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# Prevalence of Clinical and Sub-clinical Mastitis in Lactating Dairy Cows at the National Jersey Breeding Centre, Samtse, Bhutan

Dorji Tshering[1] and Kinley Gyem[2]

**Abstract**

This study investigated the prevalence and risk factors of clinical mastitis (CM) and sub-clinical mastitis (SCM) in lactating dairy cows through clinical examination and California Mastitis Test (CMT). Bacterial culture and drug sensitivity test was conducted to investigate the causal agents of mastitis and drug resistance on dairy cows. Fifty six milk samples were collected from October, 2014 to March, 2015. Prevalence of CM and SCM detected by both CMT and culture was 5.4% and 78.6% respectively. The prevalence of CM was higher in adult cows (18.2%), jersey breed (9.7%), cows with moderate parity (11.8%), high milk yielder (8%), and at late lactation stage (12.5%). The SCM infection varied among age groups showing increased infection with advancement of age. Higher infection with SCM was seen in Karan Fries breed (80%) than Jersey breed (77.4%). The SCM prevalence tended to increase with increased parity and milk yield. The infectious bacteria isolated were *Staphylococcus aureus*, *Pasteurella multocida* and *Escherichia coli* with prevalence of 89.4%, 8.5% and 2.1% respectively. *S. aureus* isolates were sensitive to most antibiotics tested except penicillin. *P. multocida* was susceptible (100%) to Gentamycin and resistant (100%) to Cloxacillin and Penicillin. *E. coli* showed susceptibility to both Gentamycin and Tetracycline, but was resistant to Amoxicillin, Cloxacillin, and Penicillin. The study indicates that there is high prevalence of SCM on the farm, which could be controlled by use of Gentamycin and Tetracycline.

**Key words:** Clinical mastitis, dairy cows, prevalence, sub-clinical mastitis

## Introduction

The word "mastitis" is derived from the Greek word "*matos*" meaning breast or udder and the suffix "*itis*" meaning inflammation (Kehrli and Shuster, 1994). Mastitis generally refers to an inflammation of the mammary gland, regardless of the cause (Pretorius, 2008). It is the most important and expensive disease of dairy industry worldwide, resulting in severe economic losses from reduced milk production, treatment cost, increased labour, milk losses following treatment, and premature culling (Sharif and Muhammad, 2009). It is manifested by a wide range of clinical and subclinical conditions, accompanied by physical, chemical, pathological, and bacteriological changes in milk and glandular tissue (Gianneechini *et al.,* 2002; Islam *et al.,* 2011). Mastitis in both clinical and subclinical forms is costly and complex disease resulting in a marked reduction in the quality and quantity of milk (Gianneechini *et al.,* 2002). Singh and Baxi (1988) reported that more than three times losses are due to subclinical mastitis (SCM) as compared to clinical mastitis (CM). Sub-clinically affected animal remains a continuous source of infection to its herd mates. Persistent infection might form a fibrous tissue barrier between organisms and antibiotic preparations, limiting their efficacy (Islam *et al.,* 2011).

Mastitis is caused by several causative agents and may be complicated by predisposing factors like poor management and hygiene, teat injuries, and faulty milking machines (Blowey and Edmonds, 1995). The causative agents of mastitis in dairy cows can be bacterial, mycoplasmal, and yeast pathogens; and the predisposing factors are known to hasten the entry of infectious agents (Islam *et al.,* 2011). Bacterial infection is the most common cause of mastitis among which *Staphylococcus* spp. are the main aetiological agents of CM and SCM (Abrahmsen, 2012; Abera *et al.,* 2013). In Asia, the major

[1]National Jersey Breeding Centre, Samtse
[2]National Jersey Breeding Centre, Samtse;
Corresponding author: dorji78tshering@yahoo.com

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aetiological agents causing mastitis in cows are *Staphylococcus aureus* Rosenbach*, Escherichia coli* Migula*, Streptococci, Corybacterium* spp., and *Kebsiella* (Ebrahimi and Nikookhah, 2005).

Globally, losses due to mastitis amount to about 53 billion dollars annually (Sharif and Muhammad, 2009). Varshney and Naresh (2004) estimated an annual loss in the dairy industry due to mastitis to 2 billion dollars in USA and 526 million dollars in India, in which SCM is responsible for approximately 70% of the losses. According to Kader *et al.* (2003), an annual economic loss of 2.11 million US dollars in Bangladesh is caused by SCM. Dua (2001) reported an annual loss of 97.63 million US dollars in India to mastitis. In Bhutan, mastitis is one of the most important diseases with regard to milk production with incidence of 24% SCM cases in the eastern Bhutan (Sharma *et al.,*1998).

Antibiotic therapy is an important tool to control mastitis in dairy herds. It minimises the risk of spreading antibiotic resistant bacterial strains in dairy herd (Abrahmsen, 2012). According to Sharif and Muhammad (2009), antibiotic treatment of SCM is recommended only during drying off period and not during lactation.

The progress report of the National Jersey Breeding Centre (NJBC), 2013–2014, showed mastitis as the highest clinical case attended during the year and there is an increasing trend. Therefore, this study was designed to estimate the prevalence of CM and SCM in lactating dairy cows of NJBC, Samtse, to understand the intrinsic factors, and to isolate and characterize the major bacterial pathogens from milk samples of mastitis infected cows.

## Materials and Method

The study was conducted at NJBC, Samtse from 1 October, 2014 to 30 March, 2015. The farm is located at 500 metre above sea level (masl) and falls in subtropical zone with daily average temperature of 23 $^{0}$C. The mean annual rainfall is 2,750 mm. The major wet season extends from May to August with the peak rainfall in June and July, whereas the major dry season is from November to January.

The study was conducted with 31 lactating Jersey cows and 25 Karan Fries of NJBC, Samtse. The animals in the farm are managed under intensive management system and housed in common shed irrespective of the breeds, age, lactation stage, number of parity, and milk production. The animals are milked twice a day using milking machine; at 3.00 A.M. and 3.00 P.M. Pre-milking and post milking hygienic practices such as udder washing and drying are practiced daily. Green grass, silage, and hay in addition to the concentrate feeds (Karma Feeds) were fed to the study animals. Supplementary feeds and 1 kg mineral mixture in 100 kg of feed in addition to concentrates and green fodder were provided when necessary. Subtropical grasses such as Napier (*Pennisetum purpureum* Schumach) and Ruzi (*Bracharia ruziziensis* Germ. and Evrard) were the main sources of green fodder to the farm animals. The animals are also let for rotational grazing for 3 hours daily from 6.00 A.M. to 9.00 A.M.

Udders of all the lactating cows were examined visually and palpated to detect possible fibrosis, signs of inflammation, visible injury, tick infestation, and swelling of the mammary lymph nodes. Information related to the previous health problems of the mammary quarters was obtained from the records maintained by the farm. Further, viscosity and appearance of milk secreted from each mammary quarter was examined for the presence of clots, flakes, blood, and watery secretions.

CM was detected by visible abnormalities in the milk and the clinical inspection of the udder. To establish prevalence of SCM; CMT was performed in all the lactating cows although the udder looked normal. Two to 3 ml of milk was stripped into individual cups in plastic paddle from each quarter and equal amount of CMT reagent added. Mixing was accomplished by gentle circular motion of the paddle in a horizontal plane for few seconds. The CMT results were recorded immediately for each teat. Reactions were graded as 0 and trace for negative, +1, +2, and +3 for positive according to Mekibib *et al.* (2010). The CMT was chosen as it is reliable than other field and chemical tests for diagnosis of SCM (Schukken *et al.,*1990; Leslie *et al.*, 2002).

The udders, especially the teat ends, were disinfected with cotton swabs soaked in 70% alcohol and allowed to dry before milk sample collection. Dust particles and other filth were removed by wiping the surface of the teats and udder with a dry towel.

Milk samples were collected from all the lactating cows in three batches and continued for entire study period. New milking cows transferred in during the period were also tested. During milk sample collection, first 1-2 drops of milk was discarded and then 10 ml of milk was collected into numbered sterilised test tubes with rubber cap. The milk samples were transported next day in ice to the laboratory at Regional Livestock Development Centre (RLDC), Tsimasham, Chukha and stored at 4 $^{0}$C on a standard bacteriological media for a maximum of 24 hours until samples were inoculated.

CMT positive milk samples were cultured according to the procedures employed by Shrestha and Bindari (2012) and Mekibib *et al.* (2010) for which the Nutrient agar, MacConkey agar and, Blood agar were used. Bacterial growth were identified and recorded after 24 –48 hours incubation at 37 $^{0}$C. Bacterial isolates were characterised by morphology, culture characteristics, gram stain reaction including shape and arrangement of the bacteria and biochemical tests.

Antibiotic sensitivity test was carried out in order to identify the most effective drugs for mastitis treatment in the study area. Tetracycline, Gentamycin, Penicillin, Cloxacillin, and Amoxacillin were used for sensitivity test using single disc in Mueller Hinton Agar prescribed by the National Committee for Clinical Laboratory Standards (NCCL). All the pathogens isolated were tested against antibiotic discs and susceptibility recorded.

Secondary data required for the study were obtained from the records maintained by the farm. Depending on the clinical inspection and CMT results, cases were categorised and scored in CMT scoring format. The age of the animals were categorised as young adults (> 3–6 years), adults (> 6 to < 10 years) and old (>10 years), and parity was categorised as few (with < 3 calves), moderate (4–7 calves) and many (> 7 calves), lactation stage was categorised as early (1–3 months), mid (4–6 months) and late (7–9 months), and likewise production was categorised as low (0–5 litres), moderate (6–10 litres), and high (> 11 litres). Data were recorded in Microsoft Excel 2007 spread sheet for statistical analyses. The analyses were done by using Statistical Package for Social Sciences (SPSS), Version 16.0.

## Results and Discussion

### *Overall prevalence of mastitis in the herd*

The CMT and culture result (Table 1) showed the overall prevalence of mastitis to be 83.9%, which is comparatively higher than those reported by Abera *et al.* (2013) and Bedada and Hiko (2011) in Government farms (66.6% and 66.1% respectively). Differences in reports of mastitis between the present study and other reports could be attributed to difference in breeds of cows, farm management practices, and level of production. However, there is high prevalence of SCM despite the farm applying recommended milking and hygeinic procedures. The only source of bacterial contamination in the farm that can be attributed to milking machines as the high prevalence of mastitis is reported in machine milked cows than hand milked cows (Blowey and Edmonds, 1995). Machine milking provides opportunities for the transmission of these organisms between quarters and cows through the milking machine itself, milkers' hands or clothes (Sudhan and Sharma, 2010). However, lower prevalence of CM (5.4%) than SCM in this study is supported by Adane *et al.* (2012) who reported that higher SCM than CM could be due to the defense mechanism of the udder and little attention given to SCM while treating clinical cases.

### *Age wise prevalence*

The distribution of CM and SCM in different age group is presented in Table 2. The prevalence of CM was highest (18.2%) in the age group of > 6 to < 10 years while SCM prevalence was highest in the age group of > 10 years. The increased prevalence of SCM with advancement of the age is in agreement with the findings of Girma *et al.* (2012). Similar observations were recorded by Mekibib *et al.* (2010), Islam *et al.* (2010), Moges *et al.* (2011), and Ayano *et al.* (2013). Elbably *et al.* (2013) explained that the increased prevalence of SCM with advancement of age in cows may be due to bad hygienic condition during calving.

### *Breed wise prevalence*

Prevalence of SCM in Karan Fries breed was found to be higher (80%) than in Jersey breed (77.4%) as shown in Table 2. This finding is in agreement with Islam *et al.* (2011) who reported mastitis infection in crossbred of Holstein Frisian to be higher than in the pure bred animals. Similarly, Moges *et al.* (2011) reported that the crosses of Zebu with Holstein Frisian cows were affected at higher rate (71.8%) than other breeds.

**Table 1.** Summary of CMT score (*n* = 56) and culture result of mastitic cows

| Forms of mastitis | CMT | | Culture | |
|---|---|---|---|---|
| | No. of positive | % positive | No. cultured | % positive |
| CM | 3 | 5.4 | 3 | 5.4 |
| SCM | 44 | 78.5 | 44 | 78.5 |
| **Total** | **47** | **83.9** | **47** | **83.9** |

*Prevalence of mastitis in relation to parity*

The result revealed that cows with moderate parity (4–7 calving) were affected more (11.8%) than the cows with lower parity, which is in agreement with the finding of Mekibib *et al.* (2010). Prevalence of SCM infection was recorded as 75%, 82.4%, and 100% at low (< 3 calving), moderate (4–7 calving), and many (> 7 calving) respectively. Increasing tendency of SCM infection was recorded with increase in parity, which is in line with the observation of Islam *et al.* (2011) and Moges *et al.* (2011) who reported an increasing prevalence of SCM with advancing parity. The high prevalence of SCM in aged-multiparous animals might be due to increase in teat patency and frequency of previous exposure (Ayano *et al.,* 2013).

**Table 2**. Prevalence of CM and SCM between intrinsic factors in the farm

| Variables | Category levels | No. of cows | CM (%) | SCM (%) | Not infected with mastitis (%) |
|---|---|---|---|---|---|
| **Age** | Young (= 3–6 years) | 42 | 1(2.4) | 32(76.2) | 9(21.4) |
| | Adults (> 6 to < 10 years) | 11 | 2(18.2) | 9(81.8) | 0(0.0) |
| | Old (> 10 years) | 3 | 0(0.0) | 3(100.0) | 0(0.0) |
| **Breed** | Jersey | 31 | 3(9.7) | 24(77.4) | 4(12.9) |
| | Karan Fries | 25 | 0(0.0) | 20(80.0) | 4(20.0) |
| **Parity No.** | Few (< 3 calves) | 36 | 1(2.8) | 27(75.0) | 8(22.2) |
| | Moderate (4–7 calves) | 17 | 2(11.8) | 14(82.4) | 1(5.9) |
| | Many (> 7 calves) | 3 | 0(0.0) | 3(100.0) | 0(0.0) |
| **Milk Yield** | Low (0–5 L) | 3 | 0(0.0) | 1(33.3) | 2(66.7) |
| | Medium (6–10 L) | 28 | 1(3.6) | 20(71.4) | 7(25.0) |
| | High (> 10 L) | 25 | 2(8.0) | 23(92.0) | 0(0.0) |
| **Lactation stage** | Early (60–90 days) | 25 | 1(4.0) | 19(76.0) | 5(20.0) |
| | Mid (91–180 days) | 15 | 0(0.0) | 12(80.0) | 3(20.0) |
| | Late (> 180 days) | 16 | 2(12.5) | 13(81.3) | 1(6.4) |

*Prevalence of mastitis in relation to milk yield*

Cows with higher milk yield showed higher prevalance SCM than low yielding cows (Table 2). The higher incidence of CM in high yielding cows in the present study could be attributed to chronic mastitic cows from the previous lactation. Chronic mastitis infections may be present in older animals resulting in an accumulated risk of CM (Levison, 2013). Prevalence of SCM was higher (92%) in high (> 10 litre) than low (33.3%) to medium (71.4%) yielders. This finding is in agreement with the reports of Kader *et al.* (2003) and Islam *et al.* (2010).

*Prevalence of mastitis at different stage of lactation*

Distribution of CM and SCM in dairy cows at different stage of lactation is presented in Table 2. Lactation stage seems to influence the occurrence of CM in the herd as the cows in late lactation stage (> 180 days) showed higher infection rate (12.5%) than early (0.0%) and mid lactation (4.0%) stages. Prevalence of SCM was highest in late lactation (81.3%) stage, which is comparable to the finding of Islam *et al.* (2010). The finding of this study is also supported by Islam *et al.* (2011) and Elbably *et al.* (2013) who stated that the prevalence of SCM could be high at late stage of lactation due to long exposure time.

*Bacterial culture*

Fourty seven samples from cows with CMT positive cases were cultured for bacterial isolations. The bacteria isolated were *S. aureus, P. multocida,* and *E. coli.* The percentage of *S. aureus* (89.4%) was significantly higher than *P. multocida* (8.5%) and *E. coli* (2.1%) in this study. The predominant bacteria causing mastitis on farm was *S. aureus,* which confirmed the findings of Abera *et al.* (2013), Shrestha and Bindari (2012) and Girma *et al.* (2012). The *Staphylococcus* spp. is an opportunistic pathogenic bacterium which survives on the skin of udder and can infect the udder through teat canal or any wound (Shrestha and Bindari, 2012). The environmental and stress factors such as shipping, co-mingling, and overcrowding are some of the predisposing factors of *Pasteurella* spp. bacterial infections (Dabo *et al.,* 2007). Prevalence of *E. coli* was comparatively lower than that reported by Shrestha and Bindari (2012). *E. coli* is an environmental pathogen and cow can be infected with it through teat canal where the management practices are poor (Blowey and Edmondson, 2011). The low incidence of *E. coli* in the farm may be due to good general cleanliness, drainage, manure disposal and use of teat dips.

*Antimicrobial sensitivity test*

All 47 positve samples were tested for susceptibility to 5 different antimicrobial discs; and the comparative efficacy of the antimicrobials is presented in Table 3. The results indicated that Gentamycin is effective antibiotic followed by Tetratcycline. Similar results were reported by Sumathi *et al.* (2008). The isolates showed highest resistance to Penicillin followed by Cloxacillin and moderate resistance was observed for Amoxicillin. High resistance to Penicillin was in agreement with the findings of Kumar *et al.* (2011) and Chandrasekaran *et al.* (2014).

*S. aureus* isolates were sensitive to most antibiotics, but showed strong resistance to Penicillin (Table 4). Similar observation was reported by Cervinkova *et al.* (2013) and Idriss *et al.* (2014) who reported strong resistance of *S. aureus* to Penicillin and Ampicillin. Higher resistance of *S. aureus* to Penicillin and Cloxacillin might be due to indiscriminate use of antibiotics and intra-mammary preparation containing the combination and broad spectrum antibiotics in the farm for more than five years. Today, the resistance to Penicillin is of concern since this antibiotic represents the main antibiotic group recommended for mastitis treatment and regular use of antibiotics for the treatment of cows may result in the spread of resistant strains (Nadeem *et al.,* 2013).

The study revealed a complete susceptibility (100%) of *P. multocida* to Gentamycin while complete resistant (100%) to Cloxacillin and Penicillin. *E. coli* tested susceptible to both Gentamycin and Tetracycline and resistant to Amoxicillin, Cloxacillin, and Penicillin. Idriss *et al.* (2014) also obtained similar result where *E. coli* showed resistance to Amoxicillin and Penicillin.

**Table 3.** Result of antimicrobial sensitivity tests on the bacterial isolates

| Bacterial isolates | No. of isolates | GEN (S/R) | TE (S/R) | AMC (S/R) | CX (S/R) | P (S/R) |
|---|---|---|---|---|---|---|
| *Staphylococcus aureus* | 42 | 38/4 | 36/6 | 19/23 | Aug-34 | Feb-40 |
| *Pasteurella multocida* | 4 | 4/0 | 03-Jan | 01-Mar | 0/4 | 0/4 |
| *Escherichia coli* | 1 | 1/0 | 1/0 | 0/1 | 0/1 | 0/1 |

The numerators show the number of sensitivity isolates, while the denominator show the number of resistant isolates among tested. GEN=Gentamicin (10 µg); TE=Tetracycline (30 µg); AMC=Amoxicilin (30 µg); P=Penicillin (10 µg); CX=Cloxacillin (10 µg); S/R=Sensitive/Resistance.

## Conclusions

High prevalence of mastitis in dairy herd is the biggest health and production problem on dairy farms. In NJBC, SCM in Jersey cows and 25 Karan Fries was higher than CM where the age, breed, parity, lactation stage, and milk yield were the important contributory intrinsic factors. *Staphylococcus aureus*, *Pasteurella multocida,* and *E. coli* were the pathogens isolated from positive milk samples among which *Staphylococcus aureus* was predominant bacteria causing mastitis in the herd. The comparative efficacies of five antimicrobials tests showed Gentamycin as the most effective antibiotics followed by Tetratcycline. Isolates of *S. aureus* showed an alarming level of resistance to Penicillin and Cloxacillin.


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