Evaluation of a Portable Somatic Cell Counter in the Diagnosis of Bubaline Subclinical Mastitis

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Abstract

The study aimed to evaluate the performance of portable somatic cell counter (PortaSCC) relative to that of the laboratory-based somatic cell counter (Fossomatic) in the diagnosis of bubaline subclinical mastitis. It determined the sensitivity and specificity of PortaSCC with Fossomatic as the reference. The agreement between the results of the two test equipments was also measured using kappa statistics. Eighty milk samples were collected from different farms. Samples were immediately processed for somatic cell count using the PortaSCC. Same samples were brought to the laboratory for the somatic cell counts. All samples were processed in triplicates. The PortaSCC has 94.12% and 87.30% sensitivity and specificity, respectively. A substantial agreement (k=0.70) between the results of the two tests was also observed. These test properties of the PortaSCC 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 remote areas under Philippine field conditions.

Keywords: mastitis, subclinical mastitis, somatic cell count, fossomatic, PortaSCC

Introduction

Mastitis is a serious health concern in the dairy industry considering the losses it can cause. The condition is related to heavy economic losses as it decreases the quality and quantity of milk. Additional costs are brought about by the needed medicines to address the problem. Additional concern is the effect of this disease to the productivity of the animal because the start of ovarian activity in post partum cows is compromised. Consequently, estrus and ovulation of infected cows are delayed (Lago et al., 2006). The condition is also associated to the occurrence of calf scouring (Mingala and Gundran, 2008).

Mastitis is either clinical or subclinical (Batavani et al., 2006). The former can be immediately detected in which hot, swollen quarter or udder with abnormal secretion is apparent. In contrast, the latter requires tests to be detected. Somatic cell count (SCC) is widely used to diagnose this condition considering its high sensitivity. It is however not as handy as the other tests like California mastitis test (CMT). Milk samples need to be brought to the laboratory for evaluation. Such set up inhibits prompt evaluation and management for affected herds located away from the laboratory. A number of dairy cooperatives are located in areas where submission of milk samples for SCC is a difficulty. The travel

and processing period consume much time. Such delay compromises production. Without evaluation, milk yield from cows previously identified as infected with subclinical mastitis will not be accepted. It is however possible that these animals had recovered and are now producing acceptable milk. Likewise, there is also a possibility that milk from currently infected animals will be included in the accepted milk harvest as they were identified as healthy before.

California mastitis test is the most common cow-side test in the Philippines provides immediate but very rough estimates of the somatic cell counts. Test results may therefore be imprecise. Thus, there is a need for a test that can provide immediate and reliable results under field conditions.

The study aimed to evaluate the performance of portable Somatic cell counter (PortaSCC) relative to the laboratory-based Somatic cell counter (Fossomatic) in the detection of subclinical mastitis using bubaline's milk. The sensitivity and specificity were computed. The degree of agreement between the results of the two tests was likewise identified.

Materials and Methods

Collection of Samples

Milk samples were collected from 80 buffaloes. The subjects were randomly selected from 10 different herds. During farm visits, farmers were asked to submit milk samples collected from each milking buffaloes. Samples were labelled for proper identification. Labels were raffled to select the samples to be included in the evaluation. Three replicates were used for each milk samples.

Methods Using the PortaSCC and Fossomatic Machine

Milk samples were immediately processed for somatic cell count using PortaSCC at the site of collection. Results were recorded after each observation. Same milk samples were used for somatic cell count using the Fossomatic machine in the laboratory. Samples were kept in a cooler prior transport to the laboratory for analysis.

Procedure for Somatic Cell Count Using PortaSCC

The PortaSCC was used according to the manufacturers' instructions (Figure 1). Moreover, to avoid measurement bias, only one person was tasked to evaluate all the milk samples. Each milk sample was thoroughly mixed prior evaluation. Using a pipette, four drops were added to the test strip sample well. Each drop was added once the prior milk drop was absorbed by the test strip. Four drops of activator solution were subsequently added to the strip. Evaluation was done after five minutes (Figure 2) (PortaCheck, 2011).

The positive sample made the test strip sample well change to a blue color. The resulting somatic cell count was based on the darkness of the blue color and was estimated by comparing the strip to the color chart (Figure 3). The color chart was divided into six categories with their corresponding estimated number of somatic cell count on each category. A somatic cell count greater than 200,000 cells mL^{-1} which is on the second to sixth category indicates that the source of the milk sample was positive with subclinical mastitis.

Figure 1 PortaSCC was used according to the manufacturers' instructions. Four drops from each milk sample were added to the test strip sample well. Each drop was added once the prior milk drop was absorbed by the test strip.

Procedure for Somatic Cell Counting Using the Fossomatic Machine

The milk samples were mixed by shaking the tube in a figure of eight motion. Sample tube containing the milk was placed in the holder in front of the machine. The somatic cell count was evaluated by the machine. Results displayed in the computer screen are recorded for comparison.

Data Analysis

A case of subclinical mastitis was defined as the presence of more than 200,000 somatic cells per mL of milk. A two by two table reflecting the results of the two tests was set up. The results of the Fossomatic machine were used as the reference in the calculation of the test properties of the PortaSCC. Sensitivity and specific of PortaSCC and their respective confidence intervals (CI) were calculated using mid-P 95% confidence interval (95% CI)

The agreement between the results of the two tests was evaluated using kappa statistics. The interpretation of the kappa results was based on the proposal of Landis and Koch (1977). Kappa statistics and their interpretation are as follows: Poor (<0.00); Slight (0.00-0.20); Fair: (0.21-0.40); Moderate: (0.41-0.60); Substantial (0.61-0.80); Almost perfect (0.81-1.00)

Figure 2 Evaluation was done after five minutes of incubation at ambient temperature.

Figure 3 Positive sample corresponds to change in color. The resulting somatic cell count was based on the intensity of the color and estimated by comparing the strip to the color chart.

Results and Discussion

Based on the results plotted in Table 1, the sensitivity of PortaSCC is 94.11% (71.31 to 99.85). Specificity on the other hand is 87.30% (76.50 to 94.35). The derived values of this study may be comparable to the results of Dillon et al. (2012) presented in Table 2. In both studies, the sensitivity is higher than the specificity. This pattern is in contrast to the results of Leslie et al. (2006), Lam et al. (2009), and Rodriguez et al. (2009). The variation in the results of these studies may be linked to measurement bias. PortaSCC is evaluated through colorimetry. Changes in color may be interpreted inconsistently by different observers. Nonetheless, all these studies lead to the conclusion that PortaSCC is an excellent alternative to the laboratory based somatic cell counter.

It is ideal that diagnostic tests have a high specificity and sensitivity. Whether the sensitivity should be higher than the specificity or vice versa depends on the purpose in doing the test. In a huge scale of dairy production, test specificity is of more importance than test sensitivity. The former can be used as a reference to prove that the herd is free from subclinical mastitis and that the milk produced is fit for consumption.

In the case of the dairy co-operators of the Philippine Carabao Center, high sensitivity is a preference. A standard herd and health management program is being discussed to the farmers. However, deviation from the management protocol is sometimes observed. Hence, health status may be compromised. Such may be linked to the belief of some co-operators that they can keep their buffaloes healthy by following their usual practice which may be different from the standard program. Thus, the ability of the test to detect the disease is more important.

Table 1 Cross tabulation of the PortaSCC and Fossomatic machine results.

PortaSCC Results	Positive	Negative	Total
Positive	48	24	72
Negative	3	165	168
Total	51	189	240

Table 2 Comparison of the observed test properties of PortaSCC in detecting subclinical mastitis.

References	Sensitivity	Specificity	Technique
Current study	66.67%	98.21%	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

Claiming that a test is a good alternative of the gold standard used is arbitrary. The study of Thekisoe at al. (2005) showed a 49% and above 60% sensitivity and specificity, respectively, of loop-mediated isothermal amplification (LAMP) relative to the mouse inoculation in detecting *Trypanosoma evansi* in experimentally infected pigs. Nonetheless, their assertion that the LAMP procedure is a good alternative to mouse inoculation was accepted by the scientific community.

The high sensitivity observed in this study justifies the use of the test in identifying which animal is infected with subclinical mastitis. Being able to provide immediate results, cows can be managed better, treated earlier, and return to the healthy herd. Currently, around 6% of the animals tested with PortaSCC may have false positive results. However, a protocol may be developed to confirm through Fossomatic. For instance, PortaSCC results that lie in the border of either healthy or infected may be re-evaluated at the laboratory. On the other hand, as PortaSCC is based on colorimetry, the evaluator may somehow improve in interpreting the results of the test. Consequently, the sensitivity may be increased. Such is likewise expected in identifying which animal is really healthy.

Other than the excellent test properties of the PortaSCC, its portability is another advantage. The kit is small and light and does not require electricity to operate. Thus, it is very much suited for field evaluation without compromising the accuracy of the results. It is better than CMT which is being utilized in the regular screening for subclinical mastitis.

A substantial agreement (k=0.70) between the results of the two tests was also observed. This strengthens the claim that PortaSCC is a good alternative for the laboratory-based SCC.

Conclusions

The PortaSCC is a good surrogate for the laboratory-based somatic cells counter. The PortaSCC has 94.12% and 87.30% sensitivity and specificity, respectively. A substantial agreement (k=0.70) between the results of the two tests was also observed.

These test properties of the PortaSCC 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 remote areas under Philippine field conditions.

Other than the adoption of the test for field evaluation, it is recommended that one person for every cluster of herds be trained to use the PortaSCC. Since herds are located distant to each other, it will be an inconvenience for one person to evaluate milking cows in all areas. Milk samples

may not be submitted as it will defeat the purpose of adopting the PortaSCC for evaluation. As presented earlier, a handy and reliable test must be used at the farm level to have immediate results. Thus, if there will be three or more evaluator, the tasked will be lighter.

Moreover, a regular bacterial culture, isolation, and antibiotic sensitivity test should be conducted to facilitate effective treatment.

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