

## Assessment of sub-clinical mastitis and its associated risk factors in dairy livestock of Lamjung, Nepal

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### ABSTRACT

**INTRODUCTION:** Mastitis is one among the top three threats faced by dairy farmers. The study was carried out to assess sub-clinical mastitis, management practices and associated risk factors for mastitis.

**MATERIALS AND METHODS:** A cross sectional study was conducted in Chandreshwor and Archalbot VDCs of Lamjung district taking 63 dairy livestock randomly each from a herd along with questionnaire survey to respective owner. Ten ml of milk sample from each quarter was taken in a sterilized syringe for further laboratory investigation. California Mastitis Test (CMT) was performed at farmer's shed. Organisms were identified based on colony characteristics, Gram's staining and various biochemical tests.

**RESULTS:** On CMT, subclinical mastitis was 46.1% (n=29) and 30.15% (n=76) on animal and quarter basis respectively, however, culture showed, 28.6% and 24.2%. Streptococcal mastitis was the commonest (11.1%) followed by coliform (9.5%) and staphylococcal (7.9%). Mastitis was highest in left fore quarter (34.92%) followed by left hind (31.76%), right hind (28.57%) and right fore (25.39%). Coliform & Staphylococcal mastitis was highest in left fore and right hind quarter respectively. Most of dairy animals (86%) were on zero grazing, 30% (n=19) of the farmers had forage trees and only 29% (n=18) had known about subclinical mastitis. The average milk production was 3.5 ±1.47 liters. Subclinical mastitis was associated significantly (p<0.01) with livestock yielding more than 3 lt per lactation.

**CONCLUSIONS:** There was high prevalence of subclinical mastitis in dairy livestock at Lamjung due to poor management, unhygienic shed, and little knowledge on subclinical mastitis.

**KEY WORDS:** Subclinical mastitis, Management, dairy animal, Quarter, Lamjung

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### INTRODUCTION

Livestock production, one of the major components of Nepalese mixed farming system contribute 31% in the national Agricultural Gross Domestic Product (AGDP)<sup>1</sup>. Buffalo milk production contributes nearly 72% (1,031,500 MT) and cattle milk contributes nearly 28% (413,919 MT) in the fiscal year 2008/09. Total milk production in Lamjung district in the year 2008/09 was 13099 MT with 3519 MT cow milk and 9580 MT buffalo milk<sup>2</sup>. The total number of milking cattle and buffalo in Lamjung district were 7,991 and 19,969 in the fiscal year 2009/10 as described by report District livestock Service Office, Lamjung<sup>3</sup>. Mastitis is one among the top three threats faced by the farmers in terms of economic loss, our cattle and buffaloes could definitely not stay apart. Traditional farming knowledge has further made the conception of this disease narrow and eventually the losses go unnoticed. Approximately 10% of total value of milk sales is lost each year as a result of decreased milk production, increased milk replacement cost, discarded milk, drug costs, veterinary fees and labor costs. The cost of clinical mastitis has also been estimated to be \$107 US per clinical episode with over 70% of the cost associated with decreased milk production and milk withheld from the market, over 20% with drugs, veterinary costs and replacement costs, and the remainder with labor<sup>4</sup>. In Nepal, according to Ng et al<sup>5</sup> largest proportion of losses in milk results from decreased milk production i.e., Rs. 4287 or USD 63 per buffalo per lactation. Milk loss was found 11% of average total lactation yield. Expenditure in medicine accounts 34% of the total treatment and management cost.

In most countries, surveys in dairy herds indicate that prevalence of infection of mastitis pathogens is about 50% of cows and quarter infection rate of about 25%. The average annual incidence rate of clinical mastitis, calculated as the number of clinical quarter cases per 100 cows at risk per year including the dry period in individual herds ranges from 10-12% in most herds but higher values, ranging from 16-65% occur in some herd<sup>6</sup>. Dhakal et al<sup>7</sup> reported the maximum numbers (16%) of clinical mastitis in July, when temperature and humidity were highest. Coagulase negative *Staphylococci* (CNS) such as *Staphylococcus albus* and *S. epidermidis* were the predominant organisms associated with subclinical mastitis. However, CNS and *Coliforms* are predominant in clinical mastitis.

Animals raised by the farmers are facing constant risk due to poor management practices, lack of

risk due to poor management practices, lack of proper nutrition, disease outbreaks and several other factors. So, animal health and proper management practices are the most important components for rearing the livestock. The feeding system in Nepal differs from region to region. Rice straw is the main crop by-product fed during winter and maize stovers during the rainy season. Lactating animals are given about 1.5 kg of homemade concentrate feed daily during the lactation period. Commercial feed was only purchased by those farmers who are rearing improved breeds<sup>8</sup>. Thus this study primarily focuses on mastitis and related risk factors. The objective of this study is to assess the prevalence of sub clinical mastitis (SCM) in dairy cattle and buffaloes, quarter wise distribution of SCM, isolation, identification of common mastitis pathogens and their associated risk factors like management, lactation stage, and milk production per lactation.

### MATERIAL AND METHODS

#### Study Site

This study was conducted in Archalbot and Chandreshwor VDCs of Lamjung district, which are pocket area of milk production. The Laboratory analysis of the collected milk samples was carried out at Bacteriology unit of the National Avian Laboratory (NAL) Bharatpur and Veterinary Teaching Hospital (VTH), Chitwan.

#### Questionnaire Survey

To assess the management aspects and its possible risks on SCM, questionnaire survey was conducted with each farmer. Individual cattle & buffalo from farmers' shed were selected and other relevant information was recorded.

#### Sampling Technique and Laboratory Examination

Samples were collected from the dairy animals. Samples representing all the wards of Archalbot and Chandreshwor V.D.Cs were collected. Out of them 30 animals from each V.D.Cs was selected using purposive random sampling method. About 10 ml of milk sample from each quarter was collected in a sterilized syringe. They were numbered and marked as left front (LF), left hind (LH), right front (RF) and right hind (RH) respectively. The diagnostic tools used for mastitis were California Mastitis Test (CMT), Cultural Examination and Biochemical Tests. All the samples were subjected to cultural

examination on Nutrient agar, Mac Conkey agar plate & EMB media. They were incubated at 37°C for 24 hours. Cultural isolates were identified on the basis of colony characteristics, Gram's staining and biochemical tests as Oxidase, Catalase, IMViC test and Motility test mentioned by Quinn *et al*<sup>9</sup>.

**Table 1.** Biochemical test used for identification after culture

Bacterial isolates	Oxidase	Catalase	IMViC test	Motility test
<i>E. coli</i>	-	+	++--	+
<i>Klebsiella</i> spp	-	+	----	+
<i>Staphylococcus</i> spp	-	+	----	-
<i>Streptococcus</i> spp	-	-	----	-

IMViC: Indole, Methyl red, Voges-Proskaur, Citrate; (-) = Negative; (+) = Positive

## RESULT

During this study period, out of 63 animals 46 were in early lactation and 17 were in late lactation period. Among them, 8 were of first parity, 16 of second parity and 39 were belonged to third parity or more. Most of the animals were found to be stall fed. Only 14% were found on grazing system and rest 86% were on zero grazing system. In total, only 5% of the animals were supplied with regular minerals and vitamins, 24% were with the occasional supplementation but rest 71% had never been provided with any kind of vitamins and minerals. Of the total, only 30% (n=19) had fodder trees in their land. Those with fodder trees didn't suffer scarcity of feed during winter but those without were found suffered. All the household were acknowledged with regular deworming of their animals in every 6 month. The average milk production per lactation was found 3.99± 0.18 lt with highest frequency for 4 liters per lactation. Less numbers of farmers did have knowledge on mastitis and more (71.4%) were found without any knowledge. Regarding SCM, table 2 describes the prevalence.

The research showed that prevalence rate was similar in almost all the quarters. The prevalence was found highest in left fore which was followed left hind, right hind and right fore. The geographic and quarter wise distribution of mastitis is

demonstrated below in figures.

The prevalence of subclinical mastitis was found higher in Chandeshwor (34.5%) compared to that of Archalbot (23.5%). The result of bacterial isolates showed that the highest prevalence was of Streptococcus (42.6%) followed by *E. coli* (32.7%) and Staphylococcus (24.6%) on quarter basis. The SCM in animal basis in different location is mentioned in the Table 4.

## Relation between prevalence of sub clinical mastitis and amount of milk production

Prevalence of Subclinical mastitis was significantly higher (73%) among the dairy animals producing less than 3 litres compared to dairy animals producing 3 or more than 3 litres (7.7%). The pearson's chi-square value 29.194 gives p value less than 0.05 that signifies for high yielding animals (>3 lts) are more susceptible to be infected with mastitis.

## Relation between prevalence of sub clinical mastitis and stage of lactation

Prevalence of subclinical mastitis was higher among the dairy animals in late lactation (58.8%) compared to dairy animals in early lactation (41.3%). The chi square test ( $\chi^2 = 1.54$ , i.e.  $p = 0.216 < 0.05$ ) showed the association between the stage of lactation and prevalence of mastitis is non-significant. This means that the stage of lactation could not be determining factor for mastitis in Lamjung.

## DISCUSSION

The prevalence of SCM in cattle and buffaloes of Lamjung district was 28.6% and 24.2% on animal basis and quarter basis respectively during cultural examination. The result obtained by Dhakal *et al.* (2002) showed the prevalence rate of SCM to be 56% of cattle with 35% of the quarters and 44% of buffaloes with 27% of the quarters which is far greater than the present result on animal basis but similar on quarter basis. Thapa<sup>10</sup> had reported the prevalence rate to be 72% by cultural examination in Gitanagar VDC of Chitwan which was far greater than our result. Upadhayaya<sup>11</sup> reported the prevalence rate of SCM to be 48.3% quarter and 68.3% cattle

**Table 2.** Prevalence of subclinical mastitis in dairy animals of Lamjung district

CMT Result	Number of animals(63)			Number of quarters(252)		
	Archalbot	Chandreshwor	Total	Archalbot	Chandreshwor	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Negative	19(55.8)	15(51.7)	34(53.9)	81(69.8)	95(69.8)	176(69.8)
Positive	15(44.1)	14(48.3)	29(46.1)	35(30.2)	41(30.2)	76(30.2)
Total	34	29	63	116	136	252

**Table 3.** Quarterwise distribution of mastitis based on CMT

Location Teat	Archalbot		Chandeswor		Total
	Positive	Negative	Positive	Negative	Prevalence n (%)
Left fore	10	24	12	17	22 (34.9)
Left hind	8	26	12	17	20 (31.8)
Right fore	8	26	8	11	16 (25.4)
Right hind	9	25	9	20	18 (28.6)
Total	35	121	41	65	76 (30.2)

**Table 4.** Distribution of different types of subclinical mastitis on animal and quarter basis

Culture Report	Number of animals (63)			Number of quarters (252)		
	Archalbot	Chandreswor	Total	Archalbot	Chandreswor	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Mastitis negative	26(76.5)	19(65.5)	45(71.4)	112(82.3)	79(68.1)	191(75.8)
Coliform mastitis	2(5.8)	4(13.8)	6(9.5)	5 (3.7)	15(12.9)	20(7.9)
Staphylococcus mastitis	4(11.9)	1(3.5)	5(7.9)	11 (8.1)	4(3.5)	15(5.9)
Streptococcus mastitis	2(5.8)	5(17.2)	7(11.1)	8 (5.9)	18(15.5)	26(10.3)

in Kathmandu district which was also higher than our result. However, Adhikari<sup>12</sup> reported the prevalence rate of SCM to be in 40% cattle and 32% buffaloes which was similar to our findings. On the other hand, CMT report shows that the prevalence rate of SCM in Lamjung was 46.1% on animal basis and 30.2% on quarter basis. Khakural<sup>13</sup> reported the prevalence rate of Kathmandu valley to be 17.2% on CMT basis which was quite less than our findings. However, our finding was nearby to the findings of Thapa<sup>10</sup> who got it to be 36% in Gitanagar VDC.

In our study, we found that left fore quarters (26%) and right hind quarters (26%) had the highest incidence followed by left hind quarters (25%) and then right fore quarters (23%). This result was quite contrasting to the result obtained by Dhakal *et al*<sup>14</sup> who found the incidence to be highest in left hind quarters (34.2%) followed by right hind quarters (31.6%). Our finding was in contrast with that of Jha *et al* who found that right fore quarters had highest incidence (33.9%) followed by left fore quarter (28.6%).<sup>15</sup> However, Thilager *et al* found highest incidence in the left fore quarters<sup>16</sup> which was similar to our result. Our finding was also

contrast with that of Upadhyaya<sup>11</sup> who found the incidence to be highest in right fore quarters followed by left hind quarters.

In our research study, we got that *Streptococcus* as the most predominant pathogens with 11.1% prevalence rate followed by *Coliform* (9.5%) and then *Staphylococcus* (7.9%). However, *Coliform* mastitis was the most frequent type followed by *Staphylococcal* mastitis in Chitwan in the study of Dhakal and Subedi<sup>17</sup> and Adhikari *et al*.<sup>12</sup> Similarly, Balakrishnan *et al*<sup>18</sup> and Dhote *et al*<sup>19</sup> reported *Staphylococcus* spp. being the most predominant pathogens followed by *Coliform*. Our result was contrast with the findings of Kateete *et al*<sup>20</sup> from Uganda where the major pathogen for mastitis was *Srrophylococcus* spp. Dhakal and Nagahata<sup>21</sup> from Nepal also reported *Staphylococcal* mastitis as most predominant which was also different from our findings. Upadhyaya also reported that *Staphylococcal* mastitis had the highest prevalence rate in Kathmandu district followed by *E. coli*, mixed infection and then *Streptococcus* spp. and Adhikari *et al*<sup>12</sup> found *Coliforms* most prevalent followed by *Staphylococcus* spp., *Streptococcus* spp. and *Pseudomonas* spp. However, in Archalbot VDC,

*Staphylococcus* spp. was predominant which is similar to the findings of various researchers.

Prevalence of SCM was higher in late lactation period (58.5%) compared to those in early lactation (41.3%) which contrast to the view of Radostits *et al.* Similarly, the prevalence was higher in the high milk yielding animals (73%) compared to low milk yielding animals (7.7%) which is similar to the view of Radostits *et al.*<sup>22</sup>

In the research site, 71% of the dairy animals were of exotic breed, which is quite favourable condition for increase in milk production in Lamjung district. Similarly, all the respondents were involved in agriculture and all were literate. So, there could have ample opportunity for commercialization in dairy farming. We found that 86% of the animals were stall fed and 56% were provided with both roughages and concentrate in balance manner while remaining were provided with dry straw only which was quite similar to the finding of Upadhyaya.<sup>11</sup> Rice straw was the main crop by-product fed during winter and maize stovers during the rainy season. Lactating animals were given homemade concentrate feed daily during the lactation period. Commercial feed was only purchased by those farmers who are rearing improved breeds. This finding was similar to the finding of Tulachan *et al.*<sup>9</sup> The milking and pregnant animals were fed Khole (liquid concentrate) in the morning and day time with very limited amount of green fodder during dry season and this was similar to the finding of Regmi *et al.*<sup>24</sup> Very few animals were provided with regular supplementation of minerals and vitamins. Which might be an obstacle for increase in milk production. Only 30% of the farmers had forage trees in their land which resulted that most of the farmers had scarcity of green grass in winter similar as the finding of Regmi *et al.*<sup>24</sup> Almost all of the animals were dewormed regularly which was far better situation than that of Kathmandu district as reported by Upadhyaya.<sup>11</sup>

## CONCLUSION

The dairy livestock of Lamjung were suffering a large prevalence of subclinical mastitis going unnoticed and very fewer farmers were aware of it. The streptococcal mastitis was predominant for the infectious cause followed by coliform and staphylococcal ones and the left quarters were the most infected one. The average milk production of dairy livestock was around three liters per lactation. The prevalence was higher in dairy animals producing more than three liters per lactation. Sub

clinical mastitis was found higher in late lactation stage compared to early lactation. The dairy animal management was poor. Farmers were following traditional methods of nutrition management and could not avoid the green forage scarcity at lean period. Nutrition management assessment in dairy livestock could an important part of future research. The farmers should be inspired for mastitis management, udder health management, shed management and nutrition management through training, workshops, tours, farmers' schools for hygienic milk production in commercial scale.

**CONFLICT OF INTEREST:** None to declare.

**FINANCIAL INTEREST:** None to declare.

## REFERENCES

1. Central bureau of statistics (CBS). Estimated Livestock Population and their Products (2007/08). CBS, Kathmandu, Nepal [online]. c2009. Available from: [www.cbs.gov.np/year-book-2009/images/final-chapters/chapter 2/2.10.pdf](http://www.cbs.gov.np/year-book-2009/images/final-chapters/chapter%202/2.10.pdf). Accessed November 2011.
2. Ministry of Agriculture and cooperatives (MOAC). Statistical year book of Nepal, 2010. MOAC, Kathmandu, Nepal. 2010. Available from: [www.moac.gov.np/content.php?id=234](http://www.moac.gov.np/content.php?id=234). Accessed October 2011.
3. Annual Progress Report, 2066/067. District Livestock Service Office (DLSO), Lamjung. 2010.
4. DeGraves FJ, Fetrow L. Economics of mastitis and mastitis control. *Vet Clin North Am Food Anim Pract.* 1993;9:421-434.
5. Ng L, Jost C, Robyn M, *et al.* Impact of livestock hygiene education programs on mastitis in smallholder water buffalo (*Bubalus bubalis*) in Chitwan, Nepal. *J Prev Vet Med* 2010;96: 179-185.
6. Radostits OM, Gay CC, Hinchcliff KW and Constable, PD. *Veterinary Medicine. A Textbook of the disease of cattle, sheep, pigs, goats and horses.* 10<sup>th</sup> ed. Elsevier, London. 2010.
7. Dhakal IP, Dhakal P, Koshihara T, *et al.* Epidemiological and bacteriological survey of buffalo mastitis in Nepal. *J Vet Med Sci* 2007;69:1241-1245.
8. Tulachan, PM, Jabbar MA, Saleem MA. Smallholder Dairy in Mixed Farming Systems of the Hindu Kush-Himalayas Issues and Prospects for Development. International Centre for Integrated Mountain Development (ICIMOD), Kathmandu, Nepal. 2002.
9. Quinn PJ, Carter ME, Markey B and Carter GR. *Clinical Veterinary Microbiology.* Mosby international, London. 1998.
10. Thapa P. Prevalence of subclinical mastitis in bovines in Gitanagar V.D.C. of Chitwan District, Nepal. B.V.Sc & 5555A.H. Internship report submitted to Institute of Agriculture and Animal Science, Rampur, Chitwan, Nepal. 2006.

## Subclinical mastitis in live stock in Nepal

11. Upadhyaya BP. Prevalence of subclinical mastitis in dairy cattle in Nayapati and Balambu VDCs of Kathmandu district, Nepal. B.V.Sc & A.H. Internship report submitted to Institute of Agriculture and Animal Science, Rampur, Chitwan, Nepal. 2008.
12. Adhikari CK and Dhakal IP. Prevalence of subclinical mastitis in cattle and buffaloes at different VDCs of Chitwan district, Nepal. B. V.Sc & A.H. Internship report (unpublished) submitted to Institute of Agriculture and Animal Science, Rampur, Chitwan, Nepal. 2009.
13. Khakural GP. Study on prevalence of subclinical mastitis in Kathmandu Valley. Proceedings of 1st National Livestock/ Fisheries Research Workshop 1996. 1996.
14. Dhakal IP, Neupane M, Nagahata H. Evaluation of direct and indirect measures of quarter milk from crossbred buffaloes. *Animal Sci J* 2008;79:628-633.
15. Jha VC, Thakur RP, Rai LB. Epidemiological investigation of subclinical bovine mastitis in the western hill of Nepal. *Vet Rev* 1993;8:35-39.
16. Thilagar S and Mohammed D. A clinical survey of bovine teat and udder lesion. *Indian Vet J* 1992;69:645-46.
17. Dhakal IP, Subedi K. Clinical Mastitis in different breeds of cattle and buffaloes at Chitwan, Nepal. *Journal of Institute of Agriculture and Animal Science*. 2002; 23: 65-69.
18. Balakrishnan G, Madhavan U, Dorairajan N, et al. Mastitis in cattle of India. *Indian Vet J* 2004;81:98-99.
19. Dhote SW, Kurkure NV, Kalorey DR, et al. Etiology and sensitivity of bacterial isolates from subclinical mastitis in cows from east Vidarbha. *Indian Veterinary Journal*. 1999;76:75-76.
20. Kateete DP, Kabugo U, Baluku H, et al. Prevalence and antimicrobial susceptibility patterns of bacteria from milkmen and cows with clinical mastitis in and around Kampala, Uganda. *PLoS, One*. [online]. 2013;8:e68413.
21. Dhakal IP, Nagahata H. Evaluation of California mastitis test and quarter somatic cell count estimation in buffalo milk. In: *The Blue Cross*. 2007;9:1-4.
22. Radostits OM, Gay CC, Hinchcliff KW, et al. *Veterinary Medicine. A Textbook of the disease of cattle, sheep, pigs, goats and horses*. 7<sup>th</sup> ed.. Elsevier, London, 2006.
23. Regmi, PR, Shrestha HK, Rayamajhi N, et al. Situation of dairy farming in Dhankuta. *The Blue Cross* 1999;3: 27-35.

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