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## Review Article

### Bovine Mastitis: Behavioral Changes, Treatment and Control

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#### ABSTRACT

In dairy animals, mastitis is the most common and expensive problem. It is ranked as a number one pathological condition of dairy animals. It effects the corporate and commercial dairy industry. It contributes a major economic loss in farm income both at small level that is commercial farm as well as at large scale industries like corporates dairy farms. The review article indicates the major cases of mastitis both in the form of clinical mastitis and subclinical mastitis, behavioral changes observed in animal, different treatment plans and medicines used at farm to control the mastitis and an estimation of economic losses due to mastitis. This article also revealed that sub-clinical mastitis was more important and the major changes that occurred in mastitis milk as compared to the mastitis free milk. Bacteria involved in causing mastitis, the predisposing factors involved and pathogenesis of mastitis are also discussed in current study. The behavioral changes in cows caused by mastitis are the most important signs for identification of condition. Estimation of economic losses due to mastitis, treatment plan good for mastitis and different methods to prevent the occurrence of mastitis are also discussed in the article.

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#### Introduction

In Pakistan, dairy animals play vital and important role in economy of country (Mohammadian 2011) as well as livelihood of farmer in the form of different dairy animal like cattle, sheep camel, goat and buffalo (Ali et al. 2016; Ali et al. 2017; Hussain et al. 2018; Hussain et al. 2020). These animals are reared basically to meet the consumption of milk, meat and other products in tropical and subtropical area of Pakistan (Tiwari et al. 2013; Ali et al. 2017; Batool et al. 2019). A major population of Pakistan involve in rearing buffaloes (29.9 million) and cattle (33.0 million) sharing more than 95% of the total milk production of the Pakistan (Government of Pakistan, 2007-2008). In Pakistan in arid and semi-arid region, cattle, buffalo and camel are considered as the important animals for production of milk (Ali et al. 2016; Qayyum et al. 2016). However different viral (Khan et al. 2018; Hussain et al. 2020), bacterial (Mahmood et al. 2017) fungal and parasitic agents cause different problem in dairy sector (AL-Samawy et al. 2019; Chemweno et al. 2019; Batool et

al. 2019). Among bovines, the animal that is more susceptible to mastitis is cattle as compared to buffalo and it is reported in many of the researches (Hussain et al. 2012; Qayyum et al., 2018). These problems become constraint in dairy sector affecting both productivity as well as food security for human consumption (Zafar et al. 2019; Rashid et al. 2019; Peter et al. 2020; Zeedan et al. 2020). Among these problems, infections of mammary glands are the major issues of livestock animals. Field investigations have shown that mastitis is the main issue of the most of livestock animals in Pakistan (Khan and Khan 2006). It is recorded that in buffaloes (Nili-Ravi) mastitis reduces about 57 days milk production period of each infected animal resulting in decrease of 438 kg milk during each lactation (Cady et al. 1983). The total economic loss due to clinical mastitis is estimated Rs. 240 (million) every year. Losses are encountered from disposal of milk, reduce milk production, culling, treatment and management costs, and replacements of infected animals. The economic

loss from clinically infected cases did not considered those happened by sub-clinical (less obvious) mastitis which can only be identified on the basis of estimation of milk leukocytes (milk somatic cell counts) (Table 1). Subclinical mastitis is of vital importance due to its high prevalence than the clinical infections (Youssif et al. 2020). The hidden infections of udder persist for a long period and cause huge economic loss and severely affect the quality and quantity of milk.

### Pathogenesis of mastitis

Mastitis is a disease that is caused by multiple factors in milk producing animals. Early diagnosis of mastitis and understanding of its pathogenesis is a very important (Khan et al. 2013; Brand et al. 2021). Mastitis occurs when bacteria invade the teat canal and mammary glands (Pyorala 2003). High number of bacterial populations *streptococci* and *staphylococci* are already present on the teat skin can be a cause of mammary gland infection (Abhishek et al. 2020). Mammary gland parenchyma provides optimum condition for the growth of bacteria (Patnaik et al. 2014).

**Table 1:** Severity of udder infections on the basis of scoring.

Sr. No	Scores	Description
1	Mild	When milk contains some clots and flakes
2	Moderate	Udder inflammation and changes in milk
3	Severe	Systemic illness such as pyrexia, in-appetence, lethargy and weakness)

**Source:** (Royster and Wagner 2015)

Different studies have indicated that teat sphincter is surrounded by smooth muscles which support the teat canal for immediate closure after milking (Murphy et al. 1989; Abhishek et al. 2020). It is also determined that the teat canal is lined with keratin layer and damage to this layer results in increased susceptibility of udder infections and microbial colonization (Yousaf et al. 2010).

Accumulation of fluid occurs within the mammary glands when animal comes near to parturition which leads to increase in intra-mammary pressure as a result teat canal is dilated and leakage of mammary gland secretion occurs which in result increases the vulnerability of mammary gland to infection (Paulrud 2005). At the time of milking of animals, different microbial agents present near the opening of teat sphincter and teat canal may have increased opportunity to invade mammary gland due to damage to keratin (Ibrahim et al. 2011). Furthermore, it is also determined that teat canal and teat sphincter partially remain for 1-2 h after milk removal and during this time variety of bacterial agents can easily invade the udder (Abhishek et al. 2020). As bacteria invade from teat canal, they circumnavigate the cellular and humoral defense mechanism of the body (Rainard et al. 2006; Abhishek et al. 2020). The form biofilm which causes the proliferation of bacteria and as a result bacteria release toxin and cause induction of leukocyte and epithelial cell to release cytokines, TNF Alfa, interleukin-8, IL-1, eicosanoids like PGF2 Alpha, acute phase protein and radicals (Abhishek et al. 2020).

Immune system work and causes the release of oxidants, protease that destroy bacteria and in result milk production is decreased and release of different enzyme like N-acetyl-b-D-glucosaminidase and lactate dehydrogenase occur. Dead and sloughed off epithelial, mammary cells and leucocytes come in milk and in result somatic cell count of milk is increased (Abhishek et al. 2020). As the infections persist, swelling of mammary gland occur, alveoli become damaged, extracellular components like sodium, potassium, hydrogen, and chloride enter into the mammary gland and result in breaking the blood-milk barriers which result in changing the normal value of pH, conductivity and milk taste (Zhao 2008). Mastitis can be categorized into acute, chronic, gangrenous, mycotic and different sub clinical forms. Mastitis is characterized by inflammatory response (Zenebe et al. 2014).

The intensity and severity of inflammatory reaction depend upon various factors like immune status of the host, age, parity and lactation stage along with different factors of pathogens (strain, virulence, species, and the size of inoculum) (Hussain et al. 2012; Hussain et al. 2013). In result of inflammatory response and infection release of different harmful toxin occur that ultimately result in increase in somatic cell count and tissue changes (Ibrahim et al. 2011; Hussain et al. 2012). Neutrophil, lymphocyte, macrophages and minute or a smaller number of epithelial cells constitute the somatic cell of milk (Abera et al. 2010; Hussain et al. 2012). Severity of infection vary from no visible or sub clinical mastitis to clinical mastitis causing systemic signs and ultimately leading to toxic mastitis and udder fibrosis (Hussain et al. 2012).

### Changes in Milk and blood-Biochemical Parameter and Somatic Cell Score

When mastitis occurs, in result of various type of infection, different chemicals are released from inflammatory epithelial cells (Hussain et al. 2012). These includes non-lysosomal enzyme and hydrolytic enzyme such as lactate dehydrogenase and Beta galactosidase (Hussain et al. 2012). These enzymes cause the degeneration of epithelial cell leading to decreasing the quality of milk that is lower level of fat, lactose, protein, casein and different macro and micro mineral (Hussain et al. 2012; Ashraf and Imran 2018). In mastitis, oxidation of lipid occurs and in result Malnodialdehyde (MDA) and Nitric oxide are produced. Malnodialdehyde, a product produced from oxidation of lipid, is highly carcinogenic for human as taken from mastitis animal, milk. A significant level of variation occur in concentration of different biomarkers of milk like pH, Malnodialdehyde and electrical conductivity of milk (Yang et al. 2011; Ali et al. 2016). In different research papers, it is reported that the increase in pH value and electrical conductivity is due to inflammatory response that result in increased permeability of vascular membrane and cause the leakage of different ions and salt (Hussain et al. 2012; Ali et al. 2016). Subclinical mastitis that most of the time remain in hidden form can be diagnosed by blood analysis by measuring the different levels of ions and enzymes (Table 2). In sub clinical mastitis, there are decreased levels of different physiological and chemical

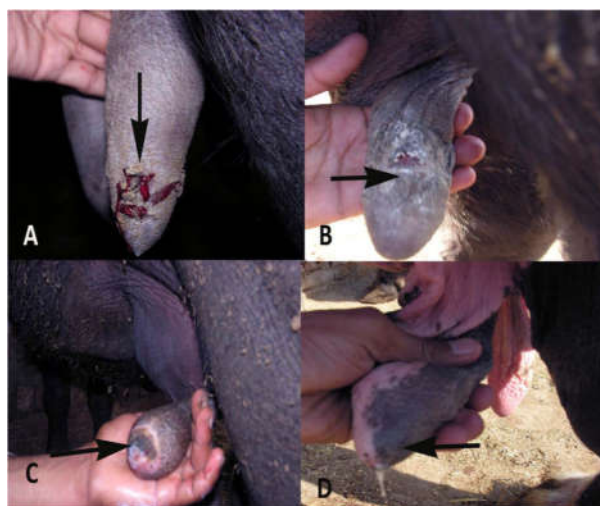
parameters like Na, K, Fe, Mg, Zn and Ca level in blood while increased levels of pH, Malnodialdehyde and electrical conductivity are observed (Qayyum et al. 2016). The researchers also reported that in sub

clinical mastitis there are increased level of different enzyme like lactate dehydrogenase, alkaline phosphatase and aspartate aminotransferase (Qayyum et al. 2016, 2018).

**Table 2:** Comparison of different milk, blood, serum and lipid oxidation profile in healthy (negative) and subclinical mastitis (mild, moderate and severe) in Cholistani cattle

Parameter		Healthy Animal	Mastitis Animal		
			Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)
Serum milk minerals (mg/dl)	Cu	0.01	0.01	0.01	0.01
	Zn	0.05	0.04	0.03	0.03
	Fe	0.07	0.04	0.04	0.03
	P	34.72	29.19	24.41	21.18
	Mg	9.84	8.81	8.40	8.004
	Ca	118.59	111.19	100.27	94.15
	K	181.82	171.49	153.72	143.93
Serum blood minerals	Na	50.67	67.47	84.31	90.91
	Zn	1.06	0.98	0.88	0.83
	P	4.88	4.19	3.97	3.49
Milk serum enzymes (IU/L)	Ca	7.95	6.57	6.2	6.0
	Lactate Dehydrogenase	167.86	264.44	477.85	777.69
	Aspartate Aminotransferase	150.17	160.33	184.69	191.32
Blood serum enzymes (IU/L)	Alkaline Phosphatase	60.79	70.8	99.9	109.86
	Lactate Dehydrogenase	376.4	514.2	556.8	637.6
	Aspartate Aminotransferase	111.1	130.9	139.6	147.6
Lipid oxidation products Malnodialdehyde and Nitric oxide	Alkaline Phosphatase	49.7	64.1	77.9	81.7
	Malnodialdehyde in milk (nmol/ml)	5.31	7.63	8.59	10.5
	Malnodialdehyde in blood (nmol/ml)	1.62	2.18	2.56	2.78
	Nitric oxide in blood (nmol/ml)	29.8	36.4	44.9	55.9

Source: (Qayyum et al. 2016)



**Fig. 1:** Various gross lesions on teats arrow: haemorrhages (A), necrosis (B), teat apex inflammation and necrosis (C) and chronic inflammation (D) in buffaloes.



**Fig. 2:** Various gross lesions on teats and udder: udder edema in cattle (A), inflammation and necrosis (B, C & D) in cattle and buffaloes.

**Table 3:** Comparison of milk somatic cell counts, hemato-biochemical alterations and milk biochemistry in healthy and subclinical mastitic she-camel

Parameter	Healthy Animal	Mastitis Animal			
		Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	
<b>Milk total and differential somatic cell count</b>	Total SCC ( $\times 10^5$ /ml)	0.97	2.68	3.61	7.20
	Macrophages Percentage	43.73	30.97	27.68	34.54
	Lymphocyte Percentage	20.08	14.64	12.64	11.5
	Neutrophil Percentage	24.51	37.98	45.41	50.31
<b>Hemato-biochemical changes</b>	Total leukocyte count	12.37	15.78	18.22	19.18
	Pack cell volume Percentage	41.12	26.92	24.72	23.9
	Neutrophils Percentage	30.81	40.87	42.05	43.88
	Monocytes Percentage	2.62	3.65	3.95	4.21
	Lymphocytes Percentage	57.9	42.44	40.15	37.61
	Hemoglobin (g/l)	12.65	8.04	7.82	7.54
	Serum total protein (g/l)	7.31	5.51	4.94	4.75
	Serum albumin (g/l)	3.86	2.75	2.56	2.42
	Alanine aminotransferase (U/L)	12.99	20.04	31.4	41.55
	Aspartate aminotransferase (U/L)	33.08	41.22	45.31	51.71
Alkaline Phosphatase (U/L)	92.6	105.77	115.77	110.15	
<b>Milk biochemical changes</b>	Milk PH	6.37	6.93	6.87	7.48
	Electrical conductivity (mS/cm)	6.21	7.37	7.77	7.93
	Fat Percentage	4.12	3.52	3.36	3.28
	Protein Percentage	3.87	2.90	2.64	2.49
	Lactose Percentage	4.15	3.46	3.3	3.21
	Solid Not Fat Percentage	9.53	7.23	6.68	6.33
	Malnodialdehyde concentration	1.41	2.51	2.65	2.70
	Aspartate aminotransferase (U/L)	8.74	9.86	11.6	12.39
	Alanine aminotransferase (U/L)	10.80	14.17	16.75	18.62
	Alkaline Phosphatase (U/L)	18.11	23.67	27.8	31.81

Source: Ali et al. (2016)

### Udder Microscopic changes (Histopathological and histochemical)

Many of the researchers had shown that there is increase number or count of somatic cell count in mastitic animal as well as increased neutrophil population is observed in mastitis animal (Hussain et al. 2012). They also revealed that there is significantly decrease count of macrophage and lymphocyte in mastitis animal as compared to healthy animal (Hussain et al. 2012). Histopathologically no gross lesion is observed in healthy animal udder examination while udder atrophy, destruction of alveoli of udder and fibrous tissue proliferation is observed in mastitis animal (EL-Hallawany et al. 2018). While on histo-chemical examination, researcher had reported that mammary gland of healthy animal was developed well and had higher activity of alkaline phosphatase as compared to mastitis animal in which both parameter were less (Hussain et al. 2012).

### Udder Gross examination

Different physical parameter of udder like teat shape, teat length, teat diameter at apex and mid of teat, teat lesion, position of udder, udder shaper and breed play an important and act as a pre-disposing factor in causing mastitis (Fig. 1 and 2). Researchers had shown that prevalence of mastitis is higher in animal having small teat and streak canal and more teat diameter as less traveling distance should be needed to pathogen to cause infection (Chrystal et al. 1999; Hussain et al. 2012). Similarly in other research, it is reported that the mastitis is more prevalent in animal having impaired teat and long udder length (Bhutto et al. 2010). They also reported that mastitis is more prevalent in animal having pendulous udder and teat or udder injuries (Bhutto et al. 2010).

### Risk Factor

There are many risk factors that help pathogens in occurring of mastitis and linked directly or indirectly. Despite of comprehensive study bovine mastitis is still frequently occurring problem at farm all over the globe (Table 3). The occurrence of mastitis is directly influenced by management practices, and many other environmental factors like feeding, housing, watering, manure removal, standard operating procedure, milking utensils and health scores of lactating animal. The incidence rate of mastitis is also influenced by animal factor like udder scoring, teat length, teat shape, teat diameter, udder length, udder position, udder shape as well as bedding material and floor surfaces (Waage et al. 2001; Compton et al. 2007; Hussain et al. 2013; Bhutto et al. 2010; He et al. 2020). Similarly another researcher reported that the type of feeding, milking method and type of housing have direct link in occurrence of mastitis in animal (Barkema et al. 1999).

Occurrence of mastitis is also associated with session and it is also reported that prevalence of mastitis is more in winter session (Nyman et al. 2007; He et al. 2020). Chrystal et al. (1999) reported that there is increase somatic cell count in animal having more teat diameter while teat shape and teat end lesion are less associated with somatic cell count. Similarly

other researchers have determined that udder injuries, care of animal before and after calving and feeding of concentrate and restraints had direct link with increase somatic cell count (Svensson et al. 2006). Moreover, it is also recorded that the occurrence of udder infections is directly linked with traditional milking practices, presence of blind quarter, feeding and washing of milking area and equipment with water (Barnouin et al. 2005). Bachaya et al. (2011) in their investigations revealed that there is a positive relationship between incidence of udder infections and animal age, parity and stage of lactation. It has been investigated that various bacteria like *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus dysgalactiae* are major etiological agents of mastitis (Bachaya et al. 2011).

### Etiology

Mastitis can be caused by bacteria, virus, fungus and parasitic infection (Table 4). The most probable causative agents of mastitis in buffaloes reported are *Staphylococci*, *Streptococci*, *Escherichia coli*, *Corynebacterium pseudomonas*, *Streptococcus dysgalactiae*, *Mycobacterium tuberculosis* and *Mycoplasma* (Khan and Khan 2006; Unnerstad et al. 2009; Jung et al. 2021). In Pakistani buffaloes, most etiological agents of mastitis have been reported due to *Staphylococcus hyicus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus capotus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus pyogenes* and *Corynebacterium* (Khan et al. 2006; Waller et al. 2009; Schabauer et al. 2018). Similarly, in some other studies, *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Staphylococcus aureus* and *Staphylococcus epidermidis* were the most common recovered gram positive pathogens from mastitis animal (Piepers et al. 2009; Roshan et al. 2022).

### Impact of mastitis on cow behavior

Mammary gland infections induce abnormal disorders/changes on the behavior of infected animals. Different pathological changes like fever, udder pain, decreased milk yield and inefficiency of the immune system have been observed by the owner of animals. It is observed that pain can vary and depends upon the severity of infections in mammary glands (Kemp et al. 2008). Various bacterial infections in the udder initiate numerous energy-demanding reactions in the target animals leading to poor performance of the body (Johnson 2002).

Studies have reported that in infected animals the energy is switched from various activities, like social contact, grooming and feeding behavior (Johnson 2002; Dantzer and Kelly 2007), and the morbid animals may change their behavioral priorities, resulting in alterations in social and environmental demands of the individuals. Different earlier studies on sickness behavior in livestock animals including cattle due to natural or experimental udder infections have indicated relatively small data on behavioral alterations indicating that udder infections may lead to decrease rumination, feed intake and changes in resting behavior (González et al. 2008; Siivonen et al. 2011).

**Table 4:** Prevalence of different bacteria in causing mastitis

Serial No	Causative agents	No of isolates	Prevalence percentage in causing mastitis
1.	<i>Staphylococci</i>	41	41%
2.	<i>Streptococci</i>	18	18%
3.	<i>E. coli</i>	6	6%
4.	<i>Staphylococci</i> + <i>Streptococci</i>	15	15%
5.	<i>Staphylococci</i> + <i>E. coli</i>	13	13%
6.	<i>Staphylococci</i> + <i>Streptococci</i> + <i>E. coli</i>	4	4%
7.	<i>Streptococci</i> + <i>E. coli</i>	3	3%

**Source:** Hussain et al. (2012)

**Table 5:** Composition of normal and mastitic milk

Constituent	Normal milk	Mastitis milk with high SSC
Fat	3.53	3.22
Lactose	4.91	4.42
Total protein	3.62	3.55
Total casein	2.81	2.31
Whey protein	0.81	Above 1.3
Serum albumin	0.02	Above 0.07
Actoferin	0.02	Above 0.1
Immunoglobulin	0.1	Above 0.60
Sodium	0.057	Above 0.105
Chloride	0.091	Above 0.147

**Source:** (Jones 2006)

Cattle exhibited typical ailments of sickness behavior (Hart 1988) on day of confirmation of clinical mastitis, showing less feed intake and competitive behaviors (Table 5). These behavioral changes have been also observed in various other experimentally induced clinical udder infections (Siivonen et al. 2011; Fogsgaard et al. 2012; Yeiser et al. 2012). There is more risk of increase somatic cell count with udder injures and feeding animal with high concentrate diet (Svensson et al. 2006).

### Treatment

Udder infections cannot be fully removed and control from the herd but the incidence of mammary gland infections can only be reduced. The major factors for control and elimination of udder infections include good husbandry practices, strict biosecurity, sanitation and proper disinfection of dairy house, teat dipping after milking, dry cow therapy and culling to infected animals from the herd. The effectiveness of the treatment protocol during the dry period is suitable to achieve the maximum milk production. Somatic cell counts and prompt identification help to treat mastitis in dairy animals. Dry animal therapy can minimize 70% of environmental streptococcal infections.

The fundamental principle to control mastitis is that either decreasing the exposure of the teat to potential pathogens or by increasing resistance of dairy animals to infection. For treating animal with sub clinical mastitis, antibiotic sensitivity testing is very crucial part in treatment especially in subclinical

mastitis. Development of antibiotic resistant is one of the major issue in treating animal all over the globe. Idriss et al. (2014) revealed that *Staphylococcus aureus* and *Staphylococcus epidermidis* showed highest sensitivity to ciprofloxacin while 95.25% resistance of *Staphylococcus aureus* to ampicillin and 94.74% resistance of *Staphylococcus epidermidis* to cephalixin were recorded. *Staphylococcus aureus* shows variation in susceptibility to different antimicrobial (Barkema et al. 2006). Similarly, some researchers reported that oxytetracycline is very effective against most of the gram positive as compared to other drugs (Kurjogi and Kaliwal 2011). While other researchers revealed that *Staphylococcus aureus* is resistant to danofloxacin and methicillin (Arslan et al. 2009; Elbayoumy et al., 2020). The infected animal can be medicated with antibiotic like enrofloxacin, anti-histaminic and NSAID like Meloxicam drugs. We can also give supportive therapy (Khan & Khan 2006). Some researchers reported that tetracycline and enrofloxacin are highly effective against *S. aureus*, *E. coli* and *Streptococcus uberis* (Idriss et al. 2014).

The infected animals were treated with Injectable drugs (Enrocin, Melonex, Anistamin, DNS 5% and Agrimin forte) and mineral mixture. The owners were also advised to perform complete frequent milking at every 4-5 hours.

On the sixth day, the infected animals were completely recovered with disappearance of clinical signs such as clear milk, free of flakes, clots and white in color.

### Treatment of mastitis during lactation

Therapeutic response in cows can be examined by California Mastitis Test (CMT), somatic cell count and bacteriological samples from herds suffering from contagious mastitis. Medication of subclinical mastitis with antimicrobials agents are not economical throughout lactation as price of treatment is high and had poor effectiveness (Pyörälä 2009)

### Controlling contagious mastitis

*Staphylococcus aureus* infections are the largest mastitis downside of farm animals. Most of the researchers reported that this pathogen is highly contagious and transferred from one animal to other animal very rapidly (Plat-Sininige et al. 2009; Schabauer et al. 2018). During lactation, cure rate of antibiotic therapy is incredibly low and plenty of infected animals become chronic case and had to be culled. *Streptococcus agalactiae* respond well to antibiotic therapy and may be eradicated with sensible practices of mastitis management including teat dipping and dry animal treatment. *Strep dysgalactiae* might live anywhere. It will board the mammary gland, feces and rumen, and within the barn and may solely be controlled with correct sanitation and are moderately at risk of antibiotics (Khan & Khan 2006).

### Controlling environmental mastitis

It is often achieved by reducing the quantity of microorganism to the teat end. The animal's setting need to be clean and dry. The animals have minimum or no access to mud, manure, or pools of stagnant water. Calving place must be clean. After milking, teat

dipping with an antiseptic solution is a good practice. To control mastitis during dry period, using germicidal, attempts have been unsuccessful. Proper antibiotic therapy is needed for all animals at drying off. It will help to control environment streptococci during the early dry period (Khan and Khan 2006).

### Conclusion

Mammary gland infections are the major issues of milk producing animals and mainly influence the optimum production of milk. Dairy sector is commonly and persistently affected by variety of infectious agent which led to poor milk yield and causing huge economic losses to dairy farmers. Studies have recorded that adequate and good husbandry practices, proper dry cow therapy and early diagnoses of udder infections can prevent and protect the lactating animals. Behavioral alterations may also help in early diagnoses of mastitis. The infected animals usually exhibit signs of classic sickness behavior like poor feed intake, less rumination and decreased self-grooming. The change in behavior of animals can be suitable indicator of mastitis for identification of mammary gland infections. Estimation of milk leukocytes, signs of hidden infections in udder, and early treatment therapy can be suitable for control and elimination of udder infections. It is further recorded that suitable dry cow therapy can lower approximately 70% of various environmental infections.

### Conflict of interest

No conflict of interest in this paper

### Authors Contribution

All authors contributed equally

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