Risk Factors Associated With Bovine Mastitis Prevalence, Case Study of Nyabihu and Musanze Districts

M.F. Mwabonimana[1], C. Hakorimana[2], P. Nabayo[3], J. P. Tuyishime[4]

[1]Department of Veterinary Medicine, School of Animal Sciences and Veterinary Medicine, University of Rwanda/ CAVM/ busogo- campus, p.o. Box, 210, musanze, Rwanda

[2,3,4]Department of Animal Production, School of Animal Sciences and Veterinary Medicine, University of Rwanda/ CAVM/ busogo- campus, p.o. Box, 210, musanze, Rwanda

Abstract: A cross-sectional study was conducted in Musanze and Nyabihu districts, Rwanda, 2015 on lactating dairy cows to determine the overall prevalence of bovine mastitis and identify associated risk factors. A total of 30 lactating cows were examined for mastitis using clinical examination and California mastitis test (CMT). An overall 60% prevalence of mastitis was recorded in the study area. Direct observations, standardized monitoring forms and interviews were used to identify mastitis risk factors. The prevalence of mastitis varied insignificantly (p>0.05) among milk yield, where the prevalence was recorded 50% at low (1-5 liter/day) level of milk production in lactating cows and an increase of prevalence of bovine mastitis was observed at medium (6-10 liter/day) level with 61.11% and at high (> 10liter/day) level with 75%. It was also appreciated that the udder cleanless and teat predip was insignificantly (p>0.05) influenced the occurrence of mastitis. Dairy cows with Poor Udder cleanless (80 %) were severely affected followed by medium Udder cleanless (50%) and Good Udder cleanless (33.33%) and that of teat pre dip with mastitis was highly recorded in cows with Unsatisfactory teat pre dipping (57.8%) than in cows with Satisfactory teat predipping (63.6%). The teat post dip and milking were found as important risk factors to significantly (p< 0.05) influenced the occurrence of mastitis. From this significance, prevalance of bovine mastitis and teat post dip was recorded high in cows with Unsatisfactory teat post dipping (83.33%) than in cows with Satisfactory teat post dipping (41.66%) and prevalence of bovine mastitis increases with unsatisfactory milking with 76.19% as a part of milk became a favorable medium for microorganisms growth and decreases when milking done completely with 33.33%.

Keywords: Bovine mastitis, CMT, prevalence, risk factors.

I. INTRODUCTION

Mastitis remains the most economically damaging disease for dairy subsector in our country as well as all over the world; it imposes economic losses through reduction in milk production (Korhonen *et al*, 1995); it affects animal health, early culling of diseased animals, cost of veterinary care and it has also public health importance by serving as a vehicle in the spread of diseases like tuberculosis, staphylococcal food poising and brucellosis (Radostits *et al,* 2007). Regarding these losses, different developed countries shown their interest and initiative in contributing in fighting and prevention of mastitis in dairy cows through the use of dipping solutions during milking by empowering good milking practices.

Mastitis occurs when the udder becomes inflamed because leukocytes are released into the mammary gland in response to invasion of the teat canal, usually by bacteria. These bacteria multiply and produce toxins that cause injury to milk secreting tissue and various ducts throughout the mammary gland. Elevated leukocytes, or somatic cells, cause a reduction in milk production and alter milk composition. These changes in turn adversely affect animal health, quality of milk and economics of milk production (Sharma *et al.,* 2004). Despite all scientific progress, mastitis remains prevalent in most of

ISSN 2394-7330

International Journal of Novel Research in Healthcare and Nursing
Vol. 2, Issue 3, pp: (120-129), Month: September-December 2015, Available at: www.noveltyjournals.com

the dairy herd. Today it can be estimated that nearly half of the dairy cow and buffalo population is suffering from clinical and sub clinical mastitis (Sharma *et al.*, 2007).

The prevalence of mastitis in dairy farms of Nyabihu and Musanze district is increasing by different risk factors; what is result in farm losses due Milk thrown away due to contamination by medication or being unfit to drink, a reduction in yields due to illness and any permanent damage to udder tissue, the extra labor required to tend to mastitis cows, the costs of veterinary care and medicines and the cost of reduced longevity due to premature culling.

Recently farmers continue to operate under this situation, unfortunately not only wasting money and time but also some of them decided to leave the Dairy industry because they don´t progress. Through this study determining those different risk factors and assess contribution of each of them in order to verify if there is a link between risk factors and mastitis prevalence; what will help to ensure the control by using different management practices such using dipping solution before and after milking, complete milking,…for example; from this we expect that those losses will be minimized and dairy industry will be sustained. This study aimed at determining the risk factors associated to bovine mastitis prevalence. In the light of the above, this paper identifies the risk factors and determines the prevalence of bovine mastitis in Nyabihu and Musanze districts, Rwanda with a determination to:

- To determine mastitis prevalence

- To assess contribution of risks factors to mastitis prevalence.

- To evaluate the link between selected risk factors and the prevalence of bovine mastitis.

- To train farmers/milkers on the use of dipping solution and the best milking practices to achieve reduction of mastitis prevalence.

II.   METHODOLOGY

Materials:

A cross-sectional study was conducted in Musanze and Nyabihu districts, Rwanda, 2015 on lactating dairy cows to determine the overall prevalence of bovine mastitis and identify associated risk factors. While carrying out the present study, different materials have been used including field materials such as milk samples collection (Cooler box for samples transport, Cloth towels for drying after cleaning, Permanent Marker for sterile tubes labeling, Dipping solutions (express blue and express 3) for teat dipping, Disinfectant (Cleaning solution),Tube holder Sterile tubes with tight fitting caps and Dip cups); California Mastitis Test(CMT Paddle and CMT Solution)…and Laboratory materials

➢ Culture medium preparation: Autoclave, Bain marin, Becker, Electronic balance, spatula, Heater plates, Lamina flow and Reagents(0.5% Peptone, 0.3% beef extract/yeast extract,1.5% agar, 0.5% NaCl and Distilled water.

➢ Milk samples culturing and isolation: Milk samples, Agar plate, Gloves, Sterile cotton tipped swabs, A marker for plate labeling, Gas flame, Cotton Strip ,pipettes ,Alcohol and Incubator.

➢ Identification of bacteria and bench tests: Forms for bench tests, Plates with bacteria colonies, Petri dishes, Inoculating loops, water, Gloves, Gas flame, Drooper, Alcohol, Potassium Hydroxide for KOH Test and Hydrogen peroxide for Catalase test and Rabbit (coagulase) plasma for Coagulase test.

➢ Gramstain and results identification: Reagents (Crystal violet or violet de gentiane, Safranine, Iodine, Alcohol and Water), clean slide with prepared bacteria smear, Immersion oil and Microscope.

Methods:

Sampling methods:

Sample determination:

Target Population:

The primary data for this research were collected from 15 farms chosen randomly to represent three sites; one in **MUSANZE** (Busogo site) and two sites are located in **NYABIHU** District (Nyiragikokora and Arusha sites) with dairy cows under three months of lactation; which are healthy and no clinical mastitis to check for subclinical mastitis at farm

level and collecting information regarding selected risk factors associated with the prevalence of bovine mastitis in those 3 sites.

➢ **Sample size and Sampling methods:**

The sample size was determined based on the farmer compliance and conditions to be fulfilled with study animals such as Crossbreed lactating cows in early lactation and in state of welfare. We used stratified sampling method; the research enrolled 30 cows from 15 farmers into the DDM (Dairy dynamic management) program and followed over the course of 8 weeks. Each quarter was sampled in its own tube to get one hundred and twenty samples every week which was repeated in period of four months to get total of 960 samples, samples was collected and transported by ice boxes to laboratory.

Table 1 : Number of samples during the study period

Total Cows selected	1cow/week		30/week		30/4weeks		30/8 weeks	
30 cows	4 samples	RR:1s	**120 samples**	RR:30s	**480 samples**	RR:120s	**960 samples**	RR:320s
		LR:1s		LR:30s		LR: 120s		LR: 320s
		RF:1s		RF: 30s		RF: 120s		RF: 320s
		LF:1s		LF: 30s		LF: 120s		LF: 320s

Detection of mastitis:

Physical examination of the udder:

We first examine the udder through visually and then palpation to detect abnormalities (fibrosis, cardinal signs of inflammation...); for cows with clinical mastitis we also proceed on other examination (Rectal temperature and Auscultation) to check systemic involvement.

Preparation of udder and teats:

The udder, especially teats was cleaned and washed with tap water, dried with a dry towel and dipped using dipping solution before and after sample milk collection to avoid sample contamination and afterward entry of germs in the udder.

Sample collection, handling and storages:

Milk samples were collected by a standard milk sampling techniques (NMC, 1990). To reduce contamination of teats ends during sample collection, the near teats first followed by the far once. After discarding 3 first stream of milk, sample milk was collected into sterile test tube placed in racks for ease handling and transported in an ice (cooler box) to the laboratory and stored at 4^0C for a maximum of 24 hour until inoculated on a standard bacteriological media (Biru, 1989; NMC, 1990).

California Mastitis Teat (CMT):

CMT results was used to diagnose subclinical mastitis considering the nature of coagulation and viscosity of the mixture (milk and CMT reagent), which show the presence and severity of the infection, respectively (Harmon, 1994).

III. DATA COLLECTION

Data were collected through visits on the field. Monitoring forms were formulated and reviewed, and all information relating to the study objectives was recorded. All the collected data including milk output, milking interval, lactating period, dietary factors and hygienic condition were recorded. Also others data related to animal health such as temperature, heartbeat, lameness, status of feces and appetite was recorded to ensure that selected dairy cows still in good state of welfare and farmer compliance was also taken into account. All the data collected including type of husbandry system, age, parity udder and milk abnormalities (injuries, blindness, swelling, milk clots and other abnormal udder secretion, etc.) was recorded. Depending on clinical inspection, CMT results cases were categorized as either positive or negative; Positive cases were further categorized as clinical and subclinical mastitis. Milk yield was categorized as low (1-5liter/day), medium (6-10liter/day) and high (>10 liter/day). In Hygienic conditions; udder clean less was categorized as good, medium and poor; teat predip was categorized as Satisfactory and unsatisfactory also Post dip was categorized as

Satisfactory and unsatisfactory. Also milking was categorized as Satisfactory when done completely and unsatisfactory when cows are partially milked.

Treatment and analysis of data:

Descriptive statistics and SPSS (Statistical Package for the Social Sciences) analysis were computed to determine mastitis prevalence, association of risk factors with mastitis prevalence, and relative importance of the risk factors on mastitis prevalence, respectively.

Laboratory works: Bacteriological isolation and characterization:

Milk samples were bacteriologically examined according to the procedures employed by Quinn *et al.,* (1990). The sample milk was inoculated in blood agar based enriched with 7% defibrinated bovine blood. The inoculated plates was incubated aerobically at 37^0C for 24 to 48 hours, then after identification of the bacteria on primary culture was made on the basis of colony morphology, hemolytic characteristics, Gram stain reaction including shape and arrangements of the bacteria, catalase, KOH and Coagulase tests; Staphylococci was identified based on Coagulase test.

IV. RESULTS AND DISCUSSION

RESULTS:

The results of our study show that from all the milk samples of 30 lactating cows screened for bovine mastitis using the CMT test, 60% of cows showed positive reaction to the test, which express that they were affected with bovine mastitis.

Table 2: Prevalence of mastitis in lactating cows in Nyabihu and Musanze district as influenced by some selected risk factors

Total number of examined cows	Variable	Category level	Nº of examined cows	Infected cows from CMT	% of Infected cows from CMT	Non infected cows from CMT	% of Non-infected cows from CMT
30	**Milk Yield**	Low (1-5 liter/day)	8	4	50%	4	50%
		Medium (6-10 liter/day)	18	11	61.11%	7	38.88%
		High (>10 liter/day)	4	3	75%	1	25%
	Udder Clean less	Good	12	4	33.33%	8	66.66%
		Medium	8	4	50%	4	50%
		Poor	10	8	80%	2	20%
	Teat predip	Unsatisfactory	19	11	57.8%	8	42.2%
		Satisfactory	11	7	63.6%	4	36.4%
	Teat post dip	Unsatisfactory	18	15	83.33%	3	16.66%
		Satisfactory	12	5	41.66%	7	58.33%
	Milking	Unsatisfactory	21	16	76.19%	5	23.8%
		Satisfactory	9	3	33.33%	6	66.6%

The table 3 above show the risk factors selected for calculation of the overall prevalence of mastitis in the study area. Those risk factors are: milk yield, udder cleanless, teat predip, teat post dip and milking. The results for CMT test show that the percentage of cows with mastitis infection is 60% and overall percentage of cows without mastitis infection CMT results account is 40%.
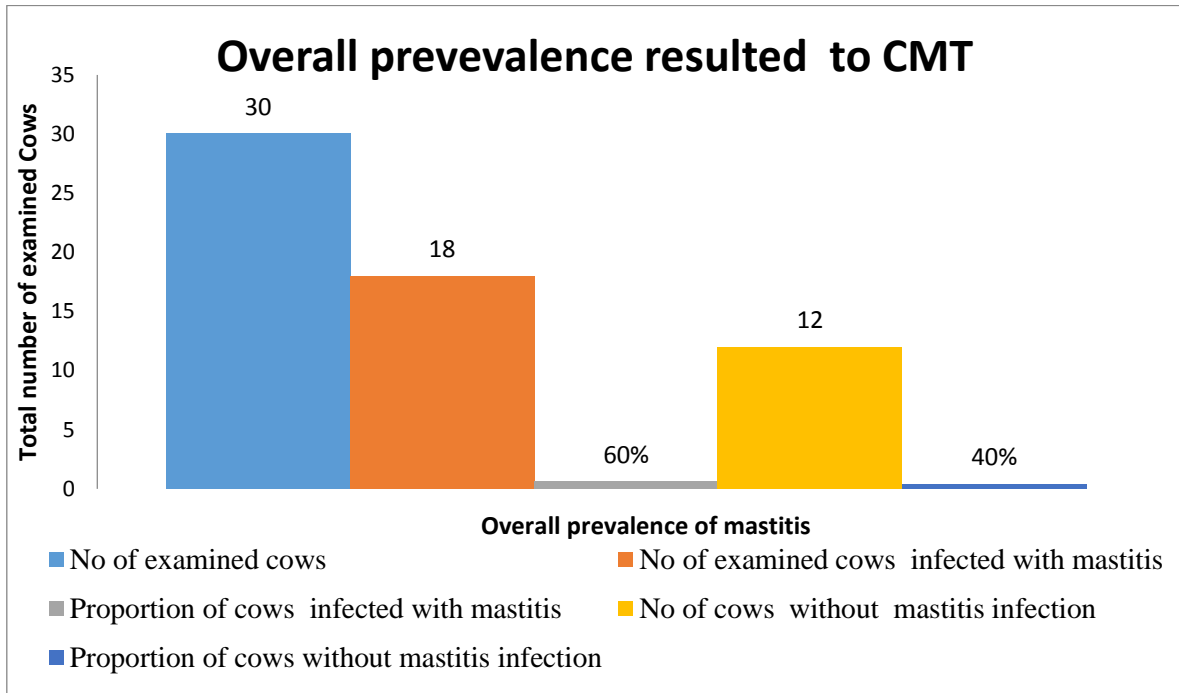
ISSN 2394-7330

International Journal of Novel Research in Healthcare and Nursing
Vol. 2, Issue 3, pp: (120-129), Month: September-December 2015, Available at: www.noveltyjournals.com

Figure 1: CMT Results with the number of examined cows

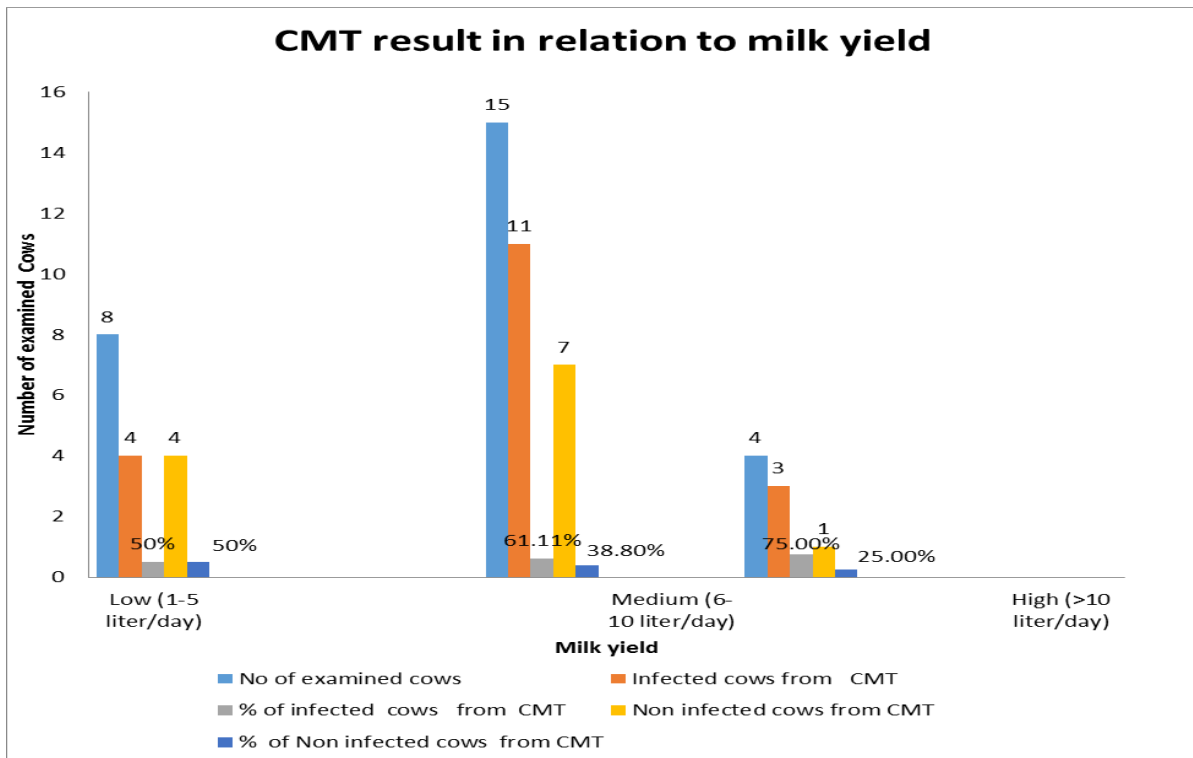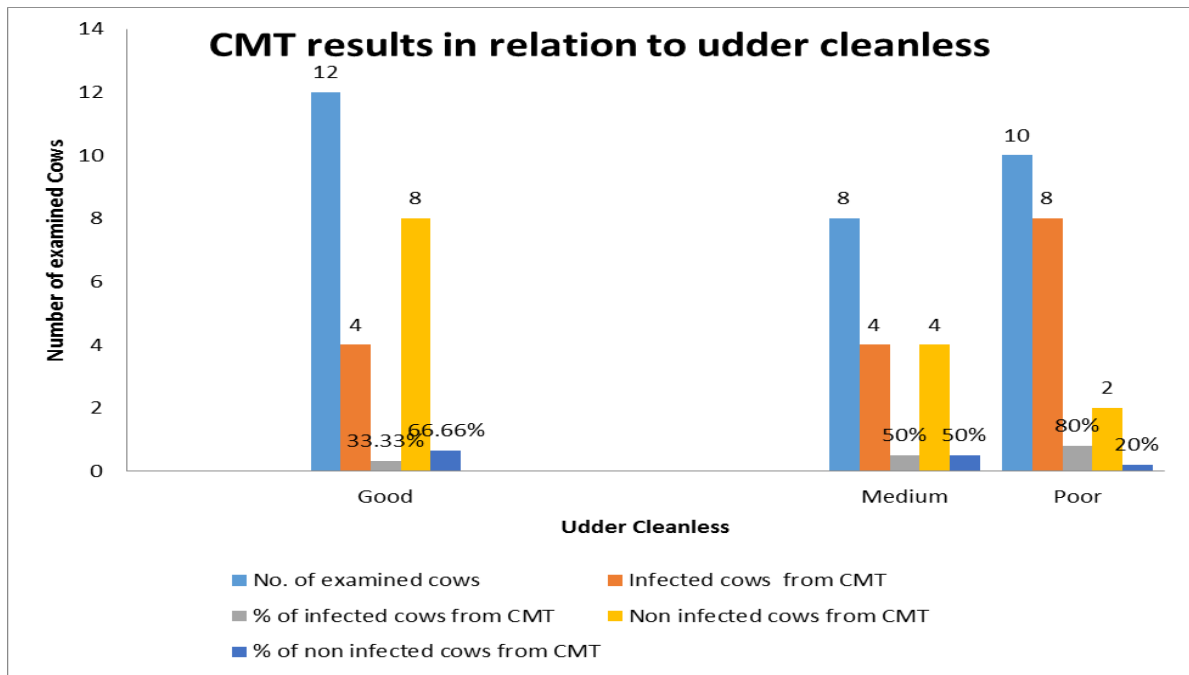**Prevalence of mastitis in relation to Milk yield:**

Figure. 2: CMT result in relation to milk yield

The figure no.5 show that the prevalence of bovine mastitis increases with milk yield. the prevalence was 50% at low(1-5 liter/day) level of milk production in lactating cows and an increase of prevalence of bovine mastitis was observed at medium(6-10 liter/day) level with 61.11% and at high (> 10liter/day) level with 75%. However milk yield became insignificant (p > 0.05) when tested with SPSS.

Prevalence of mastitis in relation to Udder Cleanless:

Figure 3: CMT results in relation to udder cleanless

The figure no.6 reveal that the association of mastitis occurrence with udder Cleanless in the group of lactating cows was illustrated that the high prevalence (80%) of bovine mastitis was recorded at the poor hygienic conditions, followed by those having medium/moderate hygienic conditions (50%) and low prevelance observed from the group with good hygienic conditions of the udder(33.3%). Bovine mastitis and udder cleanless was insignificantly (p>0.05) associated.
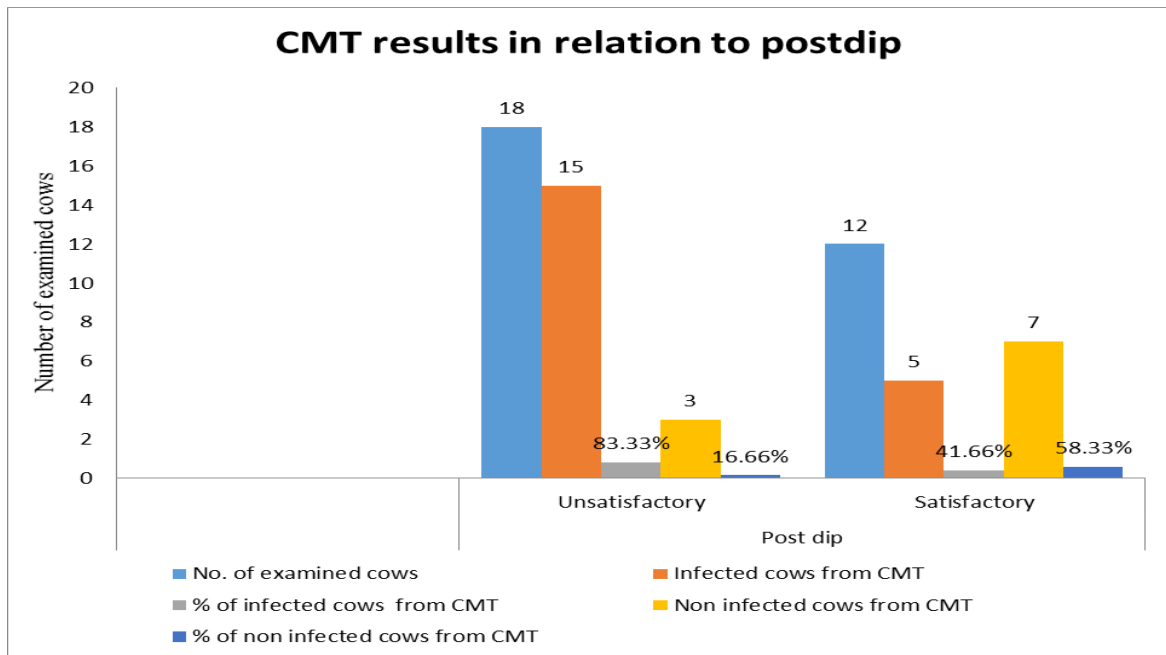
Prevalence of mastitis in relation to teat Predip:
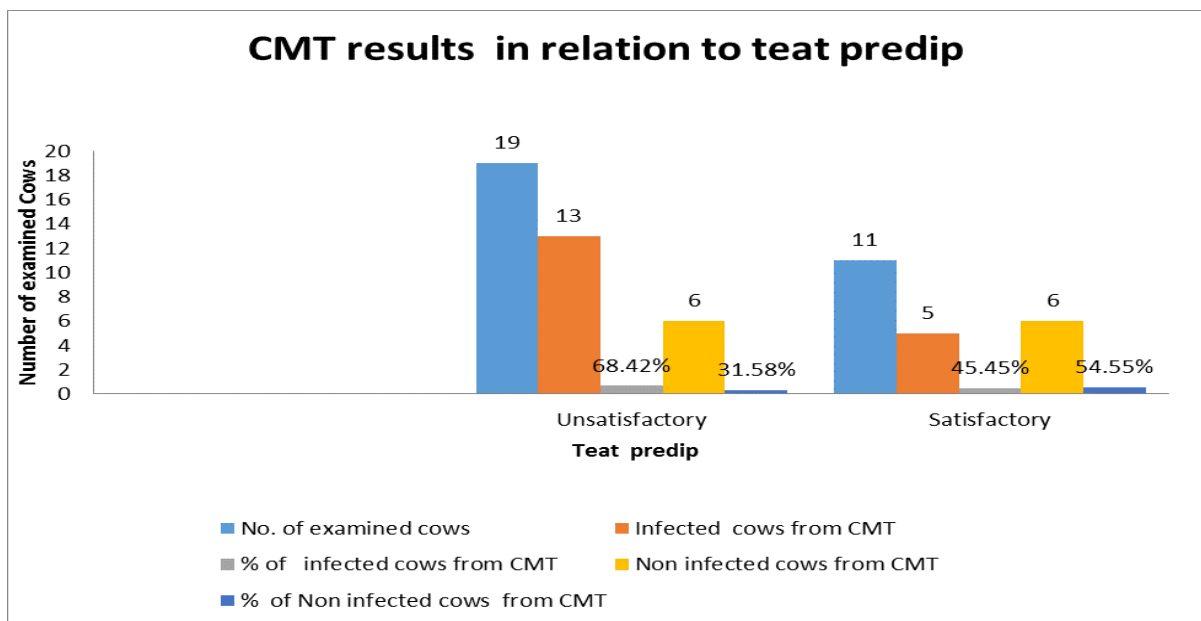
Figure 4: CMT results in relation to teat predip

The result in figure 7 show that the occurrence of bovine mastitis and teat pre dip was highly recorded in cows with Unsatisfactory teat pre dipping (57.8%) than in cows with Satisfactory teat predipping (63.6%). However teat predip became insignificant (p > 0.05) when tested with SPSS.

Page | 125

Prevalence of mastitis in relation to teat Post dip:

Figure 5: CMT results in relation to post dip

The result in the figure 8 reveal that the occurrence of bovine mastitis and teat post dip was recorded at high rate in cows with Unsatisfactory teat post dipping (83.33%) compared to cows with Satisfactory teat post dipping (41.66%). Bovine mastitis and teat post dip was significantly ($p<0.05$) associated.

Prevalence of mastitis in relation to Mlking:
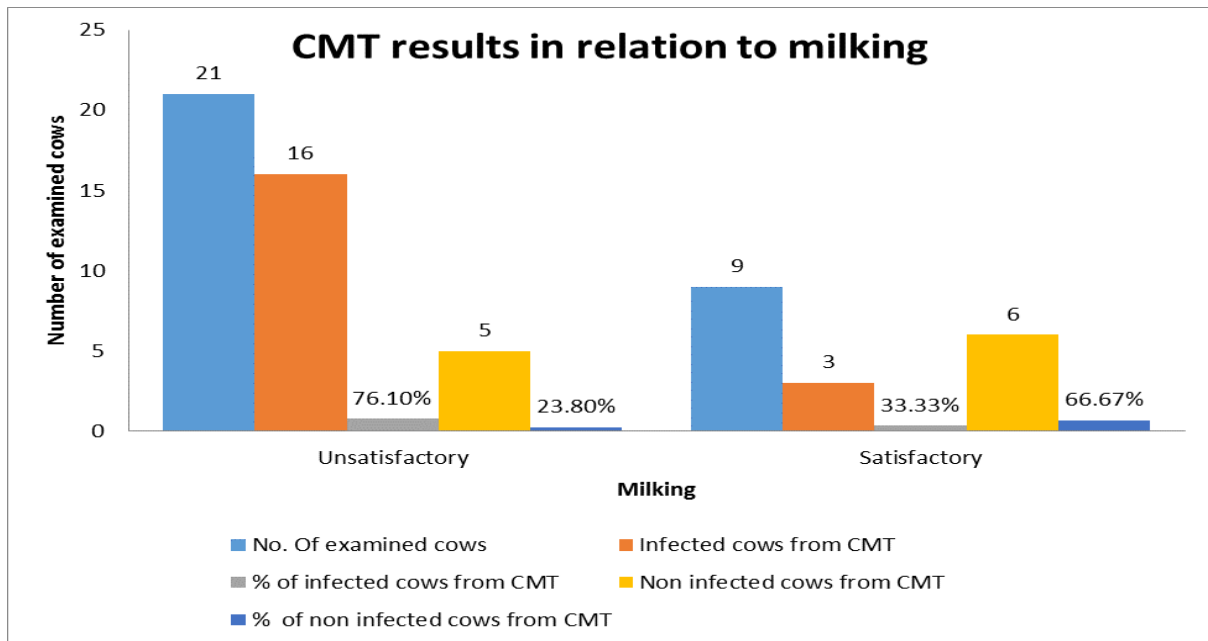
Figure 6: CMT results in relation to milking

The above figure 9 indicates the prevalence of mastitis in relation to milking during the period of study. It shows us that the prevalence of bovine mastitis increases with unsatisfactory milking with 76.19% as a part of milk became a favorable medium for microorganisms growth and decreases when milking done completely with 33.33%. Bovine mastitis and milking was significantly ($p<0.05$) associated.

ISSN 2394-7330

International Journal of Novel Research in Healthcare and Nursing
Vol. 2, Issue 3, pp: (120-129), Month: September-December 2015, Available at: www.noveltyjournals.com

DISCUSSION:

The results of this study revealed an overall prevalence of 60% in the study area. This result agrees with the findings by Mdegela *et al.,* (2004), who recorded an overall prevalence of 61.2% in small holder dairy and pastoral cattle herd in the urban and peri-urban areas of the Dodoma municipality in central Tanzania. Also who find the 71.0% of prevalence of mastitis at cow level in Dairy Farms of Holeta Town, Central Ethiopia (76/107), out of which 22.4% (24/107) and 48.6% (52/107) were clinical and subclinical, respectively. This results accompanied with Laboratory analysis through KOH, Catalase test ,coagulase test and Gram stain then results showed that Staph Spp were major infectious agent with 96.51%. Other studies elsewhere in Africa also reported Staphylococcus Spp as the most frequently major causative agents isolated. For example, Mekibib *et al.,* (2010) and Ahmed *et al.,* (2009) found that the most frequently major causative agents isolated were Staphylococcus aureus, Streptococcus agalactiae and Escherichia coli from the positive CMT samples in Ethiopia and Egypt respectively.

The current finding of the study is slightly higher than 39.5 reported by Ntampaka (2010) at Masaka dairy farms and 45.3% and it is close to that of Habimana *et al.,* (2014) who worked on risk factors of mastitis in Musanze district. However, it was higher than the works of Sori *et al.* (2005), and lower than those of Lakew *et al.* (2009) and Mekibeb *et al.* (2010) who reported mastitis with prevalence of 52.78 in and around Sebeta, 65.6 and 71.0% in their respective studies. The observed difference in the prevalence of mastitis among these studies could be due to difference in managemental system (Almaw *et al.,* 2008).

Management of the herd and hygienic milking are considered important risk factors for Sub-clinical mastitis (Kivaria *et al.*, 2004; Sarkar *et al.,* 2013). The result of the present study highlights the difference in prevalence associated with: milk yield, udder clean less, pre dip, post dip and milking. The prevalence of bovine mastitis was high (75%) in cows producing > 10liter/day, medium (61.11%) in cows producing 6-10 liter/day and low (50%) in cows producing 1-5 liter/day). These results agreed with the reports of Kader *et al.,* (2003) who reported a high prevalence in high yielding cows than low to medium yielders. However, from SPSS analysis the present result became insignificant. The occurrence of bovine mastitis and udder cleanless was insignificantly (p>0.05) associated. The prevalence of bovine mastitis was highest in Poor Udder cleanless (80 %) followed by medium Udder cleanless (50%) and Good Udder cleanless (33.33%). Bovine mastitis and udder cleanless was insignificantly (p>0.05) associated. The high prevalence of bovine mastitis was attributed with unsatisfactory teat predip high (68.42%) and satisfactory was (45.45%). However bovine mastitis and teat predip was insignificantly (p>0.05) associated. This attribution of high (83.33%) prevalence to unsatisfactory was been also remarked in satisfactory teat post dip (41.66%) but in contrast Bovine mastitis and teat post dip was significantly (p<0.05) associated. The present study agrees with Lakew *et al.,* (2009) and Sori *et al.,* (2005) who reported that the cows at farms with poor milking hygiene standard are severely affected than those with good milking hygiene practices. This might be due to absence of udder washing, pre dip/post dip, milking of cows with common milkers and using of common clothes and milking equipment, which could be vectors of spread especially for contagious mastitis. In contrast to over milking, which may increase the risk of intramammary infection, satisfactory milking in this present study was attributed to low (33.33%) prevalence than unsatisfactory (76.10%) milking.

V. CONCLUSION AND RECOMMENDATIONS

Mastitis is still the main problem as reduced milk production quantitatively and qualitatively, and affects public health through a number of milk borne infections like tuberculosis, brucellosis in humans. This study attempted to determine the prevalence and risk factors of bovine mastitis in dairy cows of Musanze and Nyabihu district. It study revealed considerable prevalence of mastitis with the association of selected risk factors such as milk yield, udder clean less, teat predip, post dip and pathogenic factors in Nyabihu and Musanze districts. Inadequate hygienic condition trough poor udder clean less and unsatisfactory teat dipping of dairy cows, unsatisfactory milking procedure, poor animal health service and lack of proper attention to health of the mammary gland were important for the high prevalence of mastitis factors in the study area. The results of this study reveal the existence of bovine mastitis within 18 out of 30 cows involved. The overall prevalence registered was 60% in the study area accompanied with Laboratory analysis through KOH, Catalase test ,coagulase test and Gram stain then results showed that Staph Spp were major infectious agent with 96.51%. The California Mastitis Test (CMT) shown that there is a considerable association between some risk factors and the occurrence of bovine mastitis in the study area. Generally, the present study revealed that

mastitis is an important problem and a serious threat for dairy industry in the study area. Therefore, the farmers should be aware of the impact of the disease and adopt good milking practices by using teat dipping solutions and establish adequate farm management system.

REFERENCES

[1] Adequate housing with proper sanitation and regular Gonzalo, C.; Ariznabarrta, A. and Carriedo, J.A. (2002): Mammary pathogens and their relationship to somatic cell count and milk yield losses in dairy ewes. J. Dairy Sci. 85:1460.

[2] Ahmed Abdel-Rady and Mohammed Sayed *(2009). Epidemiological Studies on Subclinical Mastitis in Dairy cows in Assiut Governorate. Veterinary World, Vol.2 (10):373-380 www.veterinaryworld.org.

[3] Almaw G., Molla W., Melaku A. (2009): Prevalence of bovine subclinical mastitis in Gondar town and surrounding areas, Ethiopia. Live Res. Rural Dev. 2:7.

[4] Biru, G. (1989): Major bacteria causing bovine mastitis the mammary gland were impor tant for the high and their sensitivity to common antibiotics. Eth. J. Agri. prevalence of mastitis factors in the study farms.Sci., 11: 4-6.

[5] Habimana J.B *et al., (*2014): "The study of prevalence and its association with selected risk factors of bovine mastitis in Musanze district."

[6] Hamann J., Burvenich, C., Mayntz, M., Osteras, O. and Halder, W. (1994). Machine-induced changes in the status of the bovine teat tissue with respect to new infection risk. In ―Teat Tissue Reactions to Machine Milking and New Infection Risk‖. Chapt 2 of IDF Bulletin 297, International Dairy Federation, Brussels, Belgium.

[7] Kader MA, Samad MA & Saha S (2003), Influence of host level factors on prevalence and economics of sub-clinical mastitis in dairy cows in Bangladesh. Indian Journal of Dairy Science 56, 235-240.

[8] Kivaria FM, NoordhuizenJPTM, Kapaga AM 2004: Risk factors associated with subclinical mastitisinsmall holder dairy cows in Tanzania. Tropical Animal Health and Production 36581-592.

[9] Korhonen H. and L. Kaartinen (1995). Changes in the composition of milk induced by mastits. In: The bovine udder and mastitis. Sandholm, M., T. Honkanen-Buzalski, L. Kaartinen and S. Pyörälä (eds.), University of Helsinki, Finland. 76-82 p

[10] Lakew M., Tolosa T., Tigre W., (2009): Prevalence and major bacterial causes of bovine mastitis in Asella, South Eastern Ethiopia. Trop. Anim. Health Prod. 41:1525-1530.

[11] Mdegela R H, Kusiluka L J M, Kapaga A M, Karimuribo E D, Turuka F M, Bundala A, Kivaria F, Kabula B, Manjurano A, Loken T and Kambarage DM (2004). Prevalence and determinants of mastitis and milk borne zoonoses in smallholder dairy farming sector in Kibaha and Morogoro districts in eastern Tanzania. Journal of Veterinary Medicine B, 51: 123-128.

[12] Mekibib, B. , Furgasa, M. , Abunna, F . , Megersa, B. and Regassa, A.* (2010). Bovine Mastitis: Prevalence, Risk Factors and Major Pathogens in Dairy Farms of Holeta Town, Central Ethiopia.www.veterinaryworld.org Veterinary World, Vol.3 No.9.

[13] N.M.C. (1990): Microbiological procedures for the mastitis in Danish organic dairy herd. J. Dairy Sci. 64: diagnosis of bovine udder inf ection, 3rd ed. NMC, 23.22. Workineh, S. Bayleyegn, M. Mekonnen, H. And Arlington pp, 1-15.

[14] Ntampaka Pie (2010): In vitro susceptibility of subclinical mastitis bacteria identified at masaka dairy farms to different antibacterials available in local drug stores, thesis.

[15] Potgieter, L.N.D. (2002): Prevalence and etiology of Sarkar SC, Parvin MS, Rahman AK, Islam MT (2013). Prevalance and risk factors of sub-clinicalmastitis in lactating dairy cowsin north and south regions of Bangladesh. Tropical Animal Health and Production 451171-1176

[16] Quinn, P .J. Carter, M.E. Markey, B. K. and Carter , G. R (2002): Veterinary Microbiology Microbial Diseases, Bacterial Causes of Bovine Mastitis, 8th Edition, Mosby International Limited, London, pp 465-475.

[17] Radostits O.M., Gay, C.C., Blood, D.C. and Hinchcliff, K.W. (2007). Mastitis. In: Veterinary Medicine, 9th ed., W.B. Saunders Company Ltd., London, pp. 603-687.

[18] SarkarSC, Parvin MS, Rahman AK, IslamMT 2013:Prevalance and risk factors of subclinicalmastitis in lactating dairycowsin north and south regions of Bangladesh. Tropical Animal Health and Production 451171-1176

[19] Sefinew Alemu1, Firaol Tamiru 2*, Gizat Almaw 3and Awoke Tsega 4 (2013). Study on bovine mastitis and its effect on chemical composition of milk in and around Gondar Town, Ethiopia. Vol .5(8), pp. 215-221 DOI 10.5897/JVMAH20 13.0219 © 2013 Academic Journals. http://www.academicjournals. org/JVMAH.

[20] Sharma N., Maiti S.K., and Singh R., (2004): Mastitis in dairy animals: global problem.

[21] Sharma, Neelesh, Maiti, S.K. and Sharma, K.K. (2007). Prevalence, etiology and antibiogram of microorganisms associated with sub clinical mastitis in buffaloes in Durg, Chhattisgrh State (India). International J. Dairy Sci., 2(2): 145-151.

[22] Sori H., Zerihun A., and Abdicho S., (2005). Dairy Cattle Mastitis In and Around Sebeta, Ethiopia. Inter. J. Appl. Res. Vet. Med. 3: 332-38.