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# Mammary gland pathologies in the parturient buffalo

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ABSTRACT

Parturition related mammary gland pathologies in the buffalo appear to be low on accord of anatomic (longer teat length, thicker streak canal) and physiologic (lower cisternal storage of secreted milk, lower milk production) differences with cattle. Hemolactia, udder edema and hypogalactia usually occur in the buffalo due to physiologic changes around parturition however mastitis involves pathologic changes in the udder and teats; the incidence of mastitis is however lower compared to cattle. The incidence and therapy of hemolactia, udder edema and hypogalactia are mentioned and the risk factors, incidence, diagnosis, therapy and prevention for mastitis in buffalo are also described.

## 1. Introduction

Mammary gland pathologies such as mastitis are less frequent disorders among parturient buffaloes[1]. The low incidence of mammary gland pathologies appear to be because of anatomic and physiologic difference of buffalo with cattle[2]. The parturition related mammary gland disorders recorded for buffalo include hemolactia, udder edema[3], hypogalactia[4] and mastitis[5]. Mastitis has been widely recorded in buffaloes at nearly all geographic locations with incidence ranging from 2% to 60% and Staphylococcus spp being the most frequent causative microbes[5]. Diagnostic approaches for clinical and subclinical mastitis in buffalo utilize criterion mentioned for cattle and somatic cell counts are the most frequent test for the diagnosis of subclinical mastitis in buffalo. In this review the parturition related mammary gland pathologies described include hemolactia, udder edema, hypogalactia and mastitis.

## 2. The bubaline mammary gland and milk secretion

The bubaline mammary gland emerges in prenatal fetuses at 90-109 days of pregnancy and comprises 4 mammary anlage with a centrally located sprout embedded into mesenchymal tissue[6]. These develop into fully differentiated duct system of the mammary gland at 251-254 days of gestation[6]. At birth the mammary gland consists of a small teat with gland cistern and extensive duct system which develop progressively during the prepubertal period with significantly higher glandular tissue being formed at lactation[7]. Before parturition under the influence of hormones and other factors the bubaline mammary glands enlarge and are fully functional and developed before parturition[8]. During early lactation the cellular junctions in the mammary alveoli are (19.78±0.99) nm wide and (174.16±22.36) nm long and the gap junctions between external and internal nuclear membranes were (36.39±1.75)

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nm in fully lactating cells[9].

The teat dimensions and milk outflow in buffalo is similar to cattle yet there are subtle differences in some aspects of morphology and function. In Murrah buffaloes the fore and hind teat length ranged from 5 to 14 cm and 8 to 16 cm, respectively[2]. The teat girth (thickness) ranged from7 to 14 cm and 8 to 16 cm, respectively[2]. Upon milk ejection there was more than a 10% increase in the teat length and teat girth [2]. Buffaloes had a mean total cisternal area (teat and gland) of around 22 $cm^2$ for a single quarter which is less than half of what is seen in cows (40–45 $cm^2$) and the cisterns were significantly larger in early compared to late lactation. The cavity area in the teat and gland regions was similar[2]. The teat canal length in buffaloes was around 3 cm and was much longer compared to the 0.5 to 1.5 length reported in cows[2]. Comparative abattoir studies in cattle and Egyptian buffaloes indicated that teat canals were 30 to 40% longer in buffaloes compared to Holstein cows[10]. It has also been recorded that the epithelial thickness of the buffalo streak canal was about 10% greater and the thickness of the sphincter was 13 % greater compared to cross–bred Holstein cows[7].

In buffalo cisternal milk fraction was lower than reports in cattle and goat (20% to 40%) and was only 5% of the total milk[2]. There was a close correlation between the cisternal area measured using the ultrasound technique and the cisternal milk yield[2]. During milk ejection, teat length and circumference, and the cisternal area in the ultrasound cross sections increased significantly[2]. Buffalo teats were flaccid and empty due to the small cisternal fraction of milk prior to milk ejection, while during milk ejection; there was a remarkable increase in teat dimensions[2].

Studies have indicated that buffaloes store only a small fraction of milk in the cisternal cavities after a milking interval of 10 to 11 h[2]. In cows it has been demonstrated that an 8–h interval is optimum for measuring the cisternal size and cisternal fraction of milk[11]. Most of the milk synthesized between milling's in buffalo is stored in the alveolar lumen and the small ducts and there is very little drainage of milk into the cisternal cavities. The completeness of emptying the udder during milking of buffaloes is entirely dependent upon timing of oxytocin release in response to stimulation and its sustained release through the entire milking. In buffaloes, milk ejection follows the same neurohormonal mechanism as in cattle, sheep, and goats where adequate stimulation cause an increase in blood oxytocin levels in one minute[2].

## 3. Blood in milk (Hemolactia)

Blood in milk is a state of physiological hyperemia of the mammary gland that is occasionally seen in buffalo towards the end of gestation and for a short period just after parturition[12]. This admixture of blood in milk normally persists no longer than 14 days. Failure to milk out the udder may precipitate this condition at any stage during lactation[12]. In latter cases, the udder invariably becomes turgid and more or less diffusely reddened. Immediately after calving it is usually the result of rupture of many small congested blood vessels or seepage of blood into teat canal by diapedesis[12]. Severity varies from large clots and frank blood to a pale pink tinge of the milk[12]. The etiology of hemolactia often lies is the method of milking, feeding of certain feedstuffs, vitamin C deficiency, intramammary infusion of irritants, chronic mastitis and some infections [12].

Sometimes, the erythrocytes in the milk are sufficient enough to impart a pinkish or reddish discoloration of the milk resulting in red cream and sediment. However, more frequently there is a mere trace of blood that can be demonstrated only upon centrifugation of milk. Extensive bleeding may occur as a result of rupture of blood vessels. The presence of blood, however, imparts an insipid quality to milk. Any other hemorrhage by diapedesis is considered pathological and occurs due to damage to the epithelial lining of the teat cistern[12].

Extensive bleeding in the udder tissue may be due to penetrating lacerations, tread wounds, whip injuries, horn pokes and straining of the tissues[12]. As a result there are localized or widespread areas of blood diffusion into the connective tissue of the skin of the udder or in the wall of the cistern. In every instance of macroscopic abnormality evident on withdrawal of milk from the udder, systemic and udder diseases as well as the effect of feed should be considered.

### 3.1. Therapy

A slight admixture of blood in the milk postpartum requires no specific treatment as it naturally disappears within two weeks. If it is due to slight trauma it is not a matter of much concern. If the causative factor is/are feedstuffs, the same must be eliminated at once and light feed should be provided to animals with quarter being milked out with as much care as possible avoiding intensive stripping[12]. Supportive treatment in the form of ice packs, cold water sprays may be provided.

Extensive admixture of blood in milk warrants immediate administration of haemostatic and preantral coagulants along with intravenous administration of calcium preparations such as calcium borogluconate. Intravenous infusion of 5 mL (1%) epinephrine derivatives that hamper the flow of blood by vasoconstriction and increase the blood platelets are suggested[12]. Vitamin C preparations such as 1000 mg of ascorbic acid are suggested to be administered IM. In case of severe blood loss fluid replacements are necessary. Cold fomentations are often helpful in reducing the severity of hemolactia.

Blood in milk associated with chronic mastitis and bleeding granulation tissue first requires the control of the infection by suitable intra–cisternal medication followed

by surgical removal of the granulation tissue. To preclude the development of ichoro-gangrenous mastitis (ichorous-watery blood tinged discharge), profuse administration of the intra-cisternal antibiotics is required[12].

Milk mixed with blood should be withdrawn from human use.

## 4. Udder edema

Udder edema is a periparturient disorder characterized by excessive accumulation of fluids in the intercellular tissue spaces of the mammary gland[3]. The incidence appears to be higher in high producing first calving dairy heifers however it is less frequent in the buffalo. The risk factors for this disorder appear to be obesity, heavy milk production, lack of exercise and longer gestation period. One recent study pointed out that udder edema in buffaloes was less severe in buffaloes fed a low anionic or high anionic diets compared to those fed a low cationic and medium cationic diet during the prepartum period[13]. Udder edema is a significant risk for development of mastitis, creates difficulty in milking, predisposes the teats and udder to injuries and is a major discomfort to the buffalo[3]. The edema may rarely be extensive extending upto the naval creating difficulty in walking.

### 4.1. Therapy

Some degree of udder edema is frequent in high producing dairy cows, yet it is less frequent in buffaloes. Mild degree of edema resolves spontaneously or with cold fomentation. The more severe cases require administration of diuretics such as furosemide (1–2 mg/kg IM) and corticosteroids such as dexamethasone[3] and liver tonics[3].

Udder edema has been reported to respond favorably to homeopathic drug combinations of Phytolacca 200, Calcarea fluorica 200, Silecea 30, Belladona 30, Bryonia 30, Arruca 30, Conium 30 and Ipecacuanha 30 within 2–5 days [14]. Feeding of low anionic or high anionic diets are suggested for prevention of udder edema[13].

## 5. Hypogalactia

Poor milk production in parturient buffaloes have been documented[4] and known as hypogalactia. Milk production is an interaction of various hormones, metabolites, ruminal function and environment. Thus buffaloes under thermal stress and those with low prolactin secretion, mild deficiency of calcium and phosphorous and negative energy balance during the immediate postpartum period are likely to suffer from hypogalactia. Dietary supplementation has a profound effect on milk production in buffaloes[15]. Periparturient diseases of reproductive tract such as retained fetal membranes or metritis[16] and metabolism such as ketosis are likely to have negative effects on milk production[17].

### 5.1. Therapy

Improvement in milk production is likely with optimum nutrition[18] and absence of disease and stress. Buffaloes with low prolactin secretion are likely to benefit with IM administration of prolactinemic drugs such as metoclopramide or domperidone. Many publications have addressed the usefulness of herbal galactagogues such as Leptadenia reticulata, asparagus racemosus[4, 19–21] fenugreek and their combinations[22] on milk production improvement in buffaloes. However milk production is a complex process that involves an interplay of many hormones and other factors and thus no single therapeutic approach is likely to be successful without consideration of the management.

## 6. Mastitis

Mastitis is defined as the inflammatory reaction of the parenchyma of the mammary gland that can be of an infectious, traumatic or toxic nature[23, 24]. It is usually characterized by physical, chemical and bacteriological changes in the milk such as presence of blood, water, pus containing clots, flakes and shreds[25, 26] and by pathological changes in the glandular udder tissue[23].

The disease has been recognized in most buffalo raising countries including India[27, 28], Pakistan[13, 29–32], Iraq[33], Italy[23, 34–36], Turkey[37, 38], Nepal[39], Egypt[40], Portugal [41], Philippines[42], Spain[43] South America[44–46] and Bulgaria [47].

Similar to cattle mastitis has been classified as clinical [24] and subclinical[48, 49] forms. In the clinical form of mastitis changes in milk and inflammatory changes in the teats and udder are clinically evident[25, 50–52] whereas subclinical mastitis is not evident clinically and usually diagnosed employing a variety of direct and indirect tests such as California mastitis test, white side test, somatic cell count performed on milk[28, 53–56]. Clinical reports also classify clinical mastitis into per acute (with clinical signs of anorexia, pyrexia, decreased rumination, disinclination to move)[57, 58], acute, subacute and chronic depending upon the intensity of clinical presentation[37, 59]. A few reports depict the occurrence of gangrenous mastitis in buffaloes[60–62] yet it appears to be a clinical form of mastitis probably due to Staphylococci and *E. coli* [62]. Similar lesions were also observed in mastitis due to Clostridium perfringes in Egyptian buffaloes[51].

### 6.1. Incidence

The buffalo has traditionally been considered to be less susceptible to mastitis compared to cattle and most simultaneous studies in the two species have confirmed that the incidence of mastitis is lower in the buffalo[32, 48, 63, 64].

The buffalo udder is more pendulous in comparison to cattle and has longer teats. Buffaloes are at a higher risk of teat injuries predisposing those to mastitis. A probable reason contributing to a higher incidence of mastitis recorded in buffaloes compared to cattle in one study in Pakistan could be the different test used[65]. The buffalo has a long, narrow teat canal and a tight sphincter which can be expected to effectively prevent microorganisms from invading the udder and may be one reason for an eventual lower incidence of mastitis in buffaloes than in cattle. The pH of buffalo milk is more alkaline and the buffalo species exhibit some degree of resistance towards subclinical mastitis[44]. The well-developed circular muscles of buffalo teat along with thicker layer of keratinized epithelium of the teat canal and higher amount of keratohyalin granules in buffalo teats probably prevent the easy entry Table 1. The incidence and type of mastitis in buffaloes, tests used for identification and their efficacy in different studies of microbes in buffalo teats thus reducing the incidence of mastitis in buffalo compared to cattle[5].

**Table 1**
The incidence and type of mastitis in buffaloes, tests used for identification and their efficacy in different studies.

| Breed | Country | Type of mastitis | Tests used | Efficacy | No of samples | Incidence | Reference |
|---|---|---|---|---|---|---|---|
| Murrah | India | Subclinical | White side<br>CMT<br>SCC | 78.66%<br>82.55%<br>85.23% | 358 | – | Sharma *et al* [56] |
| Murrah | India | Subclinical | Bacterial culture | | 200 | 37% | Joshi *et al* [52] |
| Murrah | India | Subclinical | Bacterial culture | – | 415 | 26.27% | *Kumar et al* [66] |
| Murrah | India | Clinical | – | – | 781 | 12.53% | Taraphder *et al* [67] |
| – | India | Subclinical | CMT | – | | 40% | Kumar *et al* [68] |
| Egyptian | Egypt | Clinical,<br>Subclinical | Dermatonecroti<br>c test<br>Bacterial culture<br>CMT | – | – | 9.64% | Osman *et al*[51] |
| Nili Ravi | Pakistan | Clinical, Subclinical | Bacterial culture | – | 300 | – | Hussain *et al*[69] |
| Pakistani | Pakistan | Clinical, Subclinical | – | – | – | Clinical (periurban areas)–25.12%<br>Clinical (Rural areas)–19.74%<br>Subclinical–51% | Dhakal *et al* [70] |
| | India | Clinical | CMT<br>WST, strip cup, electrical resistance | – | – | – | Singh *et al* [62] |
| Nili–Ravi | Pakistan | Subclinical | Bacterial culture<br>PCR<br>CMT | 6.09%<br>19.51%<br>10.97% | 164 | – | Shahzad *et al* [71] |
| Italian buffalo | Italy | Subclinical | Bacterial culture<br>PCR | 20% | 20– | | De Carlo *et al* [72] |
| – | India | Subclinical | Culture (Bacterial and fungal)<br>Biochemical | – | 102 | 84.31% | Das and Joseph, [73] |
| – | India | Subclinical | CMT<br>MAST<br>WST | 28.3%<br>22.3%<br>19.5% | 2424 | Prevalance<br>Oct–43.5%<br>Aug.–37.8%<br>Sept.–37.6% | Patil *et al* [74] |
| – | Pakistan | Clinical<br>Subclinical | SFMT | | 370 | Overall–60.27%<br>Clinical–21.08%<br>ubclinical–39.1% | Chishty *et al* [65] |
| – | India | Clinical | Bacteriological<br>Biochemical | | 21 | 28.57% | Dhillon and Singh [75] |
| Murrah | India | Clinical<br>Subclinical | CMT<br>SCC | | 1000 | Clinical–3.1%<br>Subclinical–1.7% | Kavitha *et al* [76] |
| – | India | Clinical | Biochemical<br>PCR | | 47 | | Yadav *et al* [77] |
| Murrah | India | | PCR–(960bp–870bp–740bp–<br>610bp–Bacteriological–<br>Biochemical– | 8.57%<br>19.28%<br>29.29%<br>42.85%<br>9.37%<br>21.09% | 628 | 22.29% | Sidhu *et al* [78] |
| Murrah | India | Subclinical<br>Bacterial culture | CMT | | 85 | 48.9% | 48.9% |

(continued)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Egyptian buffalo | Egypt | Subclinical | Milk electrical resistance | | | 9.69% | Ahmed *et al* [80] |
| | Egypt | | TSCC<br>DSCC<br>Bacteriological | | 50 | | Tripaldi *et al* [81] |
| | India | Subclinical | SCC<br>Bacterial culture | 23.17%<br>29.26% | 326 | 7.05% | Pankaj *et al* [49] |
| | India | Subclinical | CMT<br>SCC<br>Milk conductivity | | 1080 | 3.7% | Basiri *et al* [82] |
| | India | Gangrenous | Strip cup test<br>White slide test<br>CMT<br>Strips<br>Electrical conductivity | | 12 | | Singh *et al* [62] |
| | India | Subclinical | MWST<br>MCMT<br>SCC | 78.86%<br>82.55%<br>85.23% | 358 | 83.24% | Sharma *et al*[56] |
| Murrah | India | Clinical | MCMT | | 1 | 8-40% | Sharma *et al* [83] |
| | Pakistan | Clinical<br>Subclinical | | | 272 | 20.98% | Mustafa *et al* [32] |
| Murrah | India | Clinical | | | | | Vishnoi *et al*[84] |
| Egyptian Buffalo | Egypt | | | | 20 | | Kholif *et al* [85] |
| | India | Clinical | | | | 2.71% | Verma *et al*[86] |
| Nili Ravi | Pakistan | Subclinical | CMT | - | - | 15.2% | Hussain *et al* [69] |
| | Brazil | Subclinical | SCC, culture | | 1896 | - | Medeiros *et al*[87] |
| Bulgarian Murrah | Philippines | Subclinical | CMT | - | - | 42.76% | Salvador *et al* [42] |
| Egyptian | Egypt | Clinical | Culture | - | 200 | 16.5% | Zaki *et al*[88] |
| | Pakistan | Clinical | - | - | 200 | 72% | Bilal *et al*[89] |
| | India | Clinical | Culture | - | - | - | Grewal *et al* [90] |
| | Egypt | SCC, Culture | - | | 30 | - | El-Sangary *et al* [91] |
| | Egypt | Clinical, subclinical | PCR, Elisa | - | - | 7% | El-Ghamy *et al* [92] |
| | Pakistan | Subclinical | - | - | 100 | - | Sharif *et al* [93] |
| | Pakistan | Subclinical | CMT, WST, WSTD, Surf test | CMT most effective followed by WST/WSTD | 880 | - | Iqbal *et al*[94] |
| Murrah | India | Subclinical | | | | 18.91% | Khate and Yadav, [63] |
| | Pakistan | Clinical, Subclinical | Surf field test<br>CMT | 66.22%<br>70.27% | - | Subclinical 41.5% | Muhammad and Rashid [95] |
| | India | Clinical | - | - | 1565 | 15.97% | Mahajan *et al* [64] |
| | India | Subclinical | CMT, SCC | - | - | 54.77% | Maiti *et al* [48] |
| | India | Subclinical | CMT MDS | 83.33%<br>80.95% | 200 | - | Ramprabhu and Rajeshwar[96] |
| Murrah, Jaffarabadi | Brazil | Clinical<br>Subclinical | CMT, Bacterial culture | - | 734 | 30.93% | Prado et al [97] |
| Murrah | India | Clinical<br>Subclinical | Bacterial culture | - | - | Clinical 18.74%, Subclinical 32.90% | Sharma and Sidhu [27] |

Inflammation of the mammary gland results from the introduction and multiplication of microorganisms in the mammary gland. The incidence of subclinical mastitis is higher in buffaloes compared to clinical mastitis[50, 65, 98] and varies with the breed, geographical location, management practices and other factors. The incidence of clinical mastitis in various studies varied from 2.7% to 40%[65, 67] (Table 1) whereas that of subclinical mastitis ranged from 1.7% to 59.64%[32, 49, 68, 74, 99] (Table 1). An exceptionally high incidence of clinical mastitis (72%) was recorded only in one study in Pakistan[89]. The incidence of subclinical mastitis varied with the type of test used for identification[53, 100], stage of lactation [101], and sample processing[54]. Single quarter affection has been more prevalent for clinical mastitis in buffalo[102] and hind quarters were more affected in many studies[24, 66, 76].

### 6.2. Risk factors

The risks for udder infections are highest around drying off and parturition in dairy animals and the prevalence of mastitis is higher during the first month after calving probably because of depression of immune function around parturition[103]. Immunosuppression if often associated with high levels of glucocorticoids in blood, a common finding around parturition and stressful conditions[103]. The phagocytic activity of neutrophils is decreased in mastitic milk[84].

Risk factors identified for the occurrence of mastitis in buffaloes include type of housing, method of milking, parity [24, 28, 69, 76, 104], month of lactation and rainfall[74]. It has been mentioned that farms with good management have a low incidence of mastitis in buffaloes. A positive association of mastitis was observed with milk yield, pendulous udder, udder depth and dirty hind legs of buffaloes at Government and private farms in Pakistan[69]. Studies in Brazil evidenced that improper milking management such as lack of teat washing was considered a significant contributor to the occurrence of mastitis in buffaloes[87]. In Philippines age and lactation length influenced the occurrence of subclinical mastitis in Bulgarian Murrah buffaloes[42]. Studies in Egypt pointed out that environmental microbes are a significant risk for mastitis and thus hygienic measures are extremely important for prevention of mastitis[88]. Calving related disorders such as retained placenta, mastitis, vaginal prolapse and dystocia are a significant risk for the development of mastitis in buffaloes[89]. High milk yielding buffaloes are more prone to develop mastitis[76, 104]. Hot humid season favors the growth of microbes in the environment thus increasing the incidence of mastitis during summer and rainy seasons[70, 105]. Close confinement[106] and use of soil as bedding material increased the incidence of mastitis in buffaloes[65, 76]. Teat injuries increase the risk of mastitis in buffalo[65].

### 6.3. Etiology

Mastitis is considered to be a multifactorial disease caused by a group of pathogenic bacteria, viruses, fungi [8, 107, 108] with bacterial mastitis being common. Many bacteria are known to result in bovine mastitis[5]. A large number of bacteria including *Staphylococci*, *Sterptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus pyogenes*, *E. coli*, *Psuedomonas*, *Proteus*, *Klebsiella*, *Diphtheroids*, *Mycoplasma*, *Clostridium perfringes* and *Listeria* have been isolated from buffaloes suffering from mastitis[40, 51, 72, 77, 109]. Some unusual microorganisms isolated from buffaloes with mastitis in Brazil and Spain[43] includes Lactococcus garviae. The most common bacterial isolates however appear to be Staphylococci[13, 26, 49] and *Streptococcus agalactiae*[24] (Table 2). Isolation of yeasts such as *Trichisporon* and *Cryptococcus* has been recorded from mastitis affected buffaloes[73]. The organisms *Staphylococcus aureus*, *Streptococcus agalactiae* and *Corynebacterium bovis* are referred as the contagious pathogens of mastitis in buffaloes[24, 104] usually transmitted from infected to clean udder, contact with infected milk and through flies[24]. Environmental pathogens such as Coliforms (*E. coli*, Klebsiella), *Streptococcus uberis*, and *Streptococcus dysgalactiae* are transmitted between and during milking [24]. In a recent review it was mentioned that although Staphylococcus aureus had been the major mastitis pathogen in the past, coagulase negative Staphylococci have been emerging to be commoner during recent years[5].

### 6.4. Bubaline ulcerative mammilitis

The occurrence of bovine herpes virus mammilitis caused by the bovine herpes virus-2 (BHV-2) was described in buffaloes for the first time in 1998[110]. It was however mentioned that the disease has been prevalent in buffaloes previously and has been named necrotic thelitis, ulcerative mammilitis and udder impetigo based on the clinical findings and not on the basis of virus isolation [111]. Later reports continued to name the disease with similar terminology[112-114]. Herpes virus mammilitis is manifested by severe ulceration of the skin of the teat and udder. The affection usually affects buffaloes during their first lactation and the first two months of lactation[110, 113]. There is acute enlargement of one or more teats, ulceration, necrosis, bluish black discoloration and sloughing of the affected teats[113, 115]. The lesions may appear with sudden appearance of multiple flattened nodules, varying from 1-4

**Table 2**

Type of mastitis and organisms isolated from buffaloes in different studies.

| Breed | Country | Type of mastitis | No of samples | Microbes isolated | Reference |
|---|---|---|---|---|---|
| Murrah | India | Subclinical | 200 | Staphylo (53.01%)<br>Strepto(30.12%) | Joshi *et al* [52] |
| Murrah | India | Subclinical | 415 | Staphylococcus | Kumar and Sharma [66] |
| Nili Ravi | Pakistan | Clinical, Subclinical | 300 | Staphylococcus aureus | Hussain *et al* [109] |
| Pakistani | Pakistan | Clinical, Subclinical | | Staphylo (48.57%)<br>Bacillus cereus (2.85%)<br>E.coli-10%<br>Micrococcus Luteus-15.71%<br>Proteus Vulgaris-4.28%<br>Pseudomonas aeruginosa-1.42%<br>Strept. Dysgalactiae-11.42%<br>Strept. Uberis-4.28%<br>Citrobacter-1.42% | Dhakal *et al* [70] |
| Nili-Ravi | Pakistan | Subclinical | 164 | Staphylococcus aureus | Shahzad *et al* [71] |
| Italian buffalo | Italy | | 20 | Mycoplasma | De Carlo *et al* [72] |
| | India | | 102 | Staphylo.-27.9%( coagulase positive)<br>Staphlo.㊀16.28%( coagulase negative) | Das and Joseph [73] |
| Jaffrabadi | India | Clinical | 3 | Candida<br>Aspergillus<br>Trichosporon<br>Cryptococcus<br>Saccharomyces<br>penicillin | Ghodasara *et al* [116] |
| | India | Clinical | 21 | Staphy-44.44%<br>Strept-11.11%<br>E.coli-22.22%<br>Bacilli-11.11%<br>Klebsiella-11.11% | Singh *et al*[117] |
| | India | Clinical | 47 | Listeria | Yadav *et al* [77] |
| Murrah | India | | 628 | Staphylococcus | Sindhu *et al* [78] |
| Egyptian buffalo | Egypt | Clinical | 83 | Clostridium | Osman *et al* [51] |
| | India | Subclinical | 326 | Staphylococcus (Coagulase positive)-15.90%<br>Staphylococcus (coagulase negative)-47.72%<br>Strept. Dysgalactiae-25%<br>Strept. Agalactiae-9.09%<br>Strept. Uberis- 2.27%<br>Staph.+strept.-13.63% | Pankaj *et al* [49] |
| Egyptian | Egypt | Clinical | 200 | E.Coli 43.8%, Staph aureus 37.4%, Mycoplasma 16.5% | Zaki *et al*[88] |
| | India | Clinical | - | E.Coli, Psuedomonas, Klebsiella, Staph aureus | Grewal *et al* [90] |
| | Egypt | | 30 | E. Coli 36.36%, Staph aureus 20.45%, Streptdysgalactiae 11.36%, Psuedomonas 4.54%<br>Staph + E Coli 18.18%, Streptdysgalactiae + E.Coli 9.09% | El-Sangary *et al*[91] |
| | India | Clinical | 21 | Staph 66.66%, Streptococcus 16.66%, E.Coli 33.33%, Bacilli 16.66%, Klebsiella 16.66% | Dhillon and Singh [75] |
| | Pakistan | Subclinical | 1483 | Staph 72.32%, Streptococcus 20.96%, Others 6.76% | Ahmad *et al*[80] |
| | Pakistan | Subclinical | 324 | Staph aureus 53.85%, Strept agalactiae 23.07%, E.Coli 15.38%, Strep dysgalactiae 3.85%, Corynebacterium bovis 3.85% | Hameed *et al*[118] |
| | India | Clinical<br>Subclinical | 5707 | Staph 38.81%, Strept 32.4%, E.Coli 11.80%, Corynebacteria 5.2%, Bacillus 1.06%, Klebsiella 2.03%, Psuedomonas 0.78%, Proteus 0.14%, Yeast 0.14% | Sharma and Sindhu [27] |
| | Brazil | Clinical | 737 | Corynebacteria 59.25%, Staph 17.65%, Micrococcus 6.37% | Prado *et al* [97] |
| | Nepal | Clinical | - | E.Coli 47.72%, Staph 44.15%, Gram negative bacilli 5.5%, Strept 2.59% | Thapa and Kaphle[119] |
| | Iran | Subclinical | - | Staph 48.55%, Lactobacillus 12.3% | Behesti *et al* [120] |

cm in diameter usually on the udder spreading to the teats. Some nodules become necrotic later and slough off[115]. There is no involvement of the mammary tissue and milk remains normal at all stages of the disease[113]. Different mastitis tests revealed negative results on testing of milk samples from buffaloes affected with ulcerative thelitis[114]. However, the lesions are painful and created difficulty in milking of the udder. Thickening of the teat skin may lead to narrowing of the teat canal[114]. Confirmation of the virus can be done by electron microscopy of the vesicular fluid or scab tissue collected from ulcerative mammilitis cases[110, 114]. Udder impetigo with identification of Staphylococcus aureus was recognized at one buffalo farm[111] and at another location with neither association[115]. The therapy of ulcerative mammilitis involves the oral administration (1200 mg) and topical application (5%) of antiviral drug Acyclovir[114]. The simultaneous administration of antibiotics, antiallergics and anti-inflammatory drugs is suggested to speed up the recovery[112, 115] and prevent secondary bacterial contamination.

### 6.5. Diagnosis

Diagnosis of clinical mastitis is easy as clinical signs such as inflammatory changes in the udder, teat and milk are evident. The diagnosis of subclinical mastitis is dependent upon a number of tests performed on the milk. In clinical mastitis inflammatory swellings appear on one or multiple teats alone or the teats and the quarter. The swellings appear suddenly at or within few hours or days of parturition and changes appear in the milk quality.

Besides bacteriological culture of the milk for identification, isolation of the microbes[29, 49, 51, 88, 121] a large number of indirect tests have been used for the detection of subclinical mastitis in buffaloes such as use of strips[74, 122], California mastitis test[42, 53, 56, 109], white side test[74], detection of electrical conductivity of milk[80, 123], estimation of acute phase proteins[124] or PCR based assays for molecular characterization of Staphylococcus aureus[71, 78]. However the most common test used in most studies appears to be the somatic cell counting[36, 53, 56, 125, 126]. A new surf field test has been mentioned for the diagnosis of mastitis in buffaloes in Pakistan[95] yet its efficiency remains to be seen on a wider scale.

In the white side test (WST) and modified white side test (MWST) 4% NaOH and 0.5% alkyl arylsulfonate combined with 1.5% NaOH react with deoxyribonucleic acid in the somatic cells to form sodium salts and produce a precipitate. Higher fat percent and larger lipid size in buffalo milk however result in lower sensitivity of the test.

The California Mastitis Test (CMT), which was developed as a cow side test for bovine milk to estimate the cell content, has been used in buffalo milk. It has been assumed that the interpretation of the test results should be the same as it is in bovine milk. In California mastitis test 3% triethanolamine sulfonate and bromocresol purple react with DNA in somatic cells rupturing the cell wall and forming a gel. The formation of a gel indicates subclinical mastitis. The scoring is qualitative and subjective. CMT reflects the SCC level quite accurately and is a reliable indicator of severity of infection. CMT results alone should not be used for immediate treatment unless the specific pathogen is known or is performed in accordance with a treatment protocol recommended by a veterinarian.

The somatic cell counts in buffalo milk are performed utilizing procedures mentioned for cattle and generally have the same proportion of samples with low and high SCC as reported for cows[127]. Briefly 10 μL of thoroughly mixed milk sample is spread over 1 cm2 marked square area on a glass slide, making a thin film. The slide is dried at room temperature, fixed in methanol for 5 min, stained with Newman-Lampert stain for 2 min and dried at room temperature. The slide is then washed thrice in tap water and dried at room temperature. Somatic cells are counted at 1 000× magnification under a microscope using oil immersion[55]. The SCC per mL is calculated by multiplication of the working factor with the number of cells counted[55]. Alternatively automated optoflurometric cell counters can be used for estimation of SCC although they are not popular among buffalo clinicians. SCC is recognized as a measure of udder inflammation and represents a sign of incorrect farm management[23]. The SCC includes the whole of leucocytes (WBC) and of the epithelial cells coming from the exfoliates of the udder existing in milk. More leucocytes mostly polymorphonuclear leucocytes (PMNs) infiltrate the mammary tissue as a response to the pathologic or stressing event[128].

Buffaloes regularly shed a small number of cells in the milk (macrophages, lymphocytes and neutrophils and cells lining the ducts) with a predominance of macrophages[129]. The cell count increases with advancing age and stage of lactation[34] with counts being higher around calving and towards the end of lactation[54, 130-132]. The milking frequency and the method of milking (hand milking/machine milking) also affects the SCC to some extent[129,133,134] and minor changes also do occur during estrus[135]. SCC is also known to increase non-significantly from 1st to 4th parity[136] and is also significantly higher during summer compared to winter months[136]. During mastitis increase in SCC is because of influx of neutrophils (PMNs) into the milk reaching 90% of the cell population[129]. The type of infection is known to affect the SCC. The highest SCC was observed in quarters

infected with streptococci[100, 137]. The severity of mastitis also affects the SCC[93].

There has been a lack of consensus on the threshold values of SCC for identification of subclinical mastitis in buffaloes. While many studies considered >200×10$^3$ cells per mL as being indicative of mastitis in buffaloes[55,100,138] others have considered at least >250×10$^3$ cells per mL[139] and >280×10$^3$ cells per mL[121]. A few recent studies have on the other hand considered >500×10$^3$ cells per mL as indicative of subclinical mastitis in buffalo[49, 140, 141]. Moreover most of the studies in buffalo have utilized milk samples from individual quarters or buffaloes except a very few that utilized composite milk samples[36] or samples from bulk milk tanks[88].

Mastitic milk has a higher electrical conductivity compared to normal milk[142]. This is probably due to tissue damage and the subsequent increase in sodium and chloride ions in milk[80, 123]. The normal electrical conductivity of buffalo milk ranged from 6.05 to 10.92 m-mhos with an average of 9.17 m-mhos and was not significantly different from cow milk. The change in electrical conductivity is one of the earliest manifestations associated with new infections making the early detection and recording of mastitis[142]. Conductivity sensors can be incorporated in automated milking machines however such systems are not popular among buffalo production systems.

The comparative efficiency of different tests have been the subject of some studies in buffalo (Table 1) and it has been pointed out that the efficiency of SCC was highest compared to other tests it should be borne in mind that no single test is 100% accurate and to improve the efficiency in herds many tests should be performed simultaneously depending upon the requirement. SCC has been considered the gold standard for the diagnosis of subclinical mastitis[129] its efficiency is improved significantly only when simultaneous organism identification is performed upon detection of mastitis. Next to SCC, CMT was considered to have high efficiency for detection of mastitis in buffaloes (Table 1). Efficiency of CMT was considered slightly higher in buffalo compared to cattle whereas SCC revealed comparable efficiency in both species.

### 6.6. Effects of mastitis on milk quality

The milk yield is decreased in mastitis and the quality of milk is also affected[143, 144]. Mastitis impairs the coagulation properties of milk and results in a poor quality of cheese and curd[54, 101]. The milk yield is negatively correlated with the total somatic cell counts[81]. Significant increase in lactose and chloride content are also observed with the increasing total somatic cell counts[81]. The rennet coagulation properties and the clotting time are increased and the curd firming time and firmness are decreased[81]. Reduction in the quality of yoghurt and cheese[50] and reduction in the shelf life of processed milk obtained from buffaloes with subclinical mastitis has also been recorded. Thus mastitis affects the milk production, composition and quality[50]. The microbial quality of the milk has public health significance [145] and hence regular checkup for the presence of subclinical mastitis and effective therapies are desirable.

### 6.7. Therapy

The therapy of mastitis involves the intramammary and intramuscular administration of antibiotics and anti-inflammatory drugs. Penicillin's have traditionally been used for therapy of clinical mastitis in buffalo however some studies have shown ciprofloxacin to be an effective therapy [73] and similar results were mentioned for enrofloxacin[70, 104, 118, 146]. A large number of antibiotics have been used employing both intramammary and IM alone or in combination administered for 3-5 days (Table 3) with many studies suggesting a concomitant use to be beneficial. The disposition pattern of drugs administered IM have been studied in buffaloes. The disposition pattern of enrofloxacin in blood and milk of buffaloes suffering from clinical mastitis revealed that the peak concentration of the drug when administered IM was attained at 1 and 6 h and the minimum inhibitory concentration (MIC) in plasma and milk was maintained up to 24 h, thus reflecting that IM administration is useful for therapy of mastitis[90]. Similarly gentamicin (3 mg/kg IM) reached a peak concentration within 2 h and was maintained up to 24 h however, the drug was useful in mastitis in buffaloes caused by gram negative bacteria such as Psuedomonas[147]. Based on disposition kinetics a single IM administration of 20 mg/kg of long acting tetracycline was found to be useful in therapy of subclinical mastitis in buffaloes[148]. The peak concentration was attained within 4 h and the MIC of the drug was maintained in milk up to 84 h [148] and the cell counts were reduced significantly. Studies on disposition kinetics of IV administration of 100 mg/kg of sulfonamides to buffaloes revealed that the excretion of sulfonamides was higher in saliva and urine than in milk thus limiting the usefulness of this drug in the therapy of bubaline mastitis.

Many publications have addressed the antibiogram of microbes associated with mastitis in buffalo with diverse results[32, 73, 149, 150]. It has also been suggested to administer the antibiotic on the basis of organism culture and *in vitro* sensitivity towards the antibiotics [109] however the time needed for the results.

**Table 3**
Therapies used for mastitis in different studies.

| Type of mastitis | Drugs used | Route of administration | Period of therapy | Reference |
|---|---|---|---|---|
| Clinical/Subclinical | Enrofloxacin 5 mg/Kg alone or in combination with Vitamin E and selenium | IM | 3–5 d | Sharma *et al*[83]; Mukerjee [153]; Zaki *et al* [88]; El-Sangary *et al* [91] |
| Clinical | Ampicillin + Cloxacillin 4 mg/Kg | IM | IM | Rane *et al*[142]; Singh *et al* [159] |
| Clinical/Subclinical | Ampicillin 80 mg + Cloxacillin 200 mg | Intramammary | 3–4 d | Rane *et al* [142]; Bulla *et al*[160] |
| Clinical/Subclinical | Dihydrostreptomycin 1 gm + Procaine penicillin 10 lac IU | IM | 3 d | Villanada *et al* [161] |
| Clinical | Ceftriaxone 2 gm + Tazobactum 250 mg every 12 h | IM + Intramammary | 5 d | Gupta and Gupta [162]; Bhikane *et al* [163]; Kumar *et al* [164] |
| Clinical (Gangrenous) | Ceftriaxone 3 gm + Tazobactum 375 mg | IM | 5 d | Patil et al[165]; Singh *et al*[62] |
| Clinical | Cefradine 500 mg | Intramammary | 2–6 d | Yousaf *et al* [166]; Yousaf *et al* [167] |
| Clinical (Coliform) | Ceftiofur | IM + Intramammary | 5 d | El-Khodrey and Osman [59] |
| Clinical (Mycotic) | Mycostatin 10,000 IU | Intramammary | 5 d | Kumar and Thakur[107] |
| Clinical | Cefquinome sulfate 75 mg | Intramammary | 5 d | Sadekar *et al*[168] |
| Clinical | Amoxicillin 3 gm + Tazobactum 375 mg | IM | 5 d | Singh *et al*[117] |
| Clinical | Cloxacillin 2.5 gm + Colistin sulfate | IM | 2–4 d | Singh *et al*[57]; Villanada *et al*[61] |
| Clinical | Cefoperazone 500 mg + Hyaluronidase 1500 IU | Intramammary | 5 d | Handekar *et al* [58] |
| Clinical | Trisodium citrate 12–30 gm in 250 mL distilled water | Oral | 3–5 d | Yousaf *et al*[157]; Dhillon *et al* [169] |

## Conflict of interest statement

We declare that we have no conflict of interest.

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