

# PRODUCTION OF QUALITY MILK FROM DAIRY ANIMALS

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## ABSTRACT

*Milk is a complete diet for human consumption. Milk is a natural fluid and is unique in physico-chemical characteristics due to presence of wide range of nutrients and minerals. Mastitis is the inflammation of parenchyma / functional milk producing tissue of the udder / mammary glands. It is the response of the mammary gland tissue to injury caused by viruses, bacteria and fungus and chemicals. Mastitis and dairy food quality are related to each other, and is the result of complex interrelationship among; environment, animal and microorganisms. The most common cause of mastitis is bacteria, which invade udder and utilize the nutrients and secrete obnoxious chemicals i.e. toxins, resulting in impaired milk composition, quality and reduced production, adversely affect the shelf life of milk, decrease nutritive value of processed milk products and is risk for public/consumer health hazards. Mastitis is at number one among major diseases of dairy animals in Pakistan. Dairy animals become susceptible to this disease during milking.*

**Keyword:** Production, Quality Milk & Dairy Animals

## INTRODUCTION

Quality and milk composition alters at many degrees due to udder inflammation. The pH of milk increases due to influx of sodium and chloride ions in the milk. Mastitis is very common due to this reason, as harmful microbial organisms utilize milk of udder for their growth and multiplication. Milk and dairy products obtained from animals having intra-mammary infections are also the source of transmission of pathogenic bacteria to humans. Clean milk can only be produced when the animals are mastitis free. The bacteriology of quarter foremilk samples of dairy cows revealed *Staphylococcus aureus* as a predominant organism followed by *Streptococcus agalactiae*, yeast and mold infections. While in case of buffaloes *Staphylococcus aureus* followed by Coagulase Negative *Staphylococcus*, *Trueperella pyogenes* (*Corynebacterium pyogenes*), unidentified *Trueperella* spp. and mixed species (Javed, Muhammad, Saqib & Hussain, 2015).

Mastitis is commonly caused by two types of bacterial pathogens; contagious and environmental. Contagious pathogens find their ways from non healthy to health quarters through milked hand during the milking process, common source of contagious pathogens is the udder of infected animal. Environmental pathogens of Intra-mammary infections are isolated in the habitat of dairy cattle and buffalo. Common contagious pathogens of the IMIs are *Staphylococcus aureus* and *Streptococcus agalactiae*, and common environmental pathogens of mastitis are *Escherichia coli*

and *Streptococcus uberis*. Zeryehun and Abera (2017) recorded prevalence of 51.8% of subclinical mastitis and identified mastitis as a major problem in their study area requiring serious attention.

## LITERATURE REVIEW

### Mastitis and Milk Quality

Clinical mastitis is exhibited with the visible abnormalities in color and consistency of milk. Clots, pus and strings of blood appear in milk, udder may be felt hard, swollen, warm, reddened, painful and edematous. While the sub-clinical infections are difficult to detect grossly because no visible signs of disease appear, apparently udder of animal may look normal. The milk may be seen as normal but the quality of that milk is very low and processing of such milk results in decreased quality milk products because lactose (milk sugar), casein (milk protein) and fat synthesis is decreased in that milk. The Somatic Cell Count (SCC) i.e. level of neutrophils (white blood cells / pus cells) is very high in milk from sub-clinically affected dairy animals. The udder and resultantly the milk harbors and spreads the micro-organisms potentially dangerous for public health. Concentrations of undesirable components i.e. enzymes; lipases (breaks down butter fat, leading to rancidity of milk), proteases, oxidases, plasmin (breaks down fibrin clot as well as milk casein) and plasminogen increase which badly deteriorate milk. The stability, flavour and shelf life of milk, fresh dairy products, and processed dairy products is affected, while the concentrations of desirable components of milk i.e. milk sugar, milk protein and fat is decreased.

Mastitis causes decreased cellular synthesis, increased cellular secretion, tissue and cellular death of alveoli of udder parenchyma (death of functional milk producing tissue of mammary gland) and increased permeability of cell membranes of mammary glands of dairy animals. Several mastitis pathogens result in production of bacterial toxins in milk and even pasteurization does not destroy all food borne pathogens of milk such as the *Mycobacterium paratuberculosis* and the *Listeria monocytogenes*. Milk obtained from a healthy and non-mastitic udder contains small number of bacteria. The presence large numbers of food borne pathogens in raw (mastitic / high SCC) milk increases the risk of ingestion and transmission of potentially harmful toxins to humans. *S. aureus*, the most common cause of contagious mastitis and primary human pathogen, produces heat-resistant enterotoxins, consumption of such milk causes nausea, abdominal cramps, vomiting and diarrhea, and are responsible for outbreaks of staphylococcal food poisoning in humans. Other *Staphylococcus* spp, i.e. Coagulase Negative Staphylococci are opportunistic pathogens and cause infections and intoxications of consumers by consuming raw milk and raw milk products.

Other harmful pathogens in mastitic milk include; *Campylobacter jejuni*, *Yersia enterocolitica*, *Salmonella* spp, *E. coli* and *S. agalactiae*. Raw milk is also a major source of illnesses caused by bacterial toxins. Some studies have reported that the *Staphylococci* is the most significant isolate of mastitis pathogen, as compared to streptococci and *Escherichia coli* (Hussain, Javed, Khan, Mahmood & Kausar, 2012). Mastitis is the most frequent and expensive disease of dairy animals

due to its serious consequences on economic losses in the quantity and quality of milk produced. Its cause is directly related to aspects of well-being, health and hygiene and sanitation of milk producing animals (Izquierdo, Liera, Cervantes, Castro & Mancera, 2017). The high ambient temperature, improper storage and improper cooling during transportation of milk result in growth of microbes in milk and lead to the production of toxins. Toxins are heat stable, cause spoilage of milk and decrease shelf life of milk, processed milk and milk products like cheese. The presence of *Escherichia coli*, an environmental coliform pathogen, in milk can cause gastro-enteritis, diarrhea and food poisoning in infants and children. In early stage of *E. coli* udder infection the consistency of milk remains normal, but with the release of endo-toxins the udder parenchyma deteriorates rapidly and mammary gland quickly produce the watery secretion in milk that distinguishes abnormal milk from normal milk.

For the treatment and control of intra-mammary infections both conventional and non-conventional practices are used. Use of antibiotics is one of the most commonly available choices for mastitis treatment. Due to very fast, rapid, quick and visible therapeutic response antibiotics are extensively used. The potential threat for drug residues in milk associated with treatment of mastitis is a significant public health concern. The consumption of milk and milk products containing antibiotic drug residues causes allergic reactions. Antibiotic residues at low levels can cause sensitization and the development of antibiotic resistance in common strains of bacteria. The frequent use of antibiotics in animals results in microbial resistance, resultantly decreasing the effectiveness of antimicrobial drugs. Bacteria which cause animal diseases are capable of causing human illness and thus decrease therapeutic effectiveness of antibiotics in humans. Mostly the antibiotics are used for the treatment of mastitis infections. The most common mastitogen i.e. *Staphylococcus aureus* is sensitive to amoxicillin and enrofloxacin. The resultant usage of antibiotics in the treatment of mastitis results in increased chances of drug residues in milk, as a result the quality of milk is decreased (Qayyum, Khan, Hussain, Khan, Avais, Ahmad & Hassan, 2016).

The researchers have elaborated that the emergence and spread of antimicrobial resistance as a result of treatment of mastitis microbes is most important and urgent matter of public interest (Kromker & Leimbach, 2017). In Pakistan most of dairy farmers overlook certain factors which increase risk of mastitis in dairy animals. These are; milk let down through calf suckling, faulty milking method, damp / wet and dirty housing, flies, bad hygiene, poor management conditions and nutritional deficiency of animals, predispose animal to mastitis (Bilal, Iqbal, Muhammad, Avais & Sajid, 2004). Marketing and grading of milk on the basis of quality i.e. on the basis of somatic cell count (SCC), lactose contents, protein contents, total solid, microbial count, drug and antibiotic residues should be done towards provision of quality milk. Mastitis is recognized as a global most costly disease of dairy animals. Mastitis is widely spread in dairy animals and causes colossal economic losses due to reduced milk yield and impairing milk quality resulting producing decreased quality dairy products.

### ***Diagnosis of Clinical Mastitis***

The clinical form of mastitis depicts gross picture of visible inflammation of udder, hardness, edematous appearance in the affected gland, altered secretions and abnormal appearance of the milk. Milk secretions may be watery, bloody, off colored, or have the appearance of clots, pus and serum.

### ***The Strip Cup or Strip Plate Test***

The presence of clinical mastitis can be detected by an easy and rapid test i.e., strip plate test. The abnormalities in milk are checked by this test. The few streams of foremilk are squirted onto the strip cup and abnormalities are noted. The milk having clots, pus and thickness is indicative of clinical mastitis.

### ***Diagnosis of Sub-Clinical Intra-Mammary Infections***

Sub-clinical IMIs remain present inside the udder without any apparent abnormality of udder and the milk. This form of mastitis can only be diagnosed by field and laboratory test by determining the milk quality. Sub-clinical mastitis is much more prevalent than its clinical form, is hard to detect and badly deteriorates milk quality (Bachaya, Iqbal, Muhammad, Yousaf & Ali, 2005; Sharif & Ahmad, 2007). The diagnosis of sub-clinical IMIs mastitis is dependent upon the indicators of inflammation. Significant physiological and pathological changes occur in the functional tissue of udder and in the milk in response to inflammation, including influx of white blood cells (referred to as somatic cells) in milk and increased flow of fluids inside the udder due to increased vascular permeability. Mastitis causes the release of hydrolytic enzymes due to process of phagocytosis, as a result the oxidative substances are released and change pH of milk, alter chemical composition and change physic-chemical properties of milk. The presence of mastitic pathogens, increased level of leukocytes (White blood Cells/Somatic Cell Count), presence of ions, altered pH, presence of hydrolytic enzymes, oxidative substances, changed electrical conductivity, decreased milk sugar (Lactose), decreased protein and decreased fat are indicative of udder infection. And resultantly are indicative of low quality of milk.

### ***Somatic Cell Count of Milk***

Measurement of somatic cell count of milk is one of the most authentic and standard test for accessing milk quality. Level of somatic cells composing of epithelial cells of udder and white blood cells of udder depict the quality of milk under modern dairy production. The neutrophils sharing 90–95% of total leukocyte count of milk increase drastically in milk as a result of even very mild infection of udder. A single cow with high SCC may not increase the bulk tank SCC by very much, however if the herd has many chronically infected animal (especially due to contagious pathogens of mastitis) then the bulk tank SCC may increase significantly. Presence of Somatic Cells (White Blood Cells/Leukocytes) in milk indicates the disease combating response in animal and is the actual index of level of inflammation in mammary gland quarters. There is no one level

of SCC at which a cow is free from intra-mammary infection but a level of less than 50,000 cell/mL of milk may be considered as a start of inflammation in advanced and developed countries. The milk having SCC below the limit of 200,000 cells/mL is considered as indicative for presence of udder infection. As cell counts increase the chance of mastitis increases.

### **Chemical Tests for Detection of Mastitis**

These tests include California Mastitis Test, Surf Field Mastitis Test, pH test, Chloride Test, Whiteside test, and Catalase Test.

#### **California Mastitis Test**

CMT can be conducted on bucket and bulk tank milk samples to determine SCC of the individual cow or of the entire herd. CMT reagent (3 % solution of Alkyl-Aryl-Sulfonate) is mixed with equal quantity of milk. The reagent mixes with milk, ruptures the cell membrane of white blood cells present inside the milk, as a result of damage of cell membrane the genetic material of leukocytes oozes out and the genetic material shrinks, and changes the viscosity of milk, the less the number of leukocytes present inside the milk the mixing of milk with the reagent results no change in the consistency of milk, with increased number of leukocytes in the milk the mixing of reagent forms a gel like mixture. The consistency of milk is directly related to the number of leukocytes in milk and is indicative of quality of milk. The test can be performed by taking milk sample from each quarter into a plastic paddle and adding equal amount of CMT reagent to the milk. Then after rotate the paddle for 10 seconds to completely mix the contents of reagent and milk. The reaction disappears within 20 seconds. The test must be read quickly while rotating the paddle (Schalm, Carrol & Jain, 1971)

#### **Surf Field Mastitis Test (SFMT)**

SFMT is discovered by the eminent scientists of Agricultural University Faisalabad, Pakistan. The test is performed by using 3% solution of household detergent, SURF (Lever Brothers Pvt. Limited, Pakistan) which is very cheap alternative of CMT reagent. A high level sensitivity and specificity has been found between SFMT and CMT. The use of SFMT is very economical and cheaper alternative of CMT. The test can be performed by adding equal volumes of SURF solution and milk in the cups of testing paddle. Swirl milk and reagent by rotating the paddle gently in horizontal position. The milk sample from affected quarter will become viscous, flocculates of varying degrees will appear in milk or will show gel formation depending upon severity of infection. The results of SFMT are graded and interpreted similar to CMT (Muhammad, Athar, Shakoor, Khan, Rehman & Ahmad, 1995).

#### **Whiteside Test**

This test is done by using 4% Sodium Hydroxide (NaOH) solution is quick, simple and inexpensive technique which can be used to detect sub-clinical mastitis. Udder and teats of animals are properly

washed and dried before collection of milk sample. About 5mL of milk is collected separately from each quarter in a clean container. Five drops of milk from each container are placed on a glass slide. One drop of NaOH (4% solution) is added and mixed with an applicator for 20-30 seconds. The results are read as Negative (N), Trace (T), 1+VE (P1), 2 +VE (P2), or 3 +VE (P3) reactions depending upon the amount of gel / flocculates formation in the sample.

### **pH of Milk**

Normal milk has pH of 6.4 – 6.8. Milk from mastitic udder is alkaline, with pH as high as 7.4. The degree of alkalinity varies depending upon the severity of inflammation. The most common method to detect pH of the milk is the use of indicators that change color at or near the normal pH. The pH should be determined on freshly drawn milk, although milk held at refrigerator temperatures for 24–48 hours may be used.

### **Chloride Test**

The Chloride Test is dependent upon the determination of an abnormal quantity of Chloride in the milk. Normal milk contains 0.08–0.14 % Chloride ( $\text{Cl}^-$ ). Abnormal milk contains a greater quantity of Chloride because of presence of inflammatory exudates. These exudates contain a considerable quantity of Chloride. The test can be performed by adding 5mL of Silver Nitrate solution to 1mL of milk followed by the addition of two drops of Potassium Chromate solution and mixing by inversion of the tube. The appearance of yellow color indicates that more than .014 % Chlorides are present in the sample, and brownish red color indicates that sample contains less than that amount.

### **Catalase Test**

Level of certain enzymes like Catalase increases in mastitic milk. Most living cells, including leukocytes, contain Catalase, which is capable of decomposing Hydrogen Peroxide ( $\text{H}_2\text{O}_2$ ) to the gaseous oxygen. The quantitative level of Catalase reveals information regarding the number of leukocytes present in a milk sample. The test can be performed by mixing 1mL of 3% freshly prepared Hydrogen Oxide in 9mL of milk in 15mL screw cap test tubes. Fill the test tube with water. Cap the tube loosely and invert it in a test tube rack placed in a shallow pan. Then incubate the test tube for three hours and note the presence of gas column. The length of gas column indicates the presence of number of Catalase positive bacteria / pathogens of mastitis in milk.

### **Electrical Conductivity**

EC of mastitic milk increases due to increased influx of Sodium ( $\text{Na}^+$ ) and Chloride ( $\text{Cl}^-$ ) ions in milk and decreased Potassium ( $\text{K}^+$ ) ions and decreased level of milk sugar (lactose) in milk. Mastitis damages udder parenchyma and blood capillary permeability of udder tissue increases. Tissue damage, leakage of constituents of blood and altered capillary permeability causes the influx of ions from blood into udder. Conductivity sensors are used in developed countries in automated

milking systems. Electrical conductivity can be associated with early detection / recording of sub-clinical mastitis. An indication of increased electrical conductivity in a specific animal further suggests the examination of temperature, udder inflammation, etc. in that animal. Concentrations of Na, K, Cl ions in milk can be measured through Flame photometry and Conductivity measurements. Hand held conductivity meters are also available and may be useful for routine screening of animals with abnormal milk.

### **Milk Lactose**

Analyzing the quantity of milk sugar (lactose) is also an indicator of milk quality. The lactose is composed of two sugars, glucose and galactose. The intra-mammary infections cause damage to the udder parenchyma and the synthesis of lactose is reduced. Studies have documented that an inverse correlation exists between the level of lactose and milk somatic cell count. Decreased lactose indicates high SCC and resultantly decreased milk quality (Ahmad, Hussain, Mahmood & Munir, 1988; Qureshi & Ahmad, 1980; Sharif, Ahmad, Bilal, Yousaf, Muhammad, Rehman & Pansota, 2007).

### **Mastitis Control Practices**

Safe and quality milk can only be produced if the dairy animals are mastitis free. Following mastitis control practices can be adapted to control mastitis. Externally to the mammary teats skin serves as the line of defense against invading micro-organisms, at the opening of the teat there is teat sphincter which closes immediately after milking, keratin composed of fatty acids forms a plug at the tip of teat which act as bacteriostatic (which inhibit the growth of bacteria) for multiplication of pathogenic microbes and prevent upward movement into mammary gland, epithelial lining of teat canal and muscular sphincter also prevent the entry of bacteria to the udder parenchyma in dry animals. Bacterium only gains entry into the mammary gland when these barriers are weak. Reducing the exposure of teats and udder to microbial agents of mastitis through efficient management and strengthening natural defenses through vitamin and mineral supplementation can prevent mastitis.

### **DISCUSSIONS**

Farmers should always check foremilk and udder for mastitis, any change in color of milk and swelling on the udder. Isolation of clinically infected animals from the healthy animals should be done immediately. The disease animals should be milked at last. The first few strips of the foremilk should be discarded as these have very high bacterial load and increase the contamination of whole milk and enhance the quality of milk. Culling of chronically infected animals should be done, as they are carriers and spreaders of disease. These non-responding animals are mostly infected with *Staphylococcus* spp. *Staphylococcus* becomes resistant to chemotherapy and causes recurrent mastitis in affected animals. Culling causes production and replacement loss of the animals to the farmers, but it is the best strategy to reduce Bulk Tank Somatic Cell Count (BTSCC) in milk for

improving milk safety and quality. (Vakkamaki, Taponen, Heikkila & Pyorala, 2017) reported that *Staphylococcus aureus* infection caused the culling of dairy animals and further recommended that in order to decrease culling rate in dairy animals due to mastitis the preventive measures should be adopted at herd level.

Good sanitation, environmental and udder hygiene, pre-milking and post-milking teat dipping in disinfectants can prevent the mastitis. Post-milking teat dip should be applied for 30 seconds. Environmental pathogens of mastitis live outside the udder e.g. in manure, wet bedding, calf mouth and muzzle, nostrils, teat skin, milkers' hand, flies and waste milk. Thoroughly wash the teats and udder with clean water to remove soil, manure and mastitis causing bacteria with sanitizing solution. Dry the udder and teat properly with clean cloth or individual paper towel to remove the bacterial residues on wet skin. Excess water on the teat is loaded with bacteria runs down to the milking utensil and results in high bacterial count and reduces the milk quality. The wet bedding in the surrounding areas and wet milking should be avoided. Provide proper bedding material such as sand, clay, sawdust, dry manure or waste to have the cows clean, dry and comfortable. Floor should be smooth and even, as uneven floors are harmful. The wet and soiled bedding should be replaced. All quarters of all dairy animals should have Dry Cow Antibiotic Therapy (DCT). DCT reduces chances of infection environmental pathogens, controls new infections during start of dry period and during start of next lactation.

Those dairy farms where milking is done through automatic milking machine management of milking machine is important. Attach milking unit with the udder and teats within one minute after the start of stimulation and shut off vacuum before removing the unit. Farmers using machine milking should know the proper working and maintenance of automatic milking machine to avoid teat injuries due to vacuum pressure. As the environment of dairy animals is mostly infected and pathogens, *Klebsiella*, *Enterobacter*, *Pseudomonas*, *Pasturella*, *Serratia* etc. thrive in manure and bedding, get entry into the teat when teat sphincter is open. Feeding the animals at the time of milking will remain animal standing for a while to allow teat sphincter to close completely after milking before lying down of dairy animals. Animal densities should be reduced during the summer, as flies during hot months may contact with the lesions on teat of infected animals and potentially carry the infection to non-infected animals. Flies breeding areas should be eliminated near dairy houses. Fly breed in decaying feed, manure, calf pens, cracks and crevices in the shed.

During the month of winter it has been seen that teat end lesions may develop due to frostbite and cold stress. Teat end lesions break the defense system of the udder and pave the way for the entry of bacteria into the udder. Teat lesions also develop due to chapping, lacerations and machine damage. Teat lesions should be avoided. Nutritional and productive requirement of the dairy animals should be calculated on the basis of milk production. High yielding dairy animals require more supplementation and concentrate ration, and are liable to mineral and nutritional deficiency,



thus lead to stress on the animal resultantly mastitis incidence may increase. Providing right kind of nutrients and supplements can prevent new intra-mammary infections (IMIs). Zinc and copper supplementation in feed reduce risk of mastitis, enhance Cellular and Humoral response of body immune system and decrease SCC in milk. Vitamin E and selenium in ration 2-3 week before calving also reduce the incidence of mastitis after calving. Feed additives of Vitamin A and beta-carotene are also effective against mastitis. The alternative methods for treatment of mastitis like use of homeopathic drugs, herbal extract of leaves, stems and roots of medical plants, etc. can also be used for the treatment of mastitis and for increasing milk quality by avoiding the chances of drug residues in milk.

## CONCLUSION

The regular screening through indirect tests for detection of mastitis should be done. California Mastitis Test (CFT), Strip-Cup Test and Surf Field Mastitis Test (SFMT) show exact health status of the udder. Positive cases should be separated, milk should be withheld and antibiotic treatment should be started immediately. SFMT is the cheapest tool for dairy farmers (costs is only Rs.5/- per 100 animals), highly sensitive, easy to perform and economical. The milk samples from infected animals should be collected aseptically in a sterile vial and processed for cultural examination to see the type of pathogen involved in mastitis. Once the antibiotic therapy has started complete the course of antibiotic treatment as directed. Discard the milk from all quarters for the length of time to avoid consumption of milk having drug residues so that problems of antibiotic resistance and allergies may be overcome. The milk should also be checked for presence of antibiotic residues at 3 to 5 days after the end of treatment.

The milk containing antibiotic residues should be discarded and not be taken into the market until the residues disappear. During the course of antibiotic therapy even the calf should not be allowed to suckle the milk for feeding. The feeding requirement of animals should be assessed on routine basis keeping in view the maintenance and production requirements of milch animals. The cheap and alternative sources of nutritional feed through adopting technologies of Silage, hay making and urea treatment of wheat straw can be very useful and economical substitute for fulfillment of protein, fat and energy requirement of dairy animals. The balanced ration with optimal amount of macro and micro minerals, along with requisite amount of fiber, dry matter and green fodder will minimize the chances of udder infection and will ensure health of dairy animals and will result in production of quality milk.

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