.7

TABLE 4. Diagnosis of subclinical mastitis by electrical conductivity and Somatic Cell Counting (SCC) of 941 individual quarter milk samples obtained from four dairy farms in northern Parana Brazil between June and July 2014./ *Diagnóstico de mastitis subclínica por conductividad eléctrica y Conteo de Células Somáticas (CCS) de muestras de leche de 941 cuartos individuales obtenidos de cuatro granjas lecheras en el norte de Paraná, Brasil, entre junio y julio de 2014.*

Diagnosis by electrical conductivity	Somatic Cell Counting (SCC)	
	< 100,000 cell/ml	> 100,001 cell/ml
Subclinical mastits (%)	44.3	18.3
Normal milk (%)	16.8	20.6

Therefore, it can be concluded that the EC evaluation through the portable EC meter tested, which can be a direct test, showed a high proportion of results differing from SCC and CMT results, considering the latter an usual subclinical mastitis indicator. This fact caused a low correlation with routine tests used by producers in Brazil. So, even CMT presents some limitations like the failure to detect some types of infection and the need for a well-trained person able to execute the test, it interprets the results and decide, what action should be taken. The California Mastitis Test still shows to be the cheapest and most accessible and efficient test for the brazilian producer in comparison to the EC portable meter tested.

REFERENCES

1. Bytyqi H, Zaugg U, Sherifi K, Hamidi A, Gjonbalaj M, Muji S, Mehmeti H. Influence of management and physiological factors on somatic cell count in raw cow milk in Kosova. Vet Arhiv. 2010;80(10):173-183.

2. Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Regulamentos técnicos de produção, identidade e qualidade do leite tipo A, leite cru refrigerado e leite pasteurizado, Instrução Normativa Nº 62, 2011.

3. Ruegg PL, Reinemann DJ. Milk quality and mastitis test. 2002. Accessed in Apr. 27, 2014. http://www.milkquality.wisc.edu/wp-content/uploads/2011/09/milk-quality-and-mastitis-diagnostic-tests.pdf

4. Biggadike HJ, Ohnstad I, Laven RA, Hillerton JE. Evaluation of measurements of the conductivity of quarter milk samples for the early diagnosis of mastitis. Vet Rec. 2012;150(21):655-658.

5. Ilie, LI, Tudor L, Galis AM. The electrical conductivity of cattle milk and the possibly of mastitis diagnosis in Romania. Lucrãri ªtiin³ifice Medicinã Veterinarã. 2010;43(2):220-227.

6. Beloti V, Tamanini R. Propriedades organolépticas e físico químicas do leite. In: Leite: Beloti V. Leite: obtenção, inspeção e qualidade. Londrina: Ed. Planta. 2013; pp.51-108.

7. Zafalon LF, Nader Filho A, Oliveira JV, Resende FD. Electrical conductivity and chloride concentration of milk as auxiliary diagnostic methods in bovine subclinical mastitis. Pesquisa Vet Brasil. 2005;25(3):150-163.

8. Sarikaya H, Bruckmaier RM. Importance of sampled milk fraction for the prediction of total quarter somatic cell count. J Dairy Sci. 2006;89(11):4246-4250.

9. Janzekovic M, Brus M, Mursec B, Vinis B, Stanjko D, Cus F. Mastitis detection based on electric conductivity of milk. *J Miner Materials Characterization Engineering*. 2009;34(1):39-46.

10. Woolford, M.W., and J.H. Williamson. 1982. The electrical conductivity of milk as a diagnostic of subclinical mastitis. In Proceeding Conference Dairy Production from Pasture. Anim Res. Stn. Hamilton, New Zealand, p. 114.

11. Fernando RS, Rindsig RB, Spahr SL. Effect of length of milking interval and fat content on milk conductivity and its use for detecting mastitis. J Dairy Sci. 1981;64 (4):678-682.

12. Fernando RS, Spahr SL, Jaster EH. Comparison of electrical conductivity of milk with other indirect methods for detection of subclinical mastitis. J Dairy Sci. 1985;62(2):449-456.

13. Fernando RS, Rindsig RB, Spahr SL. Electrical conductivity of milk for detection of mastitis. J Dairy Sci. 1982;65(4):659-664.

14. Hamman J, Zecconi A. Evaluation of the electrical conductivity of milk as a mastitis indicator. International Dairy Federation. 1998;334: 5-26.

Recibido: 9-11-2015.
Aceptado: 3-5-2016.