International Journal of Applied Research 2017; 3(7): 862-867

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2017; 3(7): 862-867
www.allresearchjournal.com
Received: 08-05-2017
Accepted: 10-06-2017

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Comparative study of Porta SCC, udder check and dramanisk tests effectiveness on subclinical mastitis detection in Rwanda

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Abstract
A cross-sectional study was conducted in Musanze district, Busogo, Gataraga and Cyuve sectors with aim to compare the efficient of three on farm tests (Porta SCC, Udder Check and Dramanisk**)** in the subclinical mastitis detection. Six farms were randomly selected and a total of 30 lactating cows free of signs of mastitis were examined using those three on farm tests. Descriptive statistics, SPSS (Statistical Package for the Social Sciences) version 16 analysis were computed to determine mastitis prevalence and correlation between the results of three on farm tests. The results showed that the prevalence was of 70%, 60% and 56.7% for Porta SCC, Dramanisk and Udder checks respectively. In addition sensitivity and specificity of each test found was (76.2%, 77.8%), (58.3%, 88.9%), (61.5%, 94.2%) for Porta SCC, Dramiński and udder check respectively, while the correlation between Porta SCC and udder check was 0.602 which is significant at ($p < 0.01$), and there was no significant correlation between Dramiński and other tests. Therefore, we can conclude that cow-side tests that employ SCC (Porta SCC) for mastitis diagnosis are more accurate than Dramiński which measure EC and udder check read LDH enzymes level in milk sample. The test properties of the Porta SCC and its capability and rapidity to provide accurate results rationalize its utilization as an alternative for the laboratory based cell counter in evaluating milk samples from herds in rural areas under field conditions and help fast decision making in dairy management.

Keywords: Porta SCC, Udder Check and Dramanisk Tests, Effectiveness, Subclinical Mastitis, Lactating Cows

1. Introduction

Bovine mastitis is a major disease affecting dairy cattle worldwide and which results from the inflammation of the mammary gland. The severity of the inflammation can be classified into subclinical, clinical and chronic forms, and its degree is dependent on the nature of the causative pathogen, the age, breed, immunological health and lactation state of the animal. Sub-clinical mastitis is difficult to detect due to the absence of any visible indications, and it has major cost implications. Chronic mastitis is a rare form of the disease but results in persistent inflammation of the mammary gland.

Mastitis has been and continues to be recognized as one of the major disease problems concerning the dairy industry. It is also one of the most costly diseases confronting the dairy farmer and is a global problem as it adversely affects animal health, quality of milk and economics of milk production and every country including developed ones suffer huge financial losses. Estimating economic losses resulting from mastitis becomes an extremely difficult task because of the many levels of infection and other factors [14]. Currently, milk quality payments are based on somatic cell counts (SCCs), and elevated levels result in reduced payments. This, in addition to reduction in milk volume and treatment costs, significantly affects farm incomes [17].

Rwanda dairy subsector is the major player in the livestock industry ([14]). National milk production increased almost three-fold from 98 567 metric tonnes in 2000 to 158 764 metric tonnes in 2008 [16], and further increased to 445 000 metric tonnes in 2012 (MINAGRI, 2012). There was a glaring shortage of milk, further worsened by many setbacks including shortage of land, limited availability of feeds, unsuitable cattle breeds for high milk production, lack of funding and high prevalence of disease.

According to [3] mastitis is a major disease problem that blights the dairy subsector [2]. stated that mastitis is the most complex and costly disease of dairy cows occurring throughout the world. Mastitis causes direct economic losses in several ways, including reduction of milk yields, condemnation of milk due to bacterial or antibiotic contamination, treatment costs, higher than normal culling rates and occasionally deaths [19]. It has been shown that mastitis reduces the quality and quantity of milk, leading to losses of margins as high as $83.37 per cow per year [6].

Bovine mastitis is a significant disease of dairy herds, having a large adverse effect on farm economics; due to a reduction in milk production and treatment costs [20]. There are a large number of methods of detection currently in use, in order to monitor udder health performance. Changes in milk composition as a consequence of mastitis have contributed greatly to the diagnosing process. In Rwanda, an overall prevalence of 60% was revealed accompanied with Laboratory analysis through KOH, Catalase test, coagulase test and Gram stain then results showed that Staph Spp were major infectious agent with 96.51% [12].

Rapid and accurate diagnosis is helpful for making decisions about cows suspected of being infected with mastitis. Numerous methods are used to identify mastitis infections. Those requiring the submission of milk samples to a laboratory have been criticized as too slow for on farm decision making. To overcome this hurdle, indirect measures of the somatic cell counts (SCC) such as the California Mastitis Test (CMT) have been used for many years. Recently, several new on-farm tests have been introduced. Hence, a laboratory analysis

was undertaken to compare Porta SCC, Udder Check and Dramanisk tests with an attempt to:

a) To detect subclinical mastitis by using Dramanisk and determine the prevalence
b) To detect subclinical mastitis by using udder check and determine the prevalence
c) To detect subclinical mastitis by using Porta SCC and determine the prevalence.
d) To analyse and compare results of prevalence obtained for each test.

2. Material and methods

2.1. Study area

This study was performed using milk samples from 120 quarters from 30 cows, collected from six dairy farm selected randomly in three sectors of Musanze district (Cyuve, Gataraga and Busogo), to compare the three on farm test Porta SCC® milk test, Uddercheck™ and Dramanisk mastitis detector based on their effectiveness. Data from 30 cows samples was used in a statistical analysis.

2.2. Methods

2.2.1. Sample selection

The primary data were collected from 6 farms selected in three sectors of Musanze district (Cyuve, Gataraga and Busogo), by randomly sampling considering that the most accessible unit are selected. Cattle to be included in the sample were selected based on the following criteria: lactating, healthy, not having any clinical signs of mastitis and all quarters functioning; and a total of 30 lactating cow was selected.

2.2.2. Sample size determination

The sample size was calculated using Javeau (1985) who advised that a good sample size should be at least between 20-30% of the population. In this study, we used 30% in determining the sample of dairy cattle involved in the study, from each farm. Sample size was calculated by using the following formula: $n=\sum (30\% N*Ni/N)$

Where: N: total number of cow in six farms selected.
Ni: number of cow in each farm
n: sample size

Table 1: Population and sample size

Farm	Dairy cattle	N	Dairy cattle Ni
F1	27	8	
F2	36	10	
F3	10	3	
F4	7	2	
F5	13	4	
F6	10	3	
Total	103	30	

Source: Primary data

2.2.3. Data collection

During this study, cross-sectional study design was used in which the six farm selected from Three Sectors of Musanze District (Cyuve, Gataraga and Busogo) were visited once for data collection. A questionnaire was used to collect basic information from farmers, specifically including farm location and cow identification; records on daily farm management were also taken. Each cow was observed and scored for dirtiness and teat-end condition and checked for clinical mastitis visually. Samples of milk from the four quarters were tested using electrical conductivity (Dramanisk® Mastitis Detector); enzymes LDH (Uddercheck TM) and Somatic cell counts (Porta SCC) and we have used these tests as indicated by manufacturers, the results of each test readings are summarized in table 2.

Table 2: summarizing reading results of each test

Testa	Reading and interpretation	Conclusion
Porta SCC	Digital reader display intensity of color on test strip in number and the obtained number is multiplied by 1000000 to have exact somatic cells.	Scc> 200000, this indicated positive results, cow has mastitis. Scc <200000 indicated negative results.
Dramiński	Dramiński display the number which are interpreted on whole udder basis, if there is reading variation of 50 in quarters and all quarters having reading value above 300.	Variation of 50 indicates the case of mastitis.
Udder check	Reading below 290 for older cows(above 9 years) Dipstick was introduced in milk sample and degree of color change was scored (-), (+), (++) and (+++).	For older cows, indicate the increase of infection of subclinical mastitis (-) negative indicated no mastitis (+), (++) and (+++).positive indicated positive at different level of infection

3. Data analysis

Data analysis information and data from questionnaires were chronologically encoded in Microsoft Excel sheets. The Statistical Package for the Social Sciences (SPSS) version 16 was used to analyze the data. Descriptive statistics, Chi-square analysis and logistic regression were computed to determine subclinical mastitis prevalence in relation to each test used. Different formulas we have used**:**

1. Prevalence = Σ Condition positive/ Total population examined
2. Sensitivity and specificity have been calculated by Porta SCC as gold standard as it has been proved to be highly accurate by (Ruegg, 2009, Salvador *et al.* 2014).

Sensitivity or true positive rate (TPR)

$$TPR = TP/P = TP/(TP + FN)$$

Specificity (SPC) or true negative rate

$$SPC = TN/N = TN/(TN + FP)$$

Where
TP = true positive
TN = *true* negative
FP = false positive
FN =false negative
(*Fawcett et al., 2006)*

4. Results

4.1. Prevalence of mastitis based on results of each test

The results of our study in figure 6 reveal the prevalence summary of subclinical mastitis for 30 cows tested using the three on farm tests.

Mastitis prevalence summary from the three test

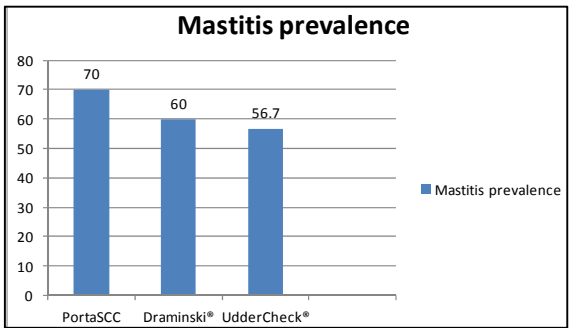

Fig 1: Mastitis prevalence summary for each test

The results of our study in figure 5 show the comparative summary of the prevalence of subclinical mastitis. There is variation of mastitis prevalence related to Porta SCC, Dramiński and udder check used, with 70%, 60% and 56.7% respectively.

4.2. Comparison between tests (significant difference)

Table 3: Correlation between tests

Correlations	Uddercheck TM Udder decision	Porta SCC Udder decision	Dramiński decision
Uddercheck TM Udder decision			
Pearson Correlation	1	0.602**	0.522**
Sig. (2-tailed)		0.000	0.003
N	30	30	30
Porta SCC Udder decision			
Pearson Correlation	0.602**	1	0.505**
Sig. (2-tailed)	0.000		0.004
N	30	30	30
Dramiński decision Pearson Correlation Sig. (2-tailed) N	0.522** 0.003 30	0.505** 0.004 30	1

**. Correlation is significant at the 0.01 level (2-tailed).

Interpretation: There was significant correlation between UddercheckTM and Porta SCC but no correlation between Dramiński.

Table 4: Porta SCC compared to Dramiński

Porta SCC Udder decision *	Dramiński decision Cross tabulation		
YES	*Yes*	No	Total
Count	16	5	21
% within Porta SCC_Udder_decision	76.2%	23.8%	100.0%
% within Draminski_decision	88.9%	41.7%	70.0%
% of Total	53.3%	16.7%	70.0%
Count	16	5	21
NO			
Count	2	7	9
% within Porta SCC_Udder_decision	22.2%	77.8%	100.0%
% within Draminski_decision	11.1%	58.3%	30.0%
% of Total	6.7%	23.3%	30.0%
Total Count % within Porta SCC_Udder_decision % within Udder check % within Draminski_decision % of Total	 18 60.0 % 100.0% 60.0%	 12 40.0% 100.0% 40.0%	 30 100.0% 100.0% 100.0%

Interpretation

1. In 21 cows tested positive to subclinical mastitis based on results with Porta SCC, 16 of them were truly positive, 5 were false negative and 7 were truly negative with 76.2% and 77.8% sensitivity and specificity respectively.
2. In 18 cows tested positive to subclinical mastitis based on results with Dramanisk, 16 were truly positive while 2 were false positive with 58.3%and 88.9% sensitivity and specificity respectively.

Table 5: Porta SCC compared to Uddercheck TM Crosstab

Porta SCC Udder decision		**Uddercheck TM Udder decision**	
YES	*Yes*	No	Total
Count	16	5	21
% within Porta SCC Udder decision	76.2%	23.8%	100.0%
% within Udder check	94.2%	38.5	70%
% of Total	53.3%	16.7%	70.0%
% within Porta SCC Udder decision	76.2%	23.8%	100.0%
NO			
Count	1	8	9
% within Porta SCC Udder decision	11.1%	88.9%	100.0%
% within Udder check	5.8%	61.5%	30%
% of Total	3.3%	26.7%	30.0%
Total Count % within Porta SCC Udder decision % within Udder check % of Total	 17 56.7 % 100.0% 56.7%	 13 43.3% 100.0% 43.3%	 30 100.0% 100.0% 100.0%

Interpretation

1. In 21 cows tested positive to subclinical mastitis based on results with Porta SCC, 16 of them were truly positive while 5 were false negative with 76.2% and 77.8% sensitivity and specificity respectively.
2. In 17 cow tested positive to subclinical mastitis based on results with uddercheck,16 of them were truly positive, 1 was false positive and 8 were truly negative with 61.5 % and 94.2% sensitivity and specificity respectively.

Table 6: Dramiński compared to Udder Check Crosstab

Dramiński decision	**Uddercheck TM Udder decision**		
YES	Yes	No	Total
Count	14	4	18
% within Dramiński decision	77.8%	22.2%	100.0%
% within Uddercheck TM Udder decision	82.4%	30.8%	60.0%
% of Total	46.7%	13.3%	60.0%
NO			
Count	3	9	12
% within Dramiński decision	25.0%	75.0%	100.0%
% within Uddercheck TM Udder decision	17.6%	69.2%	40.0%
% of Total	10.0%	30.0%	40.0%
Total Count % within Dramiński decision % within Udder check % within Uddercheck TM Udder decision % of Total	 17 56.7 % 100.0% 56.7%	 13 43.3% 100.0% 43.3%	 30 100.0% 100.0% 100.0%

Interpretation

1. In 18 cows tested positive to subclinical mastitis based on results with Dramanisk, 14 of them were truly positive while 4 were false negative with 77.8% and 69.2% sensitivity and specificity respectively.
2. In 17 cow tested positive to subclinical mastitis based on results with uddercheck,14 of them were truly positive, 3 were false positive and 9 were truly negative with 75% and 82.4% sensitivity and specificity respectively.

5. Discussion

With the increasing trend in the milk production the incidence of mastitis has also swollen up, incurring great loss in terms of economic loss and future productivity of the cow. There is an ardent need to identify certain diagnostic tools to detect mastitis at its earliest. The study was conducted in Musanze district, Busogo, Gataraga and Cyuve sectors to compare the three on farm tests (Porta SCC, Udder Check and Dramanisk**)** in order to know the more efficient in the subclinical mastitis detection. The results revealed an overall prevalence of 70%, 60% and 56.7% of Porta SCC, Dramanisk and udder check respectively in the study area.

The mastitis prevalence of 60% obtained in this study in MUSANZE district is higher than the 58.6% reported by [4] in cows on a farm in Nyagatare District using the Dramiński Mastitis Detector. The difference between the two results is low; a possible reason for this difference in prevalence may be due to environmental conditions which are different in two districts as Dramiński is sensitive to environment. The prevalence of mastitis found in our study is lower than that in the studies of [8] and [10] in Slovenia and in Pakistan respectively, who used the Dramiński test and reported

prevalence values of 80%, and 65.2% respectively. However, prevalence of mastitis in our study is higher than 39% reported by [21] in New Zealand and 46.4% reported by [18] in India. Whilst the Dramiński test based on electrical conductivity is regarded as a low-precision cow-side mastitis screening test, [1] reported compatibility between the results of somatic cell counts and Dramiński tests, in which the somatic cell count detected 64.4%of subclinical mastitis in Cows whilst the Dramiński test detected 59%.

In the current study, the prevalence of Porta SCC is 70% which is higher than that of Dramiński (60%) and udder check (56. 7%), and there is no any study found done on mastitis prevalence using Porta SCC but many studies have been done to evaluate the performance of Porta SCC capability to detect the subclinical mastitis; [15] studied the performance of portable somatic cell counter (Porta SCC) relative to that of the laboratory-based somatic cell counter (Fossomatic) in the diagnosis of bubaline subclinical mastitis and found that there is a substantial agreement (k=0.70) between the results of the two tests. This means that the test properties of the Porta SCC and its capability to rapidly provide results rationalize its utilization as an alternative for the laboratory-based cell counter in evaluating milk samples from herds in remote areas under field condition. [13] Compared the Porta SCC milk test with the California Mastitis Test (CMT) and laboratory results. Data from 289 samples was used in a statistical analysis and set a threshold of 200,000 cells/mL, the agreement between the Porta SCC and lab results was 87.8% and concluded that the results of the Porta SCC were similar to lab results and a high level of agreement was identified. Based on these evidence and results of our study, it is obvious that Porta SCC is more effective than Dramanisk.

The present study findings had shown the variation in sensitivity and specificity through different test results. In 21 cows tested positive to subclinical mastitis based on results of Porta SCC, 16 of them were truly positive, 5 were false negative and 7 were truly negative with 76.2% and 77.8% sensitivity and specificity respectively. The derived values of this study showed higher specificity than sensitivity which may be comparable to results obtained by [13], [9] and [10]. This patern is in contrast with the results of [15] and [5], which showed higher sensitivity than specificity as presented in table 10.

The variation in the results of these studies may be linked to measurement bias. Porta SCC is evaluated through colorimetry. Changes in colour may be interpreted inconsistently by different observers. Nonetheless, all these studies lead to the conclusion that Porta SCC is an excellent alternative to the laboratory based somatic cell counter.

Table 7: Comparison of the observed test properties of Porta SCC in detecting subclinical mastitis.

References	Sensitivity	Specificity	Technique
Current study	76.2%	77.8%	Dramanisk and udder check
Salvador *et al*., 2014	94.12%	87.30%	Fossomatic
Leslie *et al*., 2006	74%	94%	SCC
Dillon, 2012	92%	85%	SCC
Lam *et al*., 2009	73%	83%	Fossomatic
Rodriguez *et al*., 2009	76%	94%	Electronic enumeration of cells

In 18 cows tested positive to subclinical mastitis based on results of Dramanisk, 16 were truly positive while 2 were false positive in regard to Porta SCC, with 58.3%and 88.9% sensitivity and specificity respectively. The sensitivity of (58.3%) was comparable to that of (51%) and the specificity of Dramanisk (88.9%) was much higher than that (71%) reported by [9].

In 17 cow tested positive to subclinical mastitis based on results of uddercheck,16 of them were truly positive, 1 was false positive and 8 were truly negative compared to Porta SCC with 61.5 % and 94.2% sensitivity and specificity respectively. The sensitivity of (61.5%) was much lower than that of (93%) and the specificity of (94.2%) were approximately equal to that of (94%) reported by [7].

The results of this study, showed r = 0.602 (correlation between udder check and Porta SCC) at level of significant 1%, since critical value at level of significantly of 1% is lower than r, there is a correlation. For Porta SCC and Dramanisk r = 0.505 and critical value at level of significant of 1% is greater than r, there is no correlation. The results also showed r = 0.522 that on Dramanisk and udder check, critical value at level of significant of 1% is greater than r, then there is no correlation.

The findings of this study highlighted the accuracy of the Porta SCC test. The Sensitivity was 76.2%, specificity of 77.8%. The Spearman rank association test revealed the Porta SCC and the udder check to be slightly correlated (R^2 =0.602, P = <0.01) and this results is comparable to the results of Leslie (2006) showed that The Spearman rank association test revealed the electronically counted SCC and the Porta SCC to be highly correlated (R2 = .63, P = <0.01(Leslie, 2006)). In another study, by Professor David Lee, the Porta SCC is evaluated against electronically counted somatic cells of two separate laboratories (www.portachek.com). The samples were collected at random from Holstein cows at different stages of lactation. The results from the Porta SCC were read with a digital reader same procedure as in the present study. The digital reader calculates the actual cell count according to the depth of the blue colour on the test pad.

An excellent correlation of 98% was found between the Porta SCC data and the average of the two reference labs (www.portacheck.com). Porta Check incorporated has compiled results from various countries regarding the Porta SCC testing ability in detection of sub-clinical cases. Excellent results were achieved. A high correlation of 0.88 was established between the Porta SCC and the gold standard, which was much higher than the correlation of 0.602 between Porta SCC and Uddercheck TM.

6. Conclusion

The detection and diagnosis of subclinical mastitis is an important component of mastitis control even though sub-clinical cases are difficult to detect due to the absence of visible signs, thus appropriate diagnostic tests are needed. Cow-side tests that utilize physical changes in milk samples, SCC alterations, enzymes and/or EC are widely used in mastitis diagnostics. Based on results, prevalence of population was 70%, 60% and 56.7% Porta SCC, dramaniski and udder checks respectively. In addition

sensitivity and specificity of each test found was (76.2%,77.8%), (58.3%,88.9%), (61.5%,94.2%) of Porta SCC, Dramiński and udder check respectively, and correlation between Porta SCC and udder check was 0.602 which is significant at (p<0.01) then we can conclude that cow-side tests that employ SCC (Porta SCC) for mastitis diagnosis are more accurate than Dramiński which measure EC and udder check read LDH enzymes level in milk sample. The test properties of the Porta SCC and its capability to rapidity to provide results rationalize its utilization as an alternative for the laboratory based cell counter in evaluating milk samples from herds in rural areas under field conditions.

From these results, the comparison of Porta SCC, Dramanisk and udderchech based on test results and statically analysis revealed high effectiveness of Porta SCC over other tests used.

7. Acknowledgments

Authors are grateful to the University of Rwanda, College of Agriculture, Animal Sciences and Veterinary Medicine for the financial support and the technical support rendered by CAVM/Busogo Microbiology and Biology Laboratories. Our sincere thanks also to all farmers for the cooperation and helpful during data collection.

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