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Research Article

COMPARATIVE TECHNIQUES FOR DETECTING MASTITIS DISEASE IN BOVINE MILK SAMPLES

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ABSTRACT

Objective: The objective of our study is to analyze various techniques that are applied for detecting mastitis disease in milk samples.

Methods: In this study, we collect milk samples (n=100) from different dairy farms and diagnosed through various tests. In addition, flow cytometric analysis was also performed for milk samples to analyzed lymphocytes, monocytes, and granulocytes count.

Results: The results of these studies showed that California mastitis test (CMT) showed much more positive results against mastitis disease as compared to other techniques, whereas flow cytometric results revealed that during mastitis disease; there is an enhancement in granulocytes count.

Conclusion: Out of these techniques, CMT is the most reliable and cost-effective method for detecting mastitis disease.

Keywords: Mastitis, Diagnostic, Flow cytometer, Granulocytes.

INTRODUCTION

One of the most costly global health problems related to dairy animals that are caused by various bacteria (*Staphylococcus aureus, Streptococcus agalactiae, Streptococcus zooepidemicus, Streptococcus faecalis, Streptococcus pyogenes, Streptococcus dysgalactiae, Corynebacterium bovis, Corynebacterium pyogenes, Klebsiella* sp., *Salmonella* sp., *Pseudotuberculosis, Mycobacterium bovis, Escherichia coli, Brucella abortus, Pasteurella multocida, Leptospira pomona, Pseudomonas pyocyaneus,* etc.), virus (*Vesicular stomatitis, Infectious rhinotracheitis, Foot and Mouth disease, Pox virus infection,* etc.), fungus (*Trichosporon* sp., *Aspergillus* sp., *Candida* sp., *Cryptococcus* neoformans, etc.), and Mycoplasma (*Mycoplasma bovis, Mycoplasma bovigenetelium*) that affect all breeds of dairy animals (cows, buffaloes, Sheep, Goats, and Horses) but high yielding, exotic and cross breed cows are highly susceptible to it [1-4]. This disease directly targets udder and characterized through physicochemical including microbiological changes that are reported in the milk and also observed some pathological changes occurred in the glandular tissues of the udder [1,2]. Recently, mastitis can be defined as a disease characterized by the presence of significantly increased leukocyte content in the milk from affected gland. The increased leukocytes count is in almost all cases reaction of tissue injury and is preceded by change in milk, which is direct result of damage to tissue. In other words, mastitis disease should be reported as well as detected through enormous increased of lymphocytes count into the mammary gland, usually in response to invasion by microorganisms through teat canal [5,6]. In general, mastitis disease can examined through phenotypically (external) symptoms (i.e., swelling, heat, redness, hardness, or pain of the udder) and also showed higher somatic cell count in milk samples and lowers its milk quality. The diagnosis of mastitis has come to depend largely on indirect test which depends on leukocyte content of milk [3-6].

Clinically, mastitis has been classified, i.e., Subclinical (not appreciable clinically; direct tests are applied to detect the subclinical mastitis); pre-acute mastitis (characterized by serosanguinous secretion, necrosis, and sloughing of quarters); acute mastitis (udder is swollen and turns cyanotic, the milk appears yellowish clustered or brown with flask increased body temperature and heart rate); sub-acute (variable changes in milk without gross appreciable changes in udder tissue), and chronic mastitis (terminal stage of mastitis, the udder becomes hard, and supra mammary lymph node becomes palpable) [6-10]. In this study, we evaluated and diagnosed the mastitis disease in milk samples using various tests.

METHODS

Collection of milk

In this study, 100 milk samples were collected in sterilized test tubes which are autoclaved at 15 lbs. for 20 minutes in autoclave from Baramati region. Out of them, some of them showed the presence of mastitis disease. The udder and teat is washed with warm water and air dried for disinfection whereas udder, the tip was wiped off by spirit. The first few chills of milk were discarded and milk sample collected in sterile test tube to avoid any type of contamination. Milk samples collected aseptically to determine existing infection. All samples were subjected to diagnosis of mastitis following different tests.

California mastitis test (CMT)

This test is applied and specific for leukocytes that are present in the milk. In this test, milk sample (1 ml) were taken in clean Petri plate and then add an equal amount of CMT reagent. Mix thoroughly and if gel formation will appear indicates mastitis positive reaction [11].

Bromothymol blue test (BTB) and bromocresol purple test (BCP)

In this study, milk samples (5 ml) were collected in two different test tubes. In first tube, add BTB reagent (1.6 g) BTB in 100 ml ethanol) and second one add BCP (0.9% bromocresol solution reagent). In BTB, mastitis case is positive only when color changes from green to dark blue-green color, whereas in case of BCP, dark blue or purple color reaction will appear [12].

Chloride test

In this study, milk sample (1 ml; normal milk containing 0.07% chloride content) was taken in test tube and then add small amount of silver nitrate (0.1341%) solution. Add two drops of 10% potassium chromate, if yellow color will appear it indicates positive cases of mastitis and if blue color will appear indicates no mastitis [12].

Electric conductivity

Electrical conductivity is determined in milk samples using portable electrical conductivity meter (milk checker or digital mastitis detector) and is expressed in the unit of milk seimens/cm. First, clean the conductivity meter with cotton, take 3 ml of milk sample in conductivity meter and take the reading. If electric conductivity less than 300 indicates mastitis and more than 300 mean no indication of mastitis [12,13].

Estimation of lymphocytes, monocytes, and granulocytes count

Milk (n = 5; 10 ml) samples were collected from different dairy farms. For these studies using 100 µl of bovine milk samples were taken in each falcon tube. Incubate these milk samples for 2 hr at 4°C and lysed with red cell lysis buffer and then washed with PBS (pH 7.2) and then proceed for flow cytometric analysis for the estimation of bovine milk samples in the form of lymphocytes, monocytes, and granulocytes counts.

RESULTS AND DISCUSSION

Mastitis in a difficult problem to comprehend because it is a disease caused by many factors. Microorganisms are responsible for causing infection, but for them to enter the mammary glands and establish themselves to the point that caused an infection and involved multitude factors, e.g., hygiene, housing, climate, milking machines, feed, and genetics. it is even more difficult to generalize about the relative importance of each one as certain factors affects certain microorganism. In India, no systematic study has been made in this direction [4-8]. However, the cost of management of mastitis cases a farm beside this extra labor is required for washing of milking equipment can, etc. Therefore, there is urgently need to screen out the affected animal of the herd for timely treatment or culling as the disease is contagious in nature and can spread in the whole herd.

In mastitis disease, diagnostic tests are routinely used to evaluate milk samples received from dairy farms. A number of tests were applied, i.e., bacterial counts and somatic cell count. Other tests such as CMT, BTB, BCP, and chloride test including electrical conductivity [11-13] and also observed its counts in the form of lymphocytes, monocytes, and granulocytes count using flow cytometry. These techniques are often used diagnostically to investigate milk quality problems. In this study, our group focused and tried to validate the studies related to bovine milk samples related to mastitis disease. In this study, milk samples (n =100) were collected, out of 100, samples (46) showed mastitis disease using CMT test [11] (reagent disrupts the cell membrane of somatic cells, DNA in those cells react with test reagent). This test indicates the somatic cell count and it is one of the useful and well-established techniques for detecting subclinical mastitis. The major advantage of this test, i.e., CMT which assesses the level of infection of individual quarters and also provide better results rather than providing an overall udder result. Similarly, BTB (card test papers prepared from Whatman filter paper No.1) and BCP tests were also performed in milk samples received from dairy farm. Out of 100 samples, only BTB (34) and BCP (29) test showed positive results related to mastitis disease (Table 1).

In addition, another test was also performed and determined the amount of chloride content present in milk sample [10-13]. As per the literature during mastitis disease, there is a significant decline in lactose production and enormous increase in sodium chloride concentration to maintain the osmotic pressure in milk samples [11,12]. During inflammation, chloride content is reported in milk samples of bovine animals. Out of 100 samples, only through chloride test detect only 35 samples related to mastitis disease.

One of the tests, i.e., electrical conductivity is observed in bovine milk samples, substances that are present in solution which can ionize and which there conduct an electrical current. Only if the concentration of sodium chloride rises, the conductivity rises proportionately. In this study, only 30 samples were observed mastitis disease. This test showed some advantages, i.e., useful in finding out its etiology; results are available rapidly, etc. In addition, there are various factors responsible for mastitis disease, most importantly enhancement in granulocytes count which is reported in milk samples. A number of studies suggested as well as proved that granulocytes in milk are a major source for causing mastitis disease (Fig. 1).

Due to granulocytes enhancement, it directly correlates with somatic cell count. In other words, increase in granulocytes count means somatic cell population also increases. In short, a number of contents that are present in milk samples as nutritional value, which are required for human nutrition. Due to infection (bacteria, virus, fungi, etc.) milk reduces its quality and dairy products, affects milk shelf life and flavor and deteriorates its physicochemical properties. Mastitis milk contains both pathogens and bacterial toxins and its consumption may directly or indirectly increase the risk of foodborne illnesses. Different methods have been applied for detection of subclinical mastitis. Out of these, CMT, granulocytes count and electrical conductivity test are one of the most reliable methods and used in the detection of subclinical mastitis. Furthermore, its reliability would further increase when used together with other diagnostic methods.

Table 1: Mastitis disease reported in milk samples and diagnosed through various tests

CMT	BTB	BCP	Chloride	Electric conductivity	CMT	BTB	BCP	Chloride	Electric conductivity
-	-	-	-	500	+++	+	+	+	540
+++	-	-	-	220	+++	+	+	-	560
+++	-	-	-	240	-	-	+	+	650
-	-	-	-	500	+++	+	+	+	250
-	-	-	-	400	-	-	-	-	330
+++	+	-	+	480	-	+	+	-	550
-	-	-	-	560	+++	-	-	-	200
+++	-	+	+	320	-	+	+	+	590
+++	+	+	+	260	-	-	-	+	340
+++	+	-	+	210	+++	+	-	+	250
-	-	-	-	800	+++	+	+	-	290
+++	-	+	+	400	-	-	-	-	420
+++	+	+	-	560	-	-	-	-	340
-	-	-	-	700	-	-	-	-	320
+++	+	+	+	200	-	-	-	-	540
-	-	-	-	310	-	-	-	-	650
-	-	-	-	380	+++	+	-	-	250
-	-	-	-	440	-	-	-	-	330
-	-	-	-	550	-	-	-	-	430
-	-	-	-	450	-	-	-	-	450

(Contd...)

Table 1: ***(Continued)***

CMT	BTB	BCP	Chloride	Electric conductivity	CMT	BTB	BCP	Chloride	Electric conductivity
+++	+	-	+	230	-	-	-	-	410
+++	+	-	+	290	+++	+	+	+	120
-	-	-	-	400	+++	+	-	+	120
+++	-	-	-	820	+++	+	-	-	190
-	-	-	-	590	+++	+	+	+	250
-	-	-	-	340	-	-	-	-	390
+++	+	-	+	250	-	-	-	-	400
+++	+	+	+	290	-	-	-	-	560
-	-	-	-	420	+++	-	-	+	720
-	-	-	-	340	+++	+	+	+	350
+++	-	+	+	260	+++	+	+	+	620
+++	-	+	+	290	+++	+	+	-	420
+++	+	+	+	270	+++	-	+	+	370
-	-	-	-	540	+++	-	-	-	590
++	+	+	+	560	+++	+	-	+	600
-	-	-	-	650	-	-	-	--	630
+++	-	+	-	250	-	-	-	-	430
-	-	-	-	330	-	-	-	-	650
-	-	-	-	550	-	-	-	-	530
+++	+	+	+	200	-	-	-	-	630
-	-	-	-	590	-	-	-	-	160
-	-	-	-	340	-	-	-	-	540
+++	+	+	+	250	+++	+	-	+	290
+++	+	-	+	290	-	-	-	-	360
-	-	-	-	420	-	-	-	-	450
-	-	-	-	340	-	-	-	-	720
+++	+	+	+	260	-	-	-	-	750
+++	+	+	+	290	-	-	-	-	380
+++	+	+	+	270	-	-	-	-	590
					-	-	-	-	480
					-	-	-	-	730
					46	34	29	35	30

CMT: California mastitis test, BTB: Bromothymol blue, BCP: Bromocresol purple

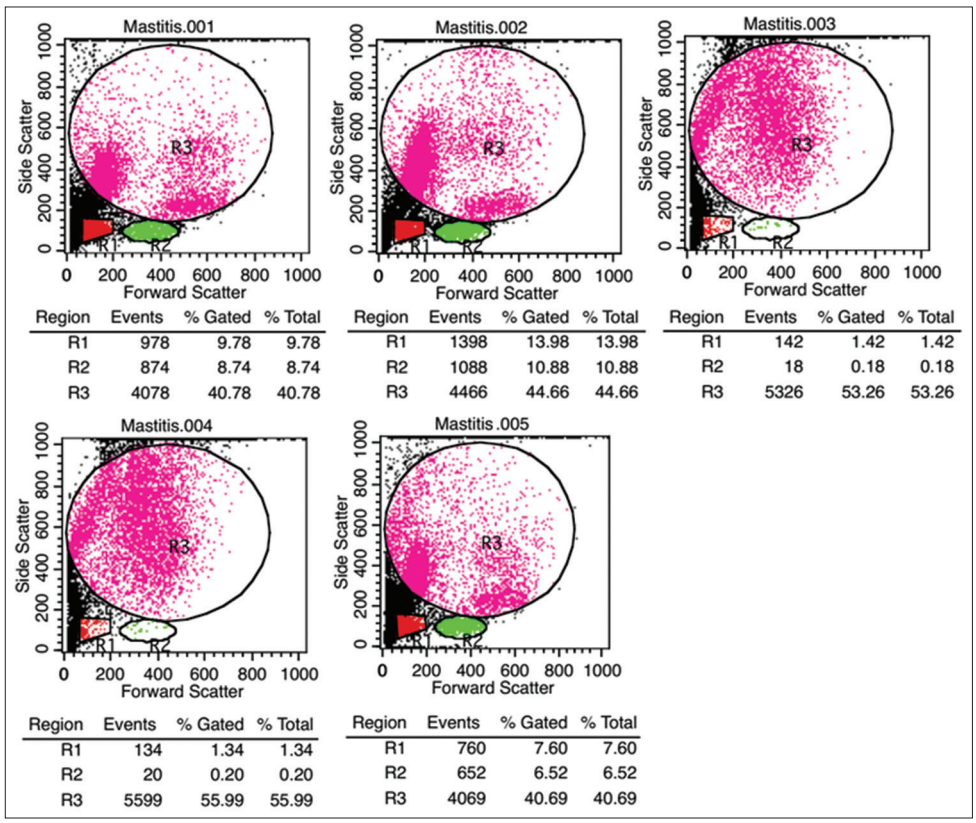

Fig. 1: Estimation of lymphocytes, monocytes, and granulocytes count using flow cytometer. Flow cytometric analysis was performed to analyze milk counts and determined forward and side scatter in the form of lymphocytes (R1), monocytes (R2), and granulocytes count (R3). Data acquisition of 10000 events and fraction or separation of cell populations representing forward and side scatter using cell quest software

CONCLUSION

This study provides various techniques for detecting mastitis disease, which is mainly supported here by enhancement of granulocytes count using flow cytometer. Thus, this study highlights the detection of mastitis disease, which is the most costly disease affecting in cattle animals. Out of these techniques, CMT, electrical conductivity, and granulocytes count (using FACS) are most reliable techniques for detecting mastitis disease in milk samples. In addition, CMT is cheap and cost effective method as compared to the rest of the techniques. If this reagent is supplied to the farmers those who are dealing with milk or dairy samples collected from bovine animals for causing mastitis disease, it will save millions of rupees for treatment against mastitis disease.

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