

Accuracy rate of mastitis on-farm test (udder check) in comparison to somatic cell count (S.C.C) in dairy herd

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Abstract

Mastitis in bovine is an important disease in milk herds that cause economical losses due to decrease of milk production and treatment costs. Somatic cell count (SCC) is one of traditional test in mastitis diagnosis. The aim of current study was to evaluate the accuracy of udder check (on-farm) in comparison to SCC in industrial livestock. Current study was conducted in a 100 bovine livestock, samples were evaluated by udder check test at farm and simultaneously for SCC measurement were sent to, Pegah pasteurized Milk Company. Then the accuracy of two tests was evaluated and compared. The result was evaluated by descriptive method in SPSS ver. 22. The results of study indicated that SCC was 2014.86×10^6 in studied bovine. In addition, SCC results demonstrate 48 percent positive and remnant was negative. Udder check test results indicate 76 percent of samples was positive which 22% was 3positive, 21% was 2positive, and 33% was 1positive and positive cases was significantly different from SCC test ($p < 0.05$). The results indicated that the udder check has a higher specificity and sensitivity in mastitis detection and successfully could detect the subclinical mastitis at farm and provide opportunity to early treatment.

Key words: Mastitis; cow; Somatic Cell Count; Udder check test; Lactate dehydrogenase

Introduction

Mastitis is the most significant disease of dairy herds, and economically important. Mastitis reduce milk production and impose treatment costs, increased labour, milk withholding following treatment, death and premature culling. Hence, using efficient techniques to identify the presence of mastitis early in the disease syndrome is very important to decrease the costs of treatment and other costs. Mastitis causes by environmental or infectious agents which some of pathogens are more virulent. The severity of the inflammation can be classified into subclinical, clinical and chronic forms (Gröhn *et al.*, 2004). Due to absence of any visible symptoms, detection of subclinical mastitis is difficult, for this reason, this type of mastitis has major cost implications (Viguier *et al.*, 2009). It was reported that 70 to 80% of losses of mastitis were due to subclinical mastitis (Reneau and Packard, 1991).

Diagnosis and detection of mastitis specially subclinical form could be perform by somatic cell counts, an indication of inflammation, measurement of biomarkers associated with the onset of the disease (e.g., the enzymes N-acetyl-b-Dglucosaminidase and lactate dehydrogenase) and identification of the causative microorganisms (Viguier *et al.*, 2009). Generally, Cell counts used for evaluation inflammatory changes. The quantification of cells in milk, or somatic cell count (SCC), is estimated using direct microscopic or electronic cell counters or by an indirect method of estimating SCC using the California mastitis test (CMT) (Donovan *et al.*, 1992).

Enzymatic activities of milk are influenced by different factors, such as stage of lactation, season, species, race, food, mastitis (Mohammadian, 2011). Immune system response against clinical and subclinical mastitis was through invasion of polymorphonuclear leukocytes and macrophages, which cause inflammation. These cells along with udder's damaged epithelial and interstitial cells secrete hydrolytic enzymes include non-lysosomal enzymes, such as lactate dehydrogenase, and lysosomal enzymes such as β -galactosidase (Oliszewski *et al.*, 2002). LDH is an enzyme that is part of the glycolytic pathway and it is known to consist of five isotopes, designated LDH1 to LDH5 (Larsen, 2005). In mastitis, LDH activity increases. The aim of this study was to evaluate changes of LDH in milk by Udder Check test and comparison the results with somatic cell counts in subclinical mastitis in dairy herd.

Materials and Methods

This study performed in 100 Holstein cow, in dairy farm in East Azarbaijan province of Iran. At the time of milking in the herd, milk samples were taken from each cow individually. The milking performed by machine. Samples taken was evaluated by strip kits Udder Check (manufactured in the USA for PortaCheck, Inc., Moorestown, N.J.) test and then to measure the somatic cells count in milk were transferred to Tabriz Pegah Dairy factory laboratory. After assessing the results of both tests the accuracy of detection of mastitis by two methods were compared.

Elevated levels of LDH (lactate dehydrogenase) in milk were detected by Udder check diagnostic kit. Lactate dehydrogenase concentration in milk is one of the most effective markers for tissue damage and is indicative of active infection. Lactate dehydrogenase levels increase generally much faster than the increase of somatic cells count, therefore, as an important indicator for early detection of breast infection. To perform the test after removing the test strip from the vial, the test strip inserted to the milk sample and then with a flick of the test strip was out the extra milk from the bar. After 2 minutes, the color of strip kit was compared by the standard color on the vial. Based on studies, the sensitivity of Udder check, 93% and specificity is 94% .

Milk of cows with subclinical mastitis classified in three stages based on Somatic cell count; low (with a somatic cell count of less than 200 Thousand per mL), intermediate (200-800 thousand somatic cell count per mL) and high (more than 800 Thousands of somatic cell counts per mL).

Statistical Analysis Data from study was analyzed using SPSS version 22.0 software. In order to compare the results of two tests used in this study, Chi-square test and for positive and negative results frequency test was used.

Results

The average amount of somatic cell counts, was above 250 thousand ($2014.86 \times 10^6 \pm 388.70$) and demonstrate the presence of infection in the studied cattle. Somatic cell count test indicated that the 48% of cases was positive in case of infection and 52% was negative. In Udder check tests, 76% of cases were positive and 24% negative. It is also noted that from 76% positive cases, 22% was 3-positive, 21% was 2-positive, 33% were 1-positive. Compare the results of two different methods using chi-square test showed a statistically high significant difference between the two methods ($p < 0.01$).

Discussion

Mastitis is a common disease in dairy herds and causes milk production decrease and blindness of teat and remove the cow from farm and economical losses to dairy herds. Subclinical mastitis is a main problem in

dairy herds, and it is not detectable by farmer and in the milking room. Subclinical mastitis, gradually contaminate the whole flock and reducing milk production and sometimes causes acute and sub-acute mastitis in flocks and blindness of teat and remove of cow (Busato *et al.*, 2000; Schrick *et al.*, 2001). Somatic cell count examination in milk, time-consuming and requires laboratory advanced equipment, so simpler techniques for detection of mastitis in cattle has been suggested and always have tried these methods as much as possible simplified, so that the farmer can perform them in diary (McCarron *et al.*, 2009).

One of the newest methods in this regard is the use of colored stripes of mastitis diagnosis which is similar to tapes used in the detection of blood glucose (Batavani *et al.*, 2003; Larsen, 2005; Viguier *et al.*, 2009). Researchers (2006) concluded that the high number of somatic cells in the milk of about 250,000 cells per milliliter or more indicates mastitis (Berry and Meaney, 2006). Researchers found in herds that their milk tank somatic cells per milliliter less than 250,000 cells show an appropriate level of breast health. According to researchers at the Pennsylvania average milk somatic cells was 331000 cells per milliliter of milk (Norman *et al.*, 2000). In the Europe from 1992 the number of 400,000 cells per ml for two consecutive three-month period is exceeded, milk production is not suitable for human consumption and is not approved by the relevant organizations (Sharma *et al.*, 2011).

The results of the 4662 teat study, from 1180 lactating cow from 36 farms demonstrated 1.24% of teat has loss their function and 39.12% of mastitis and 2.68% clinical mastitis (Souto *et al.*, 2008). Current methods used to detect mastitis include measurement of somatic cells, the enzyme analysis, and the California mastitis test (Pyörälä, 2003). In Europe the increased somatic cells to more than 200 thousand cells per milliliter of sample approved as an indicator of mastitis (Schukken *et al.*, 2003).

The researchers reported that the activity of enzymes associated with inflammation also can be used to detect mastitis as well as their results have shown that these tests are a good correlation with somatic cell count (Viguier *et al.*, 2009). LDH activity assessed by researchers has shown good results in the detection of mastitis (Hiss *et al.*, 2007)

Researchers have shown that the release of oxidizing substances and proteases cause the destruction of bacteria and some epithelial cells, as a result milk production decrease and enzymes such as N-acetyl-beta-D-Glucoseaminidase (NAGase) and lactate dehydrogenase (LDH) are released in milk (Viguier *et al.*, 2009).

Researchers indicated following incidence of subclinical mastitis in the sheep, LDH and ALP of milk will be increase and expressed that the above enzymes has a significant diagnostic role in early detection of mastitis in sheep (Batavani *et al.*, 2003). Researchers has been reported that the increase of LDH activity in the milk of infected sheep with subclinical mastitis (Nizamlioglu and Erganis, 1990). The researchers found that levels of LDH in milk of animals suffering from mastitis is higher than serum LDH, so have suggested that serum is not source of this enzyme in milk and it is likely this enzyme originated from udder parenchyma cells and fragmented leukocytes (Kato *et al.*, 1989).

In healthy cows the LDH, NAGase and somatic cells increased after delivery and 30 to 40 days after giving birth will be decrease, and they reported the increase of them was due to clinical mastitis. In addition, it was reported that the lactate dehydrogenase levels and somatic cells in cows with clinical mastitis has a very strong relationship. The results also showed that measuring the amount of lactate dehydrogenase in the diagnosis of mastitis has high (73-95%) sensitivity and (92-99%) specificity (Chagunda *et al.*, 2006).

Researchers have also expressed LDH is a cytoplasmic enzyme, which is involved in the metabolism of carbohydrates and due to damages of udder epithelial cells, phagocytes inter into the milk and causes changes in physical and chemical properties of milk (Larsen and Aulrich, 2012). It was reported that the levels of LDH activity in the milk of cows with subclinical mastitis increase, which is due to tissue abnormalities in the parenchyma of the udder (Kalantari *et al.*, 2013; Larsen *et al.*, 2010; Sørensen *et al.*, 2015; Zaki *et al.*, 2008). The researchers reported in the cow milk, the LDH correlated with the SCC (Åkerstedt *et al.*, 2011; Nyman *et al.*, 2016), and this method can be used as an indicator to identify mastitis in commercial farms (Friggens *et al.*, 2007).

Researchers found that eight days before the onset of clinical mastitis the LDH, NAGase and somatic cells levels, would be increase 56%, 30%, and 8%, respectively. This change reflects the faster increasing of LDH compared to somatic cells (Chagunda *et al.*, 2006). It was reported that the LDH activity and NAGase in milk, is an important indicator of mastitis and specially increase of LDH provides opportunity for early detection of mastitis in cows (Chagunda *et al.*, 2006).

It was demonstrated that the concentration of lactate dehydrogenase in buffalos with subclinical mastitis have dramatically increased compared with healthy buffalo, as well as the specificity and sensitivity of tests for the diagnosis of mastitis, was 95 percent. The results of the study showed significant relationship between the amount of lactate dehydrogenase and somatic cells counts (Singh *et al.*, 2016).

The results of current study showed that the udder check test which detect the amount of lactate dehydrogenase, can be used to detect subclinical mastitis with high accuracy, and also results of LDH elevated levels in clinical mastitis and its relationship with the somatic cell count is in consistent with previous studies. In addition, based on the results of this study, udder check tests can be easily to be use at the farm, and the

incidence of subclinical mastitis identify will be quick and measures for the management and treatment will be implement.

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