PREVALENCE OF SUBCLINICAL MASTITIS AND ASSOCIATIONS WITH RISK FACTORS IN BUFFALOES IN NOAKHALI DISTRICT OF BANGLADESH

Ovirup Bhushan Paul

Roll No: 0119/04

Registration No: 646

Session: 2019-2020

A thesis submitted in the partial fulfilment of the requirements for the degree of Master of Science in Epidemiology

Department of Medicine and Surgery

Faculty of Veterinary Medicine

Chattogram Veterinary and Animal Sciences University

Chattogram -4225, Bangladesh

JUNE 2021

Authorization

It is my immense pleasure to affirm that I am the sole author of this thesis. I also authorize the Chattogram Veterinary and Animal Sciences University (CVASU) to lend this thesis to other institutions or individuals for scholarly research purpose. I further authorize the CVASU to reproduce this thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for investigation.

I, the undersigned and author of this work, proclaiming that the electronic copy of this thesis provided to the CVASU Central Library, is an accurate copy of this print thesis submitted within the limits of the technology available.

Ovirup Bhushan Paul

June 2021

PREVALENCE OF SUBCLINICAL MASTITIS AND ASSOCIATIONS WITH RISK FACTORS IN BUFFALOES IN NOAKHALI DISTRICT OF BANGLADESH

Ovirup Bhushan Paul

Roll No: 0119/04

Registration No: 646

Session: 2019-2020

This is to certify that we have examined the above master's thesis and have found it to be complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

----

Supervisor

Prof. Dr. Md. Mizanur Rahman, DVM, MS, PhD
Department of Medicine and Surgery
Chattogram Veterinary and Animal Sciences University

Chairman of the Examination Committee
Prof. Dr. Azizunnesa, DVM, MS, PhD
Head
Department of Medicine and Surgery
Chattogram Veterinary and Animal Sciences University

Department of Medicine and Surgery
Faculty of Veterinary Medicine
Chattogram Veterinary and Animal Sciences University
Chattogram -4225, Bangladesh
June 2021

DEDICATED TO MY BELOVED PARENTS AND MENTORS

Acknowledgements

I want to express my deepest gratitude to almighty God for giving me strength and the opportunity to do my research and complete it successfully for the degree of Master of Science (MS) in Epidemiology in the Department of Medicine and Surgery, **Chattogram Veterinary and Animal Sciences University (CVASU)**, Bangladesh.

First and the foremost, I am indebted to **Udder Health Bangladesh** for a full time Research Assistantship (RA) to support the pursuit of my Master Degree research in the area of Epidemiology in "Climate change mitigation by a sustainable water buffalo dairy chain in Bangladesh" funded by the Swedish Research Council, Sweden and operated jointly by CVASU, National Veterinary Institute (SVA) and Swedish University of Agricultural Sciences (SLU), Sweden; Utrecht University (UU) and Wageningen University and Research (WUR) The Netherlands, University of Milan (UniMi), Italy.

I would like to express my heartiest appreciation, deepest sense of gratitude and best regards to my supervisor, Professor **Dr. Md. Mizanur Rahman**, Department of Medicine and Surgery, CVASU. His cordial supervision and motivation during the study period, important suggestions during the field work, data analysis, and thesis writing helped me a lot. It was my pleasure and a great experience to work with him.

I would like to convey my very special gratitude to **Dr. Ylva Persson**, Associate State Veterinarian, SVA, Sweden for providing cordial support and excellent supervision during the "Bacteriological Diagnostics Training" program in SVA and SLU, Sweden and the IDF Mastitis Conference in Denmark in 2019. The cutting-edge learning helped me as a pathway to complete my laboratory work and thesis. Her research experiences on mastitis were vital to making up the questionnaire and literature review.

I sincerely acknowledge the contribution of Professor **Dr. Md. Ahasanul Hoque**, Department of Medicine and Surgery, CVASU, for his guidance, critical advice, and well thought suggestions during choosing study design and continuous support during the whole study period.

Special thanks to **Dr. Md. Saiful Bari**, Associate Professor, Department of Dairy and Poultry Science, CVASU, for his scholastic guidance, critical advice, inspiration, and well thought suggestions to complete this thesis. He helped me a lot in data analysis, interpretation, and writing up, without which it was difficult to complete this work.

I would also like to thank **Dr. Gerrit Koop**, Assistant Professor, Department of Farm Animal Health, UU, the Netherlands. His critical thought in the study design and guidance to make the literature review structure was essential. Additionally, he also guided me a lot during the makeup of the clinical mastitis decision tree to improve udder health in Bangladesh.

I would like to acknowledge the support and cooperation of Shuvo **Singha** (PhD fellow at UniMi) for his guidance, excellent support and cordial advice during the questionnaire making, field visit, lab work, data analysis, interpretation and thesis writing. It was a great experience to work with him during my whole study period. A special thanks to **Dr. Sanjib Chandra Nath** and **Dr. Salma Chowdhury** (MS fellow at CVASU) for their earnest support during sample collection, data collection, and laboratory work.

Furthermore, I am very pleased to express my cordial appreciation to the buffalo farmers of the study location (Noakhali district, Bangladesh). It would not have been possible without their cordial help on the island (buffalo concentrated zone) during sample collection and for their time in filling out the questionnaire.

I want to acknowledge the authorities and all the technicians of the **Udder Health Bangladesh** laboratory at the Department of Medicine and Surgery, CVASU, for their cordial help during my research work.

The Author

Table of Contents

List of Tables

List of figures

List of Abbreviations

Abbreviation	Elaboration
%	Percentage
<	Less than
>	Greater than
≤	Less than or equal to
≥	Greater than or equal to
°C	Degree Celsius
µl	Microliter
ANOVA	Analysis of variance
BBS	Bangladesh Bureau of Statistics
BHIB	Brain Heart Infusion Broth
BM	Bulk Milk
BMSCC	Bulk Milk Somatic Cell Count
CI	Confidence Interval
CM	Clinical Mastitis
CMT	California Mastitis Test
CVASU	Chattogram Veterinary and Animal Sciences University
DLS	Department of Livestock Services
E. coli	*Escherichia coli*
EMB	Eosin Methylene Blue
FAO	Food and agriculture organization of the United Nations
et al.	And his associate
G	Geometric mean
GDP	Gross Domestic Products
h	hour
ID	Identification Number
IMIs	Intra-mammary infections
L	Liter
LRT	Likelihood Ratio Test
MALDI-TOF	Matrix-Assisted Laser Desorption Ionization Time-of-Flight

mL	Milliliter
MR	Methyl Red
MS	Master of Science
MSA	Mannitol salt agar
N	Number
NAS	Non aureus Staphylococcus
NMC	National Mastitis Council
OR	Odds Ratio
p	Probability
RE	Random Effect
ROC	Receiver Operating Characteristics
SCC	Somatic Cell Count
SCM	Subclinical Mastitis
SE	Standard Error
Se	Sensitivity
SFMT	Surf Field Mastitis Test
Sp	Specificity
Spp	Species
Str	Streptococci
SVA	National Veterinary Institute, Sweden
VIF	Variance Inflation Factor

Abstract

Bovine mastitis is one of the most prevalent and economically significant diseases affecting dairy herds globally. Mastitis causes physical and chemical changes in milk as well as pathological changes in the udder. The cost of milk production rises as a result of high somatic cell count in milk along with significant decrease in milk production. Bulk milk somatic cell count (BMSCC) from bulk milk samples is used worldwide as an indicator of monitoring farm hygiene and udder health. Bangladesh represents 1.5 million buffalo heads of which 40% are reared in the saline coastal region. Buffalo contributes up to 4% of the total milk production demand in Bangladesh. There is a substantial lack of studies on the physiological threshold of the somatic cell count on milk quality in the case of buffalo milk. The present cross-sectional study was designed to investigate the multilevel (farm, animal and quarter) prevalence of subclinical mastitis (SCM) along with the association of potential risk factors and causal pathogens in buffalo quarter milk samples in Noakhali district of Bangladesh. A total of 45 buffalo farms (n=1660): 8.9% coastal (n=4), 66.7% semi-coastal (n=30), and 24.4% inland (n=11) farms with lactating buffalo (n=183) and functioning udder quarters (n=732) were investigated during March-April, 2021 in 6 buffalo concentrated zones of Subarnachar and Hatiya upazila of Noakhali district. Small (33%), Medium (38%) and Large (29%) size herds were observed with <15 buffaloes per farm, 15-40 buffaloes per farm and >40 buffalo per farm, respectively in the study area. Among the studied lactating buffaloes (n=183), majority (58%) of the buffaloes were indigenous (95% CI: 51-65) and the rest of them were cross-bred (42%) (95% CI: 35-49) buffaloes. The Geometric (G) mean of BMSCC (413×10^3 cells per mL of milk) (95% CI: 349-488) of the studied herd was two times higher than the threshold level (200×10^3 cells/mL of milk). Overall farm, animal, and quarter level prevalence of SCM were found 86.7% (95%CI: 0.73-0.94), 47% (95%CI: 0.40-0.54), and 27% (95%CI: 0.24-0.30), respectively based on BMSCC threshold level (200×10^3 cells/mL of milk) CMT score (≥ 2 =positive). Prevalence of SCM was observed high in buffaloes those were being reared in bathan or free ranging area at both animal (71%) and quarter (75%) (P=0.02) level. Prevalence of SCM among the quarters did not differ significantly. However, a significant (P=0.05) prevalence of SCM was found in hind quarter of the buffaloes that were being reared in bathan. MALDI-TOF results of the culture samples (n=292) obtained from quarter milk samples (n=320) from the lactating buffaloes (n=183) were revealed 47% (95% CI: 0.41-0.53) (n=137) of SCM prevalence. Non aureus *staphylococci* (NAS) were the most prevalent (39.4%) (95% CI: 0.41-0.53) organisms followed by *Staph. aureus* (3.8%) (95% CI: 0.02 to 0.07), Gram negative bacteria (1.4%) (95% CI: 0.005-0.04), and other bacteria (2.4%) (95% CI: 0.01-0.05). Among the CMT positive (CMT positive ≥2) quarter samples, high prevalence of *Staph. aureus* was observed in CMT score 3 (27.27%), followed by CMT score 4 (18.18%) and CMT score 2 (9.09%). Among the NAS in the quarter milk sample, the most prevalent bacterium was *Staphylococcus chromogenes* (38%), followed by *Staphylococcus hyicus* (28%), *Staphylococcus sciuri* (16%), *Staphylococcus xylosus* (8%), *Staphylococcus epidermidis* (4%), *Staphylococcus haemolyticus* (3%), and others (3%). Buffaloes grazed or stall-fed together or in group were less associated (P=0.005; CI=0.17-0.88) for increasing BMSCC level compared to those buffaloes fed separately. Stimulating udder only by calf suckling was found associated (P=0.02; CI=-0.71-0.07) with higher BMSCC than either only hand or both. Asymmetrical teats had 2.2 times (P=0.02, CI=1.15 - 4.05) higher association with the prevalence of SCM than symmetrical teats. The about 3.2 times higher odds of SCM in high milk yield (4.1-6 L) (P=0.05, CI=1.02 -10.31) buffaloes indicate that high productive buffaloes had 3.2 times more association for developing SCM than low productive buffaloes. The identification of BMSCC level, associated risk factors and pathogens would helpful in monitoring udder health control program in buffalo farms in Bangladesh.

***Keyword*:** Bathan, Coastal regions, Bulk milk somatic cell count, Multi-level occurrence, Risk factors, *Staph. aureus*

Chapter I: Introduction

The water buffalo (*Bubalus bubalis*), a member of the family *Bovidae*, sub-family *Bovinae* and genus *Bubalus* was domesticated 3000 to 7000 years ago (Maheswarappa et al., 2022). Majority (73.77%) of the world's estimated 202 million buffaloes are populated in the South Asian subcontinent (Hamid et al., 2017b; Zhang et al., 2020). In Bangladesh, saline coastal regions (Chattogram, Patuakhali, Bhola, Khulna) possess about 40% (Faruque et al., 1990; Samad, 2020) of the total 1.5 million buffalo (DLS, 2022). Around 65 thousands buffaloes in Noakhali district under Chattogram division are distributed at Companiganj (22%), Kabirhat (16%), Shenbag (15%), Subarnachar (14%) and Chatkil (12%) upazila (LDDP, 2018).

Usually most of the farmers in the saline coastal regions are rearing their buffaloes in extensive free range farming system in islands called bathan (Hamid et al., 2017a), which in practice is about 80% in Noakhali district (Amin et al., 2015). In this farming system, buffaloes are kept mostly outside under open sky (91%), followed by under tree (5%) and roof (3%) at night (Faruque et al., 2019). In household level farming system, farmers (82%) have 1 to 3 buffaloes per household, whereas in bathan farming system farmers (73%) have 51 to 200 buffaloes in each bathan in Noakhali district (Uddin et al., 2016).

Although there is no recognized breed of buffalo in Bangladesh, they can be classified as largely indigenous (local) type with few cross-bred (local × Nili-Ravi, local × Murrah) (Saadullah, 2012; Samad, 2020). According to Amin et al., (2015), the average milk yield of indigenous buffaloes is higher than the indigenous cattle in the Subarnachar upazila of Noakhali. The indigenous buffaloes produce 2.7-2.9 liters of milk per day on average in this coastal belt (Faruque et al., 1990; Amin et al., 2015). Dairy products derived from buffalo milk have higher quality than cow milk due to the high amount of saturated fatty acids, mainly palmitic acid and unsaturated fatty acids like trans fatty acids, linolenic acid (ω3) and conjugated linolenic acid (Martini et al., 2003; Ménard et al., 2010; Zotos & Bampidis, 2014). Curd, particularly which one produced in Noakhali with buffalo milk, is a popular regional food. The curd is famed for its health benefits, particularly its cholesterol-lowering properties (Alam & Naser, 2020).

Bovine mastitis also called "mammary gland inflammation," occurs due to causes of infectious or noninfectious origin (Bradley, 2002). It is one of the most important diseases in the buffalo species, although buffaloes have been thought to be less prone to mastitis than cattle (Wanasinghe, 1985). Subclinical mastitis (SCM) is characterized by normal gland and milk appearance and increase of somatic cell count (SCC) (Fagiolo & Lai, 2007), whereas clinical mastitis (CM) is characterized by pathological changes in the udder, physical, chemical and bacteriological changes in the milk (Radostits et al., 2007; Thrustfield, 2007). Subclinical mastitis is undetectable without the milk screening tests such as SCC, california mastitis test (CMT) (Fagiolo & Lai, 2007), and considering the iceberg concept of infection, SCM is now bigger concern than CM (Gay, 2009). In Nepal, 68% of total losses in buffaloes resulted from a drop in milk production with mastitis (Dhakal, 2002; Singh, 2004). The mastitic condition is also a public health concern due to contaminated raw milk and antibiotic residues in milk from the medicine used (Andrew et al., 2009).

The prevalence of SCM is more common, and 15 to 40 times higher than CM in buffaloes and dairy cows (Khan & Muhammad, 2005; Sori et al., 2005; Roy et al., 2009; Mekibib et al., 2010). Singha et al., (2021) reported the occurrence of SCM as 42.5% and 81.6% at quarter and animal level, respectively in water buffalo of Noakhali and Bagerhat district of Bangladesh (Singha et al., 2021). However, Islam et al., (2016) and Aliul et al., (2020) recorded lower SCM prevalence such as 31.57% and 10.50% at animal level in buffaloes concentrated in coastal areas and Bhola district of Bangladesh, respectively. Several studies reported that the overall prevalence of SCM in buffaloes were 27, 15, 42, 32, and 67% (Khan & Muhammad, 2005; Hussain et al., 2013; Ali et al., 2014; Abid et al., 2018; Asghar et al., 2019) at Faisalabad, Dera Ismail Khan, Lahore and Pothohar regions in Pakistan, respectively. Similarly, prevalence of SCM in buffalo was recorded 21- 68.33% (Kashyap et al., 2019; Srinivasan et al., 2013; Kaur et al., 2018; Hardenberg, 2016; Sharma et al., 2018; Pankaj et al., 2013) in India. Dhakal, (2006) observed 21.7% prevalence of SCM at 60 clinically healthy buffaloes in India and Nepal. Salvador et al., (2012) mentioned recurrence of SCM was higher (75%) than its actual prevalence 42.7% in Philippines. Prevalence of SCM in buffaloes differ among the quarters. However, overall quarter-wise prevalence of SCM in buffalo was recorded at 37.75% quarters (Sharif & Ahmad, 2007; Abid et al., 2018). Prevalence of SCM is high in the hind quarters of buffalo compared to the front quarters and also higher on the left side than quarters on the right side as revealed in different studies (Kashyap et al., 2019; Kavitha et al., 2009; Sharma et al., 2013; Srinivasan et al., 2013; Zenebe et al., 2014; Ali et al., 2014).

Several risk factors of environmental, herd, animal and quarter level are associated with SCM in buffalo. Higher tendency of mean SCC ($P < 0.05$) was observed in the winter and spring months than in summer and fall (Moroni et al., 2006). In Bangladesh, the highest prevalence of SCM was recorded in the rainy season (11.11%) followed by the summer (10.94%), and winter (10%) seasons, respectively (Aliul et al., 2020). Asghar et al. (2019) discovered that SCM was more prevalent in commercial (77.3%) than in traditional buffalo farming (22.7%) systems. Mastitis prevalence increases with bigger herd sizes of buffalo farms, such as large (40%), medium (32%), and small herds (27%), (Ali et al., 2014). In Bangladesh, large and medium-sized farms were also found to be more susceptible to SCM in dairy buffaloes (Aliul et al., 2020). Again, the incidence of mastitis in buffaloes was found higher in soil bedding, whereas less common on sand floors than on concrete (Kavitha et al., 2009; Bartlett et al., 1992). There is an association between hygiene standards (animal cleanliness, teat cups) and mastitis in the traditional farm. Microorganisms that cause mastitis are mostly fecal and soil- borne bacteria (Thomas, 2008; De Oliveira Moura et al., 2017). However, the incidence of mastitis was found high (53.8%) in stripping, and accordingly low in the knuckling (46.2 %) method (Ali et al., 2014). Lactating buffaloes with 2nd or greater lactation number were 4.6 times at higher risk of SCM than buffaloes with the first lactation (Asghar et al., 2019).The prevalence of SCM was also reported to be the highest in the age group of 9 to 11 years (90.32%), followed by 6 to 8 years (65.78%) and 3 to 5 years (41.37%) (Kashyap et al., 2019). In Bangladesh, water buffaloes aged 7 to 18 years had the highest prevalence (13.46%) of SCM compared to those aged 3 to 6 years (7.29%) (Aliul et al., 2020). In terms of breed, crossbred buffaloes (72.30%) had the highest frequency of SCM followed by indigenous (65.62%) and nondescriptive (47.23%) breeds (Kashyap et al., 2019). Again, mastitis occurred more frequently as the parity increased (Kivaria et al., 2007; Nyman et al., 2009; Kavitha et al., 2009; Hussain et al., 2013; Moroni et al., 2006). In Bangladesh, the highest prevalence of SCM was found at 2nd parity (11.1%) followed by 10.13% at 1st parity in dairy buffaloes (Aliul et al., 2020). Dhakal et al., (2006) and Moroni et al. (2006) observed no significant ($P>0.05$)

difference of prevalence between subclinical mastitic quarters of buffaloes. Pendulous udders (P=0.002) and cylindrical shape teats (OR=2.559) in buffaloes have significant association for having SCM in buffaloes (Asghar et al., 2019).

Bulk milk somatic cell count (BMSCC) from bulk milk sample is used worldwide as an indicator of monitoring farm hygiene and udder health (Costa et al., 2020). The mean normal somatic cell count (SCC) of buffalo milk sample was observed in different study as 130×10^3 cells/ml, 160×10^3 cells/ml and 171×10^3 cells/mL (de Oliveira Moura et al., 2017; Dhakal et al., 2008; Özenç et al., 2008). Bacteriological culture findings are another useful tool for the assessment of udder health and categorization of milk samples in buffalo (Dhakal et al., 2008). Again, several studies recommended SCC value $\geq 200 \times 10^3$ cells/mL with positive bacterial cultures is likely indicative of udder infection to diagnose SCM in buffaloes (Dhakal, 2006; Tripaldi et al., 2010). However, the California mastitis test (CMT) have positive relation with the SCC to diagnose SCM in dairy river buffaloes. Almost 94% of CMT-negative quarters had somatic cells below 200×10^3/ml (Dhakal, 2006; Preethirani et al., 2015).

Mastitis can be divided into two categories depending on pathogen type: "contagious" and "environmental" (Blowey & Edmondson, 2010). More than 200 infectious agents causing bovine mastitis have been identified so far (Yong et al., 2009). A study in South India revealed that *Coagulase-negative Staphylococci (CNS (non-aureus staphylococcus:NAS))* was the most predominant (64.8%) bacteria, followed by *Streptococci.* spp. (18.1%), *E. coli* (9.8%) and *Staphylococcus aureus* (7.3%) from 190 quarters SCM buffalo milk samples (Preethirani et al., 2015). Dhakal et al., (2008) also found *NAS* as major pathogens associated with SCM from characterization of 216 quarters of 54 Murrah crossbred buffalo milk samples. Singha et al., (2021) identified *NAS* (24.7%), *Streptococcus* spp. (0.7%), *Micrococcus* spp. (5.5%), *Bacillus* spp. (2.7%), *Moraxella* spp. (1.4%), *Klebsiella* spp. (0.7%), *Arcanobacterium* spp. (1.4%), *Corynebacterium* spp. (2.1%) in 299 quarter milk samples of water buffaloes in Bangladesh. Typically, pathogen cultures are used to assess microorganisms associated with SCM (Pyörälä, 2003). MALDI-TOF is a highly effective and cutting-edge approach for bacterial identification, although it is uncommon in Bangladesh yet.

The prevalence of SCM in buffaloes causes severe economic losses like other Asiatic countries in Bangladesh. Diversified contagious and environmental pathogens along with potential risk factors in farm, animal and quarter levels are significantly responsible for developing SCM in buffaloes like other dairy animals. Usually the buffalo farmers are not aware of udder health in Bangladesh. There is a substantial lack of epidemiological studies on SCM in buffaloes in Bangladesh especially at the Noakhali district of Bangladesh which is an important buffalo zone in Bangladesh in terms of buffalo population, distribution, and popularity of buffalo milk and milk products. Thus, an investigation is necessary to determine the prevalence of SCM along with the associated risk factors and to know the distribution of pathogens in order to implement hygienic/preventative measures and control of SCM. Hence, a cross-sectional study was conducted to fulfil the following objectives:

i. To estimate the prevalence of SCM based on BMSCC in buffaloes of Noakhali district of Bangladesh
ii. To know the prevalence of SCM in buffaloes by production types and seasons
iii. To determine risk factors associated with SCM in buffaloes
iv. To know the distribution of pathogens causing SCM in buffaloes

Chapter II: Literature Review

A thorough review of relevant literatures has been performed to prepare this section. Review of domestication of buffalo, its population, regional distribution and breeds, rearing systems, production potentiality and prospects of buffalo farming, subclinical mastitis (SCM) in buffalo, impact of SCM, milk quality and relation with screening tests, distribution of pathogens causing SCM, associated risk factors, diagnosis, control and prevention, and therapeutic management have thoroughly been accomplished. The main objective of this section is to provide up to date scientific information based on past scholarly articles published in peer-reviewed journals particularly, and accordingly identify inconsistencies and justify the present research work on prevalence of SCM in dairy buffaloes at Noakhali district of Bangladesh.

2.1. Domestication of Buffalo

Buffalo originated from Asian wild buffalo, which has been domesticated since pre-historic times in Asia, particularly in indo-Pak subcontinent (Bruford et al., 2003). According to archaeological discoveries and historical evidence, it was domesticated for the first time in approximately 2500 BC in the Indus Valley, present-day India and Pakistan (Falvey and Chantalakhana, 1999). About 5000 years ago, water buffalo (*Bubalus bubalis*), a member of the family Bovidae, sub-family Bovinae and genus *Bubalus* domesticated in Iran, Iraq and indo-Pak subcontinent, whereas the swamp buffalo *Bubalus carabensis*, was domesticated after a thousand years in China and other parts of Southeast Asia (Bruford et al., 2003; Hamid et al., 2016). Due to its innate need to wallow in muddy areas and watery ponds, this animal is known as the water buffalo (Thomas, 2008). Originally from India, they were transported to Egypt by Arab invaders in the 9th century and then to Europe by pilgrims and crusaders in the middle ages for their meat, milk, and draft purpose (Saadullah, 1998). In the sixth century, Asian buffaloes were introduced to Italy, and more recently, Australia, and America (Yindee et al., 2010).

There are two subspecies of Asian water buffalo (*Bubalus bubalis*). They are called swamp buffalo (*Bubalus carabanesis*) and river buffalo (*Bubalus bubalis bubalis*) on the basis of their morphological and behavioral criteria (MacGregor, 1941; Maheswarappa et al., 2022). Swamp buffalo are distributed throughout south-east Asia, from Assam and Bangladesh in the west to the Yangtze valley of China, whereas river buffalo are available to the western region of the Indian subcontinent (Kumar et al., 2007) and have expanded west as far as the Balkans, Greece, Egypt, and Italy during recorded historical times (Cockrill, 1974). The present geographic ranges of river buffaloes and swamp buffaloes overlap in Bangladesh and eastern India (Assam). Both of the buffalo types had independently evolved in the biogeographical regions east and west of the Arakan Mountain chain, therefore this overlap was likely a result of post-domestication human activity (Udvardy, 1975). River buffaloes' population is larger than swamp buffalo populations although they differ in chromosome number, phenotypic characteristics, and geographical locations (Degrandi et al., 2014; Zhang et al., 2020).

2.2. Buffalo population

River and swamp buffaloes are around 81.5% and 18.5%, respectively of the global buffalo population. Both sorts of populations are primarily found in Asia (196 million, or 97.0% of the world's population), with considerable populations also present in Africa (3.4 million) and South America (2.0 million) (Zhang et al., 2020). Asia is home to the vast majority (97%) of the world's 207 million buffaloes (FAOSTAT, 2018). About 74.80% of buffaloes are found in South Asia, followed by East Asia 12.80% and only 8.40% are in South-East Asia within whole Asia regions (Hamid et al., 2016).The majority of buffaloes live in India (58.11%), followed by Pakistan (16.83%), China (13%), Nepal (2.57%), Bangladesh (0.75%) and Sri Lanka (0.21%) (Hamid et al., 2017). Almost 69% of the river buffaloes are found in India, while 63% of swamp buffaloes are found in China (Zhang et al., 2020). Small farmers with less than two hectares of land and five or fewer buffalo raise about 98% of the water buffalo in Asia and the Pacific region (Falvey and Chantalakhana, 1999).

In Bangladesh, the total buffalo population are 1.5 million (DLS, 2021) of which extensive free range (Bathan) farming system in saline coastal regions possess about 40% that are used as a draught animal and partially for milk and meat production (Faruque et al., 1990). In most countries, there has been a large increase in the number of river buffalo. However, this global trend conceals significant country-specific variations, as the number of heads has increased in China and Myanmar, and decreased in Malaysia, whereas in Cambodia, Indonesia, the Philippines, Thailand, and Vietnam it has stabilized over the last three to five years following an initial decrease. Swamp buffalo numbers, on the other hand, significantly decreased over the same period (Zhang et al., 2020)

2.3. Regional distribution and breeds of Buffalo: Asia and Bangladesh

The buffalo is one of the most significant livestock species, and it is mostly found in tropical and subtropical regions of the world (Das and Khan 2010). The world distribution of Buffalo reveals that most of the buffalo are found in Asia followed by Africa, America and Europe (Pasha and Hayat 2012). Asia has a long history of dairy buffalo production, particularly in south Asian countries like Bangladesh, India, Pakistan, and Nepal. Additionally, there are buffaloes in Iraq, Turkey, Thailand, China, Egypt, Denmark, Bulgaria, Romania, Germany, Macedonia, and Italy (Borghese, 2013; Hamid et al., 2016). In western Asia, which includes India, Pakistan, Bangladesh, Iran, Iraq, Nepal, and Sri Lanka, river buffaloes are primarily found. On the other hand, the Philippines, Thailand, Vietnam, China, Burma, Indonesia, and Malaysia all have swamp type buffaloes. Tamaraw or Mindoro buffaloes (*Bubalus mindorensis*), which are only found in the Philippines, and Anoa buffaloes (*Bubalus depressicornis* and *Bubalus quarlesi*), which are native to Indonesia, are two other varieties that are present in limited numbers and likewise endangered (Minervino et al., 2020).

When compared to the river buffalo subspecies, which have multiple breeds, the swamp buffalo has a stable phenotypie and is thought of as one kind (Minervino et al., 2020). There have been reports of major physical changes between water buffaloes from different countries, mostly as a result of size and body weight variation (Borghese and Moioli, 2016). The River buffalo has 50 chromosomes, with 20 acrocentric and 5 submetacentric pair divisions, whereas 48 chromosomes, including 19 pairs of metacentric chromosomes, make up the Swamp buffalo. The two subspecies can reproduce with each other and produce offspring with 49 chromosomes (Borghese, 2011). However, the Food and Agriculture Organization (FAO) has listed 123 buffalo breeds based on information submitted by various countries, 90 of which are in Asia and primarily consist of native breeds (FAO 2015). The Asian continent is home to 16 of the world's most significant river buffalo breeds, including the Murrah, Nili-Ravi, Jafarabadi, Kundi, Bhadawari, Surti, Mehsana, Toda, Nagpuri, Pandharpur, Kalahandi, Manda, Jerangi, Sambalpur, South Kanara, and Tharai. Due to their widespread distribution around the world, the breeds of the Murrah, Nili-Ravi, and Mediterranean are recognized internationally. Other notable breeds of river buffalo raised for milk and meat include the Lime breed in Nepal, the Parkote breed in Pakistan, the Anatolian breed in Turkey, the Azeri or Caucasian breed in Iran, the Khuzestan breed in Iraq, and the Egyptian breed (Moioli and Borghese, 2005; Pasha and Hayat, 2012; Maheswarappa et al., 2022). In Pakistan, Nili Ravi buffalos are 34% of the population, Kundi buffalo accounts for 21%, and the remaining buffalo are unremarkable (Khan, 2001). China has a huge variety of buffalo genetic resources, mostly swamp types. These have adapted themselves to a range of climates. All Chinese buffalo with the exception of the milking buffalo breeds such Wenzhou, Jianghan, and Fuan, are used for draught purposes (Chunxi and Zhongquan, 2001)

There is no recognized breeds of buffalo in Bangladesh, most of them are indigenous origin. Available buffaloes can be classified into two categories: i. Indigenous non-descriptive varieties and ii. migrated buffaloes from India and Myanmar (Hamid et al., 2016). Indigenous buffaloes (*Bubalus bubalis*) are found in the coastal areas and marshy land throughout the country, whereas migrated buffaloes from India and Myanmar are found in the sugar-cane producing belt of Jamalpur and Coxes's bazar district, respectively covered with riverine types. The coastal region also contains a few number of cross-bred swamp and river-type buffaloes (Faruque et al., 1990; Saadullah, 2012). However, the Meghna-Ganga and Jamuna-Brahamaputra flood plain, Sylhet haor region, and Kanihari in Trishal upazila of Mymensingh district which are thought as the buffalo pockets and have been used exclusively for milk production for hundreds of years. Most of the buffaloes are river-type, whereas swamp types are found in the country`s eastern portions (Faruque et al., 1987; Sohel and Amin, 2015). Due to border migration from India, there are also a small number of crossbred buffaloes (indigenous with Murrah, Nili-Ravi, Surti, and Jaffrabadi, etc.) around the Indian border of Bangladesh (Huque and Borghese, 2012). In 1960, Bangladesh acquired a small number of Nili-Ravi buffalo from Pakistan to serve as breeding bulls for farmers in the southern coastal region who wanted to crossbreed the buffalo. In 1990, the Department of Livestock Services (DLS) brought 100 Nili-Ravi pregnant heifers and the first lactating cows into Bangladesh from Pakistan. These animals were raised on a newly created buffalo farm in the Bagherhat district of the country's southwest (Hamid et al., 2016).

2.4. Rearing systems: Bangladesh and Asia

The practice of raising buffalo has gradually moved from backyards to industrial farms and significant business operations. The enormous demand for buffalo milk and meat has meant that the industry's development has paralleled that of the dairy cattle sector. However, buffalo breeds need to be developed, with a clear focus on the desired output, enabling this species to operate at its best under the stress of intensive production methods (Thomas, 2008). In Italy, 400,000 buffaloes are reared in an intensive system. The female buffalo cows are reared free in an enclosure (loose housing barns) and mechanically milked twice a day (Borghese, 2013). Calves are typically removed from the dam and receive colostrum through bottle feeding. After reconstituted milk is kept in a single cage one to two months after birth, which helps to avoid infections and control milk consumption. When calves are kept in multiple boxes receive milk replacers, starter concentrates and quality hay until the weaning (Borghese, 2013). In the majority of farms, common water troughs are located in each enclosure, and total mixed rations are provided from a feed mixer wagon. Buffaloes are typically classified according to their lactation stage and fed according to productivity. However, the practice of individual concentrate feeding in conjunction with in-parlor feeding is used on a number of farms (Thomas, 2008).

In Bangladesh, buffalo is used as a draught-purpose animal in the household subsistence farming system in the villages (Hamid et al., 2016). Buffalo in the tropics rely on unrestricted grazing, tethering, or stall-feeding, and free grazing, occasionally under the supervision of herders, is typical in countries with native grasslands and fallows. In locations with little available land and with cropping, tethering and stall-feeding are common practices. There appeared to be roughage restrictions for animals in the stall-feeding and tethering systems in many instances (Wanapat and Chanthakhoun, 2009). With a few exceptions related to its distinctive grazing, breeding, and water needs, buffalo rearing systems in Bangladesh are very similar to those of tropical cattle. Buffalo in the tropics rely on unrestricted grazing, tethering, or stall-feeding, and free grazing, occasionally under the supervision of herders, is typical in countries with native grasslands and fallows on a small farm with limited resources, where financial inputs are infrequently used, traditional buffalo farming is typically characterized by subsistence. According to land types and locations, three production systems are used to grow significant buffaloes: a household subsistence system, a semi-intensive system, and an extensive system in coastal saline regions, which account for around 23% of all land areas. The domestic buffaloes reared in household subsistence farming are raised in stalls and given very minimal feed while grazing for 6-7 hours in and around backyards or public lands. The herd size ranges from roughly 1-3, with the largest number being 10. The buffaloes are raised in a semi-intensive farming system that combines both free range in upper coastal land and sometimes in the household during seasonal rice cultivation. The herd size ranges from 4 to 15 animals on average. In the lower coastal region, buffaloes are raised as part of a large-scale extensive farming system known as bathan farming. Offshore islands, mudflats, chars (soil that has accreted over time), and new accretions are all part of the bathan farming system in the coastal region. The herd size ranges from 51 to 200 animals, with the maximum number being 600 and the natural grazing systems in the grasslands are the only source of feed without any additional feed supplement in bathan farming. Buffalo provides milk in low quantity producing about 800 kg per lactation while calves consume most of it, with some being sold in the market. Buffaloes in bathan farming mostly used for the production of meat, while whole milk is fed to the calf or slightly milked for sale (Hamid et al., 2016). Buffalo has its own wallow, but occasionally the whole herd of the entire village lie down together in mud wallows and ruminates until dusk. They emerge from their mud wallows around noon, covered in grey slime, and graze on roads and other aquatic plants until dusk. Once this is done, they are relocated to dry areas where they stay until dusk (Saadullah, 2012).

2.5. Production potentiality

The domestic Asian water buffalo (*Bubalus bubalis*) is an important animal resource. At least 67 countries rely on this species for draught power, milk, meat, leather, dung, hide, horns, and traction power for many thousands of years and more people depend on buffalo than any other domestic animal (FAO, 2000; Abdel-Shafy et al., 2022). All buffaloes in Europe and the Near East are of the river type, having a similar phenotypic but a range of sizes. The river buffalo's significance is based on the milk yield quality and vast amounts of milk it produces (Borghese, 2011). A minimum of 300 kg and a maximum of 280 kg body weight respectively, for adult male and female buffaloes in Egypt. In Iraq, the most common sizes of male and female buffaloes are 800 and 600 kg, with 1000 and 900 kg as the maximum according to their live weight (Hamid et al., 2016). The Swamp buffalo is mostly raised for draught purposes due to less milk production than river buffalo (Borghese & Moioli, 2016). The adult male weight of a swamp buffalo is between 325 and 450 kg, while that of a river buffalo ranges between 450 and 1000 kg (Borghese, 2011). Majority of (92%) the world's buffalo meat around 3.08 million tons comes from Asian countries in 2008. South and South West Asia provided about 78.5% of the Asian buffalo meat, where a major portion was contributed by India and followed by Pakistan (Wanapat and Chanthakhoun, 2015). Other reports reveal that India is the fourth-largest producer of meat in the world and the top exporter of buffalo meat, contributing 17% to the country's overall meat output (Hamid et al., 2016). The hide is another economic benefit provided by buffalo to humans. An estimated 850.16 thousand tons of buffalo hide were produced worldwide, and 818.37 of those tons originated from Asian countries. As a result, Asia accounted for 96.26% of all buffalo hides in 2007 (Pasha and Hayat, 2012).

The SAARC region is home to a large range of buffalo genetic material, including the internationally recognized Murrah and Nili-Ravi buffaloes, which are prized for their excellent milk production potentiality. The 68.00 MMT of milk produced by buffalo represents 56% of total milk production in India (Muehlhoff et al., 2013; Hamid et al., 2016). The SAARC countries contribute 93.19% of global buffalo milk production and 96.05% in Asia, where India and Pakistan each produce 67.99 and 23.96%, respectively (Hamid et al., 2016). Usually, a healthy water buffalo can yield 6–7 liters of milk per day in India and Pakistan (Thomas, 2008) whereas Chinese dairy buffalo breeds can produce on average 390 to1020 kg milk in 150 to 300 days (Chunxi and Zhongquan, 2001). In Italy, 50,000 buffaloes are counted each month while they are lactating, producing 2,220 kg of milk in 270 days with 8.4% fat and 4.6 % protein, despite the fact that several champions can produce more than 5,000 kg throughout lactation (Borghese, 2013). India is the largest exporter of dairy and dairy products globally. The buffalo serves as the foundation of India's dairy industry, which accounts for 67.99% of the world's production of buffalo milk (Chakravarty, 2013). The famed Murrah buffaloes are known for their excellent milk production potential. Recorded data reveals that Murrah breed produce14 –15 L of milk per day on average, with peaks of 31.5 L. The peak milk production at 31.5L recorded at milk yield competition conducted by the Government of India. Usually, elite Murrah buffalo produces more than 18 L of milk each day (Singh, 2013; Hamid et al., 2016). Globally, Pakistan is the second-largest milk producer after India and SAARC regions also. Around 67% of the milk produced annually in Pakistan comes from buffaloes, which contribute 29.78% of the milk produced worldwide. It is often called the Black Gold of Pakistan. The famous Nili-Ravi buffalo in Pakistan is another best performing buffalo in the world for milk production which can produce 2500 L of milk in 305 days lactation period. The average milk production of buffaloes is 5-7 L/day in Pakistan (Hamid et al., 2016).

Bangladesh is a country in South Asia with an agricultural economy, and livestock is an essential component of the rural economy (Hamid et al., 2016). In Bangladesh, water buffaloes are primarily indigenous non-descriptive varieties and have no recognized breed. According to reports, buffalo play an important role in the livestock economy of Bangladesh by providing milk, meat and draught power (Abdul et al., 1991). Production in villages is often based on a small herd of mixed sexes and ages, mostly for draught and breeding purposes. In a semi-intensive production system, the major reasons people rearing buffaloes are either for draught or for milk production (Saadullah, 2012). Although Bangladesh's total milk production is expected to reach 13 Mt in 2021, just 3–4% of that will come from buffalo, despite the fact that the number of buffaloes and their growth rates has both increased over the past decade (DLS, 2021).

2.6. Prospects of Buffalo farming in Bangladesh

2.6.1 Buffalo over cattle

Buffaloes offer a number of advantages over cattle, including the usage of low-quality roughages to gain more protein and increase body weight, greater disease resistance, exceptional draught capacity, and a longer life span. Compared to cattle, growing buffaloes may use coarse feed more effectively, are more disease resistant, produce more solids in their milk, and require fewer management inputs (Dubey et al., 2013). Buffaloes degrade crude protein (CP) and protein-free dry matter (DM) more rapidly than cattle (Terramoccia et al., 2000). It can produce meat and milk from the remains of both cultivated fields and open pastures because it has a far better capacity for degrading fibrous fodder than cattle, such as straw and sugarcane remnants (Bertoni et al., 2019; Bertoni et al., 2020).

Indigenous buffalo cows produce twice as much milk as indigenous cows, with higher levels of milk fat and total solids. (Faruque et al., 1990).

Since 96% of river buffaloes in the world live in Asia and are mostly raised by small-scale and landless farmers, due to different rearing systems efficiency to convert feed into milk, meat and tractive energy greatly differ within and between countries (Saadullah, 2012). Buffalo is still kept in conditions of inadequate nutrition, breeding, management, and welfare despite having the ability to produce milk and meat at outstanding levels. More than 10% of global milk output has come from water buffalo for quite some time, however, despite this fact, the true potential of these animals is rarely recognized or appreciated (Thomas, 2008). The production of buffalo should continue to be improved and made more efficient in order to meet the present and future needs of its population. It is commendable that the production efficiency of Buffalo has risen during the past few decades (Abdel-Shafy et al., 2022) The majority of people who have any financial interest in raising buffalo are extremely poor, therefore they lack the resources to foresee how the animal will affect their quality of life (Thomas, 2008).

2.6.2 Adaptability to Harsh Weather

The thick epidermis of water buffalo skin is full of melanin pigments, which give the surface of the skin its black color and heat absorbing properties. Again, sweat glands in buffalo skin are one-sixth less than in cattle skin. Therefore, water buffaloes can't sweat as well to get rid of absorbed heat (Marai and Haeeb, 2010). So, it’s require bathing during the hot summer months to maintain a normal body temperature and to prevent stress, restlessness, and nervousness brought on by solar radiation and heat stress (Thomas, 2008; Marai and Haeeb, 2010). On hot summer days, water buffaloes would feel uneasy and restless if they are not given access to freshwater or wastewater wallows (Thomas, 2008). This species' breeding expansion has been promoted due to its capacity to adapt to a variety of climatic situations, increased ability to digest low-quality pasture, and quicker growth, making it a versatile and beneficial species for sustainable livestock production (Naveena and Kiran, 2014). The buffalo's adaptability is more than Friesian cows. For instance, in two distinct studies, the adaptability of the Egyptian buffalo in a sub-tropical environment was estimated at 89.6% and 89.1%, whereas adaptability estimation for Friesian cows was found 82.9% (Marai and Haeeb, 2010).

2.6.3 Milk quality of river buffalo

The water buffalo (*Bubalus bubalis*) is an essential part of human nutrition since it provides raw meat and milk (Borghese, 2011). In some countries, River buffalo is replacing other ruminants as a producer of meat and milk. Over 50% of drinking milk in some developing nations, including India, Pakistan, Egypt, and Nepal, comes from the milk of this species, whereas in Italy, the manufacture of mozzarella cheese uses almost solely buffalo milk (Zicarelli, 2004). Chinese swamp buffalo's milk quality and quantity were increased through crossbreeding, however the nutrition of the crossbred's milk is similar to that of river buffalo like Murrah and Nili-Ravi (Ren et al., 2015).

Compared to cow, buffalo milk has a higher percentage of all components like total solids, lipid, fat, lactose, proteins, ash, calcium, tocopherol, a natural antioxidant, and vitamins A and C with lower levels of vitamin E, riboflavin and cholesterol (Han et al., 2007; Abd El-Salam and El-Shibiny, 2011; Zotos and Bampidis, 2014). Despite having a higher fat content, buffalo milk has a lower cholesterol content than cow's milk in both milk and mozzarella (Han et al., 2007). Buffalo milk has greater natural preserving properties than cow's milk since it has a two to four time higher peroxidase activity (Falvey and Chantalakhana, 1999). Due to the absence of the yellow pigment carotene, a precursor to vitamin A, buffalo milk appears to be whiter than cow milk. The presence of a bioactive pentasaccharide, gangliosides, and the blue-green pigment (biliverdin) that are not found in cow milk. Generally, biliverdin in buffalo milk convert to bilirubin during storage and acidification period. Bilirubin is an essential antioxidant during the neonatal period and also have an inverse effect on the risk of coronary heart disease in adult (Turfan et al., 2013). However, buffalo milk has even higher levels of vitamin A than cow's milk. (Thomas 2008; Abd El-Salam and El-Shibiny, 2011). Compared to bovine and sheep milk, buffalo milk contains higher amount of fat due to larger size of fat globules composed of triacylglycerols (TG: 98% of milk lipids). Triacylglycerols are esters of fatty acids and glycerols (Ménard et al., 2010). So, the high-fat content of buffalo milk makes it highly suitable for further processing of dairy products. Dairy products derived from buffalo milk has higher quality than cow milk due to the high amount of saturated fatty acids, mainly palmitic acid and unsaturated fatty acids like trans fatty acids, linolenic acid (ω3) and conjugated linolenic acid (Martini et al., 2003; Ménard, et al., 2010; Zotos and Bampidis, 2014). Buffalo milk has higher viscosity and curd tension, faster rennet coagulation, and less heat stability than cow milk (Abd

El-Salam and El-Shibiny, 2011). Due to its higher dry matter content, buffalo milk has a higher cheese yield capability than cow milk (Martini et al., 2003).

Table 2.1: Comparison of cow and buffalo milk (Falvey and Chantalakhana, 1999).

Traits (unit)	Buffalo	Cow
Total solids (%)	16.30	13.10
Fat (%)	7.90	4.30
Protein (%)	4.20	3.60
Lactose (%)	5.00	4.80
Tocopherol (mg/g)	0.33	0.31
Cholesterol (mg/g)	0.65	3.14
Calcium, Ca (mg/100 g)	264.00	165.00
Phosphorus, P (mg/100 g)	268.00	213.00
Magnesium, Mg (mg/100 g)	30.00	23.00
Potassium, K (mg/100 g)	107.00	185.00
Sodium, Na (mg/100 g)	65.00	73.00
Vitamin A, incl. carotene (I.U.)	33.00	30.30
Vitamin C (mg/100 g)	6.70	1.90

2.6.4 Meat quality of River buffalo

India accounts for 49% of the world's buffalo meat production, followed by Pakistan (25%), China (10%), and Nepal (Caizares et al., 2017). In comparison to beef, buffalo meat is a healthy nutritious substitute (Tamburrano et al., 2019). Because of its polyunsaturated fat concentration, there may be less health hazards (Sharma et al., 1986; Guerrero-Legarreta et al., 2020). Buffalo meat is superior to beef, highlighting how its higher protein content and lower fat and cholesterol content reduce the risk of cardiovascular and atherosclerotic damage in humans who consume it (Kandeepan et al., 2009; Giordano et al., 2010). Whereas consumption of intramuscular fat is not associated with the occurrence of cardiovascular and cerebrovascular disorders (Cruz-Monterrosa et al., 2020). Buffalo meat might be a source of personalized nutrition for physiological conditions like pregnancy in women and person's risk with cardiovascular disorders due to its sufficient B-complex vitamin, zinc, and cholesterol content (Ordovas et al., 2018; Tamburrano et al., 2019). Analysis of the Longissimus dorsi muscle of male buffaloes from the Campania region in Italy by Landi et al., (2016) revealed that buffalo meat has advantages for human consumption since it has a higher protein compared to beef. Tamburrano et al., (2019) has recently demonstrated that buffalo meat from the Italian Mediterranean region is superior to beef in several dietary and nutritional aspects like vitamins (B6 and B12) and minerals (Phosphorus, Iron, Zinc, Sodium, and Copper).

Studies comparing buffalo meat to standard beef products have revealed that its qualities are comparable. For instance, although river buffalo meat has a reddish color in physicochemical terms, its protein level is higher than that of beef (Robertson et al., 1983; Guerrero-Legarreta et al., 2020). River buffalo meat is tenderer than beef from *Bos indicus* crossbreds (Neath et al. 2007a; Neath et al. 2007b; Guerrero-Legarreta, et al., 2020). The lipids in buffalo meat are deposited mostly between the muscles, as opposed to cow, hence it lacks marbling and has a tendency to be a deeper shade of red than beef (Giuffrida-Mendoza et al., 2015; Hamid et al., 2016). Older buffaloes have a minor fluctuation in myoglobin content that gives their meat a gold tint (Kandeepan et al., 2013). Although lipid oxidation is stronger in the longissimus muscle of buffaloes than in beef, buffalo meat contains more saturated fats and fewer polyunsaturated fatty acids than beef (Di Luccia et al., 2003).

2.6.5 Disease prevalence of buffalo

In Asia and other places, the water buffalo is a necessary livestock animal. It has contributed significantly to the livestock sector and is tolerant of tropical temperatures. Infectious diseases prevent livestock from good reproduction, which results in severe economic loss. The majority of buffalo diseases are zoonotic and quite common in developing nations, posing substantial risks to human health (Villanueva et al., 2018). The prevalence of and possible risk factors for tuberculosis (TB) were studied in 3,917 pregnant and non-pregnant female Murrah and Mediterranean water buffaloes were studied in Brazil. The Tuberculosis prevalence was recorded 4.3% in Murrah and 4.8%

in Mediterranean breeds whereas 5% and 4.3% were recorded in pregnant and non-pregnant buffaloes, which is a dangerous public health issue for water buffalo farming (Barbosa et al., 2014).

Leptospirosis, brucellosis, bovine TB, Bovine viral diarrhea virus, and fascioliasis are anticipated to have a detrimental economic impact on the water buffalo sector in addition to their consequences as zoonosis. (Villanueva et al., 2018). Brucellosis and tuberculosis were recorded up to 474 and 604 cases respectively in buffalo in Brazil (Schwarz et al., 2021). Another study recorded an outbreak of lumpy skin disease in Asian water buffalo (*Bubalus bubalis*) along with cattle belonging to smallholder farmers in 32 villages of Madhya Pradesh, India (Pandey et al., 2022). Khademi et al. (2019) determined the prevalence of Q fever (*C. burnetii*) in 840 raw milk samples collected from water buffalos where 19.3% in buffalo and 14.6% in cattle samples were positive for *C. burnetii* in Northwest Iran (Khademi et al., 2019). The overall infection rates for *Tetratrichomonas buttreyi* and *Pentatrichomonas hominis* found 8.1% and 5.4%, respectively in water buffalo, China (Li et al., 2020).

Islam et al. (2016) conducted investigations to reveal the incidence and prevalence of different buffalo diseases in different selected areas of Bangladesh. Gastro-intestinal parasitological infestation was found high (64.2%) among studied buffaloes. *E. coli* (62.5%) and *Salmonella* spp. (29.16%) were the main causes of non-parasitic enteritis. Clinical and Subclinical mastitis were recorded in 23.68% and 31.57% of total samples analyzed, respectively (Islam et al., 2016). Aliul et al. (2020) noted a comparatively higher prevalence of subclinical mastitis (SCM) than clinical mastitis (CM) in dairy buffaloes in Bangladesh. Higher prevalence (11.5%) of SCM noticed among 139 buffaloes those have previous history of post-purturient diseases. The prevalence of SCM in dairy buffaloes is 11.0 2% and 10.29% in poor and medium health conditions, respectively (Aliul et al., 2020).

2.7. Mastitis: its relevance and classification

Bovine mastitis, which is referred to as "mammary gland inflammation," can have either an infected or a noninfectious origin (Bradley, 2002). It is one of the most significant infectious diseases of dairy herds affecting the global dairy industry as a result of the decreased quantity and quality of milk yields. It also has negative effects on the chemical and cytological makeup of milk. Furthermore, it might lead to the existence of bacteria and other infectious agents that could be dangerous to human health (Costa et al., 1997a; Beheshti et al., 2010) Traditionally, mastitis has been divided into two categories depending on pathogen type: "contagious" and "environmental" (Blowey and Edmondson, 2010). Both of these categories are further divided into two subcategories like subclinical and clinical forms of mastitis. Depending on the duration and the persistence of clinical indications, both of subclinical and clinical forms of mastitis can be further divided into an acute and chronic form (Adkins and Middleton, 2017; Paul et al., 2019; Rollin et al., 2015). Clinical Mastitis (CM) is characterized by physical, chemical and usually bacteriological changes in the milk (Paul et al., 2019). Whereas, SCM is characterized by normal gland and milk appearance and an increase of somatic cell count (Fagiolo and Lai, 2007).

2.8. Incidence of mastitis

There are some traits that may increase the chance of developing mastitis in buffalo. For instance, in comparison to cattle, the udder is more pendulous and the teats are longer (Krishnaswamy et al., 1965). The buffaloes, on the other hand, have a long, narrow teat canal, which may be predicted to stop the invasion of microbes (Moroni et al., 2006). A different study reported incidence of mastitis is lower in buffalo than in cattle. The incidence of SCM in buffalo and cattle represents 24.4% and 43.9%, whereas incidence of CM is 24.8% and 54.7% respectively (Ståhl and Lind, 2003). However, the incidence of mastitis in buffaloes have a decreasing tendency with the following parturition. The occurrence of mastitis in buffalo decrease as the number of calving increase. A higher incidence (51.6%) of CM was found during their 1st calving, with a subsequent reduction in the 2nd calving (Dhakal et al., 2007). In the province of Salerno, Galiero et al., (1996) examined 13 farms and observed that contagious pathogens are mostly responsible and have a marginal role in SCM. Singha et al., (2021) reported the occurrence of SCM in 299 active udder quarter samples of water buffalo is 42.5% and 81.6% at quarter and animal level, respectively.

2.9. Subclinical mastitis

The most prevalent type of mastitis is subclinical mastitis (SCM), an asymptomatic inflammation of mammary tissue. The prevalence of SCM is 15 to 40 times higher than CM (Khan and Muhammad 2005; Sori et al., 2005; Roy et al., 2009; Mekibib et al., 2010). SCM is a bigger concern than CM considering the iceberg concept of infection (Gay, 2009). SCM is undetectable without the screening tests such as somatic cell count, California mastitis test, and bacterial agent detection (Fagiolo and Lai, 2007). As a result, several consequences are involved when dairy animals are suffering from SCM, whereas milk production losses won't be addressed either. In addition, cows suffering from SCM have a 3.32 incidence risk ratio for CM (Compton et al., 2007). However, due to the anatomical and physiological characteristics of the teat, traditionally buffalo are less prone to mastitis than cattle (Wanasinghe, 1985; Thomas, 2008). Buffalo's long, thin teat canal may be thought to help them resist microbial invasion. According to Krishnaswamy et al., (1965), the teat sphincter of buffalo has smoother muscle fiber, acting as a better barrier to microbial infiltration than the teat sphincter of cows.

2.9.1 Prevalence of SCM

Table 2.2: Worldwide overall prevalence of SCM in water buffalo

Countries	Overall prevalence (%)	References
	82	Singha et al.,(2021)
Bangladesh	32	Islam et al., (2016)
	11	Aliul et al., (2020)
	67	Asghar et al., (2019)
	32	Abid et al., (2018)
	42	Ali et al., (2014)
	44	Ali et al., (2011)
Pakistan	27	Hasina et al., (2018)
	15	Hussain et al., (2013)
	60	Mustafa et al., (2011)
	51	Sharif and Ahmad, (2007)
	27	Khan and Muhammad, (2005)
	68	Kashyap et al., (2019)
	26	Srinivasan et al., (2013)
India	21	Kaur et al., (2018)
	20	Kaur et al., (2015)
	29	Hardenberg, (2016)
	34	Sharma et al., (2018)
	29	Pankaj et al., (2013)
Nepal	22	Dhakal, (2006)
Philippines	43	Salvador et al., (2012)
Iran	27	Beheshti et al., (2011)
Egypt	6	EL-Naker et al., 2015)
Brazil	19	Costa et al., (2000)
	31	Bonini et al., (2007)

%= Percentage

2.9.2 Impact of SCM

Bovine mastitis is one of the most prevalent and economically significant diseases affecting dairy herds globally. It results in significant financial loss to dairy farmers (Seegers et al., 2003). Every year, the US dairy business loses over $2 billion, whereas similar effects are seen in Europe and other nations also (Donovan et al., 2005, Denis; Wedlock et al., 2009). Mastitis that develops early in the lactation stage reduces production over the long run (Bachaya et al., 2011; Nazifi et al., 2011), whereas SCM increases the chance of developing CM in succeeding lactations and resulting in the early culling of animals (Parker et al., 2007). According to epidemiological studies, bovine mastitis is a significant dairy health issue, and it causes a variety of physical and chemical changes in milk as well as pathological changes in the udder (Radostits et al., 2007; Thrustfield, 2007). Therefore, the cost of milk production rises as a result of an increase in milk SCC, a significant decrease in milk production, the expense of veterinary care and the early culling of mastitic animals (Vink, 1995; Seegers et al., 2003).

Mastitis is also the most expensive disease in the buffalo species, despite the fact that buffalo have been thought to be less prone to mastitis than cattle (Wanasinghe, 1985). Some studies have revealed comparable mastitis frequency rates for both species (Kalra and Dhanda, 1964; Badran, 1985; Bansal et al., 1995). In Nepal, 68% of total losses in buffaloes resulted from a drop in milk production with mastitis (Dhakal, 2002; Singh, 2004). Effective mastitis control is crucial from both the consumer and processor's perspectives, as well as for farmers who may suffer financial losses (Kavitha et al., 2009). This is because milk from affected animals may contain organisms that are potentially pathogenic to humans, and processing such milk could result in inferior fermented products (BILAL, 2004). Whereas the mastitic condition is also linked to threats to the public's health from contaminated raw milk and antibiotic residues left over in milk from the medicine used (Andrew et al., 2009)

2.10. Pathogen distribution of SCM

Diversified pathogens are potentially responsible for having SCM. Findings of pathogens causing SCM tabulated according to several previous studies (Table 2.3).

Table 2.3: Distribution of pathogens causing subclinical mastitis in buffalo

(%)	**1***	**2***	**3***	**4***	**5***	**6***	**7***	**8***	**9***	**10***	**11***	**12***	**13***	**14***	**15***
	Pakistan		Iran	Brazil		Italy			India						Nepal
Staphylococci spp.	28.3		48.5	41	50.6	17.7	17	37	44		39		15.9	46.3	1
CNS										59		78.4	47.7		
S. aureus		45								31					11.1
S. albus															33.3
S. epidermidis															11.1
E. coli	16.2	18			2.47				18		5			11.1	2.8
Pseudomonas spp.	13.3														
Bacillus spp.	12.4	14	7		7.4							2.1		5.6	5.6
Streptococci. Spp.	7.5			32	13.6			39	35		31			20.3	
Str. agalactiae		23					13						9		
Str. dysgalactiae												0.7	25		
Str. uberis												12.3	2.3		
Str. fecalis												1.6			
Salmonellae	7.2														
Corynebacterium spp.	6.6		8	14	13.6	59.2	59	24		10	25				
Klebsiella spp.	5.2				2.5									3.7	
Enterococci	3.2														
Lactobacillus spp.			14												
Micrococcus				9	1.2	6.4									19.3
Enterobacter spp.					1.2		2.8								
Clostridium spp.												0.6			5.6

1* Ali et al., (2011); 2* Khan and Muhammad, (2005); 3* Beheshti et al., (2011); 4* de Oliveira Moura et al., (2017); 5* Cunha et al., (2022); 6* Bonini Pardo et al., (2007); 7* Costa et al., (1997b); 8* Fagiolo, (2007); 9* Guha et al., (2012); 10* Kaur et al., (2018); 11* Kaur et al., (2015); 12* Moroni et al., (2006); 13* Pankaj et al., (2013); 14* Srinivasan et al., (2013); 15* Dhakal et al., (2008)

2.11. Diagnosis of SCM

Direct measurements such as somatic cell count (SCC), electrical conductivity (EC), bacteriological culture findings are helpful for the assessment of udder health and categorization between SCM and CM milk samples of buffalo reported in the previous study (Dhakal et al., 2008). California Mastitis Test (CMT) performed best when compared to the gold standard of SCC. In order to diagnose SCM in dairy river buffaloes, Isloor et al., (2015) suggested combining the CMT with the SCC will be the best option to diagnose of SCM. Subclinical mastitis was detected by the SCC, EC, CMT, bromothymol blue test, and N-acetyl glucosaminidase test in 48.4%, 40.0%, 45.8%, 61.1%, and 61.6% of the cases, respectively (Preethirani et al., 2015).

2.11.1 Indirect measurements

2.11.1.1 CMT

Leucocytes and epithelial cells, which are referred to as somatic cells, are regularly assessed in the milk of cows by the California Mastitis Test (CMT), which was developed by Schalm and Noorlander, in 1957. It is based on how an anionic surfactant interacts with the DNA of the somatic cells present in the milk samples. The thickness of the produced gel increases in direct proportion to the likelihood of mastitis (Thiers et al., 1999; Xia, 2006). There are authors who think that CMT can play a significant role as a field diagnostic of buffalo subclinical mastitis due to its simplicity, speed, and affordability (Vianni et al., 1990). In Brazil, Bonni et al., (2007) found 30.93% of SCM positive in buffalo quarters milk samples with CMT. However, other studies in Brazil recorded 14.5%-16.8% of SCM positive in buffalo milk samples (Costa et al., 1997b; Oliveira, 1997; Bonini Pardo et al., 2007). In another study, Dhakal et al., (2006) observed positive relation of CMT positive samples with the SCC value in buffalo milk samples. Almost 94% of CMT-negative quarters had somatic cells below 200×10^3/ml. When compared to CMT negative quarters, the mean SCC of the positive quarters was significantly higher ($P<0.01$) (Dhakal, 2006). Beheshti et al., (2011) found little consistency between CMT and culture test findings. Positive CMT and SCC findings were obtained at 34.82% and 45.77%, respectively from collected buffalo milk samples. The specificity and sensitivity of the CMT test in detecting SCM were 31.79%; and 46.43%, respectively (Beheshti et al., 2011). However, several earlier findings revealed that CMT tests show lower SCM positive than bacteriological culture in milk samples (Dhakal, 2006; Karimuribo et al., 2006; Özenç et al., 2008; Beheshti et al., 2011).

2.11.2 Direct measurements

2.11.2.1 SCC

Microbial contamination alters the content of milk and makes it less suited for processing and consumption. The somatic cell count (SCC) is a helpful indicator of milk quality and subclinical mastitis. There are currently no quality requirements for buffalo milk, however, research study indicates that it has a lower SCC than cow or bovine milk in all normal, SCM, and CM sample samples (Dhakal, 2004; Moroni et al., 2006). The mean normal SCC of buffalo milk sample observed in the different studies is 130×10^3 cells/ml 160×10^3 cells/ml; 171×10^3 cells/mL (Dhakal et al., 2008; Özenç et al., 2008; de Oliveira Moura et al., 2017). In subclinical mastitic buffaloes, a few studies have shown positive correlation between SCC and bacterial count (Dhakal, 2006).

Table 2.4: SCM positive SCC values on buffalo milk samples by different studies

Country	SCC value (cells/ml)	References
Italy	$<400 \times 10^3$	Galiero et al., (1996)
	221.28×10^3	Tripaldi et al., (2003)
	$\geq 200 \times 10^3$	Moroni et al., (2006)
	$\geq 200 \times 10^3$	Tripaldi et al., (2010)
Brazil	63,610	Ceron-Munoz et al., (2002)
	259×10^3	de Oliveira Moura et al., (2017)
Nepal	$\geq 200 \times 10^3$	Dhakal, (2006)
	799×10^3	Dhakal et al., (2008)
Sri Lanka	140×10^3	Silva, (1994)
Turkey	130×10^3	Özenç et al., (2008)

2.11.2.1.1 SCC and udder health

Somatic Cell Counts (SCC) are not only acknowledged as a global indicator of udder inflammation (Smith, 2002), but they also serve as a warning indication for improper farm management, inadequate milk quality, overall discomfort, and mammary stress (Galiero and Morena, 2000). Somatic cell count is therefore used to evaluate farm management, udder health status, and milk appropriateness for human consumption (Reichmuth, 1975; Harmon, 1994). Additionally, SCC has been used to diagnose mastitis in buffaloes. In fact, earlier researches (Dhakal et al., 1992; Singh and Ludri, 2001) suggest that an SCC value of above 200,000/mL is likely indicative of udder infection. The yield of milk and lactose is negatively impacted by high SCC in buffaloes (Ceron-Munoz et al., 2002). According to Tripaldi et al., (2003) SCC between 100 000 and 200, 000/ml result in increased milk yields as well as superior chemical and technological properties. Moroni et al., (2006) reported 63% of quarters of collected buffalo milk samples are infected and the SCC value of these infected quarters was >200,000 cell/mL, whereas 98% of quarters with SCC <200,000 below this threshold were uninfected in his study. According to these numbers, the sensitivity and specificity were 99.1 and 100%, respectively (Moroni et al., 2006). Similarly, several studies recommended SCC value ≥200 000/ml with positive bacterial cultures to diagnose SCM in buffaloes (Dhakal, 2006; Tripaldi et al., 2010). Again, in another study, Dhakal IP et al. (2008) reported mean SCC in normal, subclinical, and clinical mastitic milk is 171 x 10^3, 799 x 10^3, and 6039 x 10^3 cells/mL, respectively from 216 quarters of 54 Murrah crossbred buffaloes (Dhakal et al., 2008). According to Smith et al. (2002), a quarter of Holstein cows producing milk with >200 x 10^3cells/mL is classified as sub-clinically mastitic, while quarters with 100 x 10^3 cells/mL are classified as healthy.

2.11.2.1.2 SCC and milk quality

SCC is typically used to maintain milk's hygienic standards and is particularly useful as a sub-clinical mastitis indicator. In addition to decreasing milk yield, mammary epithelial inflammation alters milk composition, which has an impact on cheese-making characteristics, yield, and composition. There have been some studies on cow milk (Tripaldi, 2005). However, there is a lack of studies on the physiological threshold of the somatic cell count or the effects of somatic cell count on milk quality in the case of buffalo milk (Esposito et al., 1997; Tripaldi et al., 2003). Regarding the hygienic and sanitary qualities of buffalo raw milk, Italian rules only imposed a restriction for the overall bacterial count; there was no limit set for the somatic cell count. Furthermore, similar to cow's milk the European Union Directives (46/92 and 71/94) specified a limit of 400,000 cells/mL for SCC in manufacturing buffalo raw milk products (Moroni et al., 2006). High SCC degrades milk proteins severely, which has an impact on the production of mozzarella cheese and its quality. It also affects the quality and shelf-life of pasteurized milk (Ma et al., 2000).

2.11.2.2 Electrical conductivity

Mastitis damage mammary epithelia which alter the equilibrium of sodium, potassium, and chloride ions in milk leading to variation in the conductivity of milk (Kitchen, 1981). A rise in sodium and chloride contents results in increased Electrical conductivity (EC) in the milk (Biggadike, et al. 2002). However, changes in EC occur prior to the development of visible clinical signs of mastitis (Milner et al., 1996). The absolute EC score is the reading obtained directly from the EC meter, whereas the differential EC score is the difference between the highest and lowest absolute EC score (Musser et al., 1998). Dhakal IP et al. (2008) measured EC values in milk from 216 quarters of 54 Murrah crossbred buffaloes. EC values are significantly higher in buffalo milk samples from the infected udder. The study recommends that a cut-off value of 3.7 mS/cm is the optimal balance between sensitivity and specificity for EC score-based mastitis diagnosis in buffaloes. Clinical mastitic milk's mean of the highest absolute and differential EC scores differs significantly ($P<0.001$) from normal milk's. The study also suggests that EC scores significantly rise in the first month following calving compared to the mid and late lactation periods ($P<0.05$) (Dhakal et al., 2008).

2.11.2.3 Isolation and identification of bacteria

2.11.2.3.1 Bacterial culture

Bacteriological cultural findings are one of the useful tools to diagnose SCM in milk samples. Bacterial count has a stronger correlation ($r=0.621$) between average SCC/ml and bacterial number in foremilk samples of Murrah buffaloes, whereas bacterial number has been in increasing trend with SCC (Dhakal, 2006). The quarter-wise percent prevalence on the basis of SCC (11.04%) found as similar to bacteriological examination (11.65%). Dhakal et al., (2008) examined the presence of bacteria in 216 quarters of 54 Murrah crossbred buffaloes to characterize milk samples. *Coagulase-*

negative Staphylococci (CNS (non-aureus staphylococcus:NAS)) were found as major pathogens associated with SCM, while CNS and coliforms predominated in CM. Among environmental pathogens *Bacillus* spp., *Streptococcus* spp. and *Clostridium* spp. found from the SCM quarter milk sample (Dhakal et al., 2007; Dhakal et al., 2008). Another study notified *Staphylococcus aureus* is one of the most important pathogens causing mastitis in dairy cows and in Mediterranean buffaloes (Guccione et al., 2014). Isolation in pure culture revealed that Staphylococcus spp. (41%) had the highest frequency, whereas Staphylococcus epidermidis species accounts for 18%, followed by *Streptococcus* spp. (32%), where *Streptococcus uberis* (13.6%) had the highest prevalence, and *Corynebacterium bovis* (*C. bovis*) (14%). *Micrococcus* spp. (9%) and *Escherichia coli* (4%) microorganisms were also identified in buffalo milk samples (de Oliveira Moura et al., 2017).

2.11.2.3.2 Matrix Assisted Laser Desorption Ionization Time-of-Flight

The identification and characterization of *staphylococci spp.* can be performed using physical techniques such as (MALDI-TOF). DNA microarrays have been created in recent years not only to study the expression of several genes in tissues but also to genotype microorganisms and conduct epidemiological research. The MALDI-TOF method is based on the gentle ionization principle, whereas ion production safeguards the integrity of the sample, such as bacteria. It generates single-charged ions that make data interpretation simple (Everley et al., 2008). It is a quick replacement for conventional identification techniques for blood culture specimens (Clerc et al., 2013; Nagel et al., 2014). Compare to biochemical tests on blood cultures because of their capacity to deliver data more quickly while preserving a comparable sensitivity and specificity (Kohlmann et al., 2015; Scott et al., 2016).

Bacterial isolates are re-cultured in blood agar and just one pure colony is discovered on a MALDI-plate, it appears to produce a high percentage of accurate species identifications (Sauer et al., 2008; Ndahetuye et al., 2019). Then, using the MALDI Biotyper software, isolates on MALDI plate were analyzed. Mass spectra were compared to 4613 spectra in the MALDI Biotyper database and library (Sauer et al. 2008; Ndahetuye et al., 2019). A score of 2 suggests a likely species identification, a score of 2 but 1.7 implies a likely genus identification, and a score of 1.7 indicates a doubtful identification (Ferroni et al., 2010; Nonnemann et al., 2013; Scott et al., 2016).

To understand the true situation of SCM in dairy farms, a diagnosis of SCM is crucial. For the purpose of this study, a number of diagnostic techniques, including CMT, Bulk milk somatic cell count (BMSCC), conventional bacterial culture, and MALDI-TOF, will be applied to identify SCM and Intra-mammary infection (IMI).

2.12. Risk factors influencing SCM

Several risk factors related to health, management and bio-security measures in farm, animal and quarter levels are responsible in the case of SCM in both commercial and traditional dairy buffalo farming systems. Animal level factors such as lactation stage, number of lactations, body mass index, udder shape and milk yield; different management practices like udder preparation; and bio-security like a source of animal, manure removal and deworming notified as potential risk factors of SCM ($OR>1$; $P<0.05$). Quarter-level variables are also significantly associated ($P<0.05$) with SCM such as teat shape and position, teat dipping status, and udder preparation, (Asghar et al., 2019). The prevalence of mastitis in dairy animals is strongly influenced by environmental and management factors along with general preventive practices (Nyman et al., 2007; Steeneveld et al., 2008).

2.12.1 Environmental risk factors

2.12.1.1 Heat stress

The best or optimum climatic conditions for growth and reproduction in buffaloes are 13–18 °C of air temperatures, 55–65 % of average relative humidity, 5-8 km/h of wind speeds and moderate sunlight levels (Payne and Tech, 1990). Exposure to high ambient temperatures in tropical and subtropical regions is the main factor limiting buffalo productivity in hot climatic regions (Marai et al., 1995; Marai and Haeeb, 2010). When high ambient humidity is present, the effects of heat stress are exacerbated. As a result, it causes a sequence of abrupt alterations in the biological processes of the body, which impairs the animal's ability to reproduction performance (Marai et al., 2002; Marai et al., 2006; Marai and Haeeb, 2010). Sodium and Potassium requirements for milk production appear to be different in thermally stressful conditions compared to thermally neutral conditions (Beede et al., 1985). It has been found that daytime temperature and humidity affect mastitis infection in dairy animals (Lescourret et al., 1995; Steeneveld et al., 2008, Breen et al., 2009).

2.12.1.2 Season

Buffaloe's calving in summer (128,130 cells/ml) and spring had the highest SCC in comparison with calving in winter (78,180 cells/ml) and autumn season (Sahİn et al., 2017). In contrast, Moroni et al., (2006) reported the mean SCC increased ($P < 0.05$) and higher tendency in the winter and spring months (December to May) than in summer and fall (June to November) (Moroni et al., 2006). Similarly, another study mentioned, that SCM prevalence is higher in winter months (40.90%) comparatively than summer months (25.71%) (EL-Naker et al., 2015). According to Aliul et al., (2020), the rainy season (11.11%) had the highest prevalence of SCM followed by summer (10.94%), and winter (10%) seasons respectively. Similarly, during the 1st four months of lactation, rainy seasons have an association with higher infection rates. After the fifth lactation, calving buffaloes that are machine milking were observed to have a high rate of IMI in the late spring and early summer (Özenç et al., 2008). The highest incidence of CM was recorded in the summer (37.3%), followed by the autumn (31.7%) season in Nepal (Dhakal, 1997; Dhakal et al., 2007).

2.12.2 Farm level risk factors

2.12.2.1 Farming system

Farming system is one of the important risk factors in the SCM of buffalo. Traditional backyard farming in rural areas; semi-intensive, intensive and extensive free-range farming in coastal saline regions are the common type of farming in buffalo in the tropical region of Asia. In commercial farms, Asghar et al., (2019) found a high prevalence (77.3%) of SCM than subsistent (22.7%) buffalo farming. Asghar et al., (2019) also discovered that prevalence of SCM in commercial farms (77.3%) is higher due to lack of proper management and biosecurity measures than in traditional buffalo farming (22.7 %). In Pakistan, the highest prevalence of SCM in buffalo was recorded at individual backyard farming (58%) followed by peri-urban area (42%) in small holding types and the lowest prevalence at organized farms (32%) where the least management condition are practices (Ali et al., 2011). Similarly, another study also reported the highest prevalence in peri-urban (25.12%) than rural (19.74%) areas in Pakistan. It might be due to the carelessness of farm owners, thereby less animal care and an unhygienic milking system by laborer in peri-urban areas increase the incidence of mastitis (BILAL, 2004). Again, the prevalence of SCM also depends on housing type. Rahman et al., (2014) revealed that kaccha types of farms (38.6 %) had the highest prevalence followed by semi-pakka (32.7 %) and pakka types of farms (28.7 %) (Ali et al., 2014).

2.12.2.2 Herd size

Mastitis in buffalo is typically contagious, therefore as the herd size grows the prevalence of the mastitis also increases (Allore, 1993; BILAL, 2004). According to Ali et al., (2014), there is a much higher chance of mastitis in bigger herd sizes of buffalo farms, with the highest prevalence in large (40%), medium (32%), and small herds (27%) respectively (Ali, Rahman et al. 2014). In Bangladesh, large and medium-sized farms were also found to be more susceptible to SCM in water buffaloes (Aliul et al., 2020). Similarly, another study also reported the prevalence of mastitis was higher in large (52.6%) herds followed by medium (46.5%) and small (40.2%) (Abid et al., 2018). So, it can be concluded that mastitis cases must be screened at the herd size and quarter levels in order to control the SCM.

2.12.2.3 Water

The body temperature of buffaloes in the hot sun could only be kept normal in the shade or by wallowing (Marai and Haeeb, 2010). In shade or in the wallow, buffaloes cool off quickly, radiate heat efficiently, but buffaloes cool off more quickly than cattle in the shade. Experience has particularly demonstrated that wallowing is not necessary (Marai and Haeeb, 2010). However, water buffaloes need to immerse themselves in water to maintain body temperature during the hot summer months, which impacts feed intake, reproduction, milk production behavior, and disease control (Nayak and Mishra, 1984). This wallowing behavior of buffaloes was found to have a substantial impact on both clinical and sub-clinical mastitis, with a relative risk of 1.2 and 1.01, respectively (Kavitha et al., 2009). Research reports revealed that water buffaloes that bathe in contaminated surface water have a high prevalence of bovine fasciolosis, tick infestation, CM, and FMD (Khan et al. 2009; Elahi et al., 2017). Teat ends are proven to remain open for about an hour after milking (Neijenhuis et al., 2001). Buffaloes that bathe in sewage within 30 minutes of post-milking are more likely to be subjected to bacterial penetration in the teat ducts, which could cause an IMI infection ($p \leq 0.01$), resulting in greater economic losses (Elahi et al., 2017; Elahi et al., 2018). This raises the possibility that water buffaloes bathing in a common pool could spread a variety of contagious diseases from sick animals to healthy ones, which may increase the exposure to mastitis (Harwood et al., 2014; Elahi et al., 2017). So, wallowing tanks should be cleaned and disinfected at least once a week to prevent the spread of various contagious diseases or they can bathed with hose pipe.

2.12.2.4 Floor

According to data the prevalence of mastitis in buffalo is influenced by the type of floor (Brick, cemented, and Kacha). Most cases of mastitis were reported when keeping animals on brick floors (BILAL, 2004). Cleanliness conditions of a farm including the kacha and cement floor and using the proper bedding materials may reduce the occurrence of mastitis (Ali et al., 2014). Good management of bedding conditions significantly lower the incidence of mastitis, according to previous research reports (Hoare and EA, 1972; Faull and Hughes, 1985). Several research studies mentioned bedding as another risk factor. The incidence of mastitis was higher when the soil was utilized as bedding material, and it was less common on sand floors than on concrete (Bartlett et al., 1992; Kavitha et al., 2009).

2.12.2.5 Feeding

The buffaloes graze in free-range areas, making them more susceptible to SCM due to ill health and other postpartum diseases. Water buffaloes raised in free-range grazing areas had a prevalence of SCM of 10.99% compared to 5.56% in stall feeding areas (Aliul et al., 2020). Again, another study reported the prevalence of mastitis was higher in buffaloes that practice both stall-fed and grazing (OR=.69) than in those who were simply stall-fed (Hussain et al., 2013).

2.12.2.6 Hygienic practices

Hygienic practices have a significant impact on the rate of mastitis in the herds as well. Mastitis occurred at a rate of 31% and 22.2% in farms with moderate and good hygiene standards, respectively. Farms with low hygiene conditions had the highest rate of mastitis (46.8%) (Ali et al., 2014). Another study revealed that the prevalence of SCM in buffalo is linked with animal cleanliness and high microbial counts in the teat cups, both of which indicate poor hygiene (de Oliveira Moura et al., 2017). The absence of basic hygienic practices increases the risk of mastitis on traditional buffalo farms. The microorganisms that cause mastitis on traditional farms, are mostly fecal and soil-born bacteria, which is a clear indication of the association between hygiene standards and mastitis (Thomas, 2008).

2.12.2.7 Milking methods

Milking methods are one of the important risk factors for having SCM in buffalo. Proper milking methods can help to lower the occurrence of mastitis (Nickerson, 1990). Several studies reported that compared to dairy cows, buffaloes have longer, thicker, and longer teat canals which are important to consider during machine milking (Saxena, 1973; Sastry et al., 1988a; Thomas, 2004). But a higher prevalence of whole hand milking (72.7%) was noticed compared to machine milking (13.6%) at dairy buffalo Farms (Asghar et al., 2019). Usually, there are three types of hand milking practices observed in case of dairy buffalo production such as full-hand method, stripping and folded thumb or knuckling or fisting method. Grasping the teat with all five fingers and press against the teat is full hand method, whereas pressing down the teat with thumb and fore finger is stripping and bend the thumb against teat is called knuckling or fisting method. The prevalence of mastitis was higher in case of folded thumb method compare to full hand milking and induced milk let down through suckling calves (BILAL, 2004). Ali et al., (2014) did not find any significant ($p>0.05$) relation between the incidence of mastitis with the effect of milking methods in their study. However, the incidence of mastitis was high (53.8%) in stripping, and accordingly low in the knuckling (46.2 %) method. Another study revealed that comparing the full-hand approach to knuckling instead of stripping, a high incidence of CM and SCM is observed in knuckling method. For instance, the relative risk found 2.8 and 2.2 for both CM and SCM respectively in case of knuckling method of milking, which exhibit a very high positive correlation between the knuckling method and mastitis (Kavitha et al., 2009).

2.12.3 Animal level risk factors

2.12.3.1 Age of the animal

Age of the animal or lactation number has significant positive correlation ($R^2=0.772$) with the incidence of mastitis. When the number of lactation or age of buffaloes increased, the incidence of mastitis also increased (Ali et al., 2014). Several studies also reported a significant association of mastitis with the age of dairy cattle (Valde et al., 2004; Nyman et al., 2007). SCM prevalence was reported the highest in the age group of 9 to 11 years (90.32%), followed by 6 to 8 years (65.78%) and 3 to 5 years (41.37%) (Kashyap et al., 2019). In Bangladesh, dairy buffaloes aged 7 to 18 years had the highest prevalence of SCM (13.46%), compared to those aged 3 to 6 years (7.29%) (Aliul et al., 2020). Another study also reported similar results, stating that mastitis prevalence increases with age (Radostits et al., 2007). Growing older and being more susceptible to SCM may be related to increased milk production, becoming pendulous udders that are more vulnerable to injuries, and decreasing the trend of immunity with older ages. Also, damage to the protective barrier of the teat sphincter or orifice in aged buffaloes leads to the entry of bacteria (Kashyap et al., 2019).

2.12.3.2 Breed

The prevalence of SCM in buffalo is comparatively higher in crossbred (12.5%) as compared to local breeds (10.42%) (Aliul et al., 2020). Several earlier studies showed that crossbred buffaloes are more vulnerable than other breeds, which may be caused by decreased immunity as a result of milking stress. Crossbred buffaloes (72.30%) had the highest frequency of SCM in terms of breed, followed by indigenous breeds (65.62%) and non-descriptive breeds (47.23%) (Kashyap et al., 2019). While Shrinivasan et al., (2013) observed that SCM prevalence in Murrah, Surati, and nondescript breeds was 15.33%, 5.83% and 4.85% respectively. In contrast, Hussain, Javed et al. (2013) observed no significant difference in SCM between Nili-Ravi and cross-bred buffaloes in case of frequency analysis. But the odds ratio (OR=0.78) in the Chi-square test reveals SCM significantly ($p>0.3$) higher in cross-bred buffaloes than Nili-Ravi (Hussain et al., 2013).

2.12.3.3 Body condition score

A significant association of body condition score (BCS) with the prevalence of SCM has previously been reported (Kivaria et al., 2007), while another study reported a non-significant association (Fregonesi and Leaver, 2001). BCS at medium (OR=2.765) and at good (OR=2.086) levels have an association with having SCM in commercial buffalo farms (Asghar et al., 2019).

2.12.3.4 Parity

The parity has a significant positive association with on the onset of SCM and the lactation number of buffaloes (Beheshti et al., 2011). Mastitis in buffalo occurred more frequently as the parity increased (Moroni et al., 2006), which also observed in other studies(Kivaria et al., 2007; Kavitha et al., 2009; Nyman et al., 2009; Hussain et al., 2013). SCM was significantly more common (P=0.01) in buffaloes with their third or later lactation number (88.6%). Lactating buffaloes with 2nd or greater lactation number were 4.6 times at higher risk of SCM than buffaloes with the first time (Asghar et al., 2019). This could be a result of increased exposure to the milking process, particularly hand milking, which also increases the risk of infection to pathogenic microbes (Neave et al., 1969). However, it has also been noted that there is a non-significant difference in mastitis between animals of different parity (Slettbakk et al., 1995). Another study conducted in Pakistan reported that at the third lactation most of the mastitis cases were found both in peri-urban (19.00%) and rural (22.98%) areas (BILAL, 2004). In Bangladesh, the highest prevalence of SCM found at 2nd parity (11.1%) followed by 10.13% at 1st parity in dairy buffaloes (Aliul et al., 2020).

2.12.3.5 Lactation stage

The stage of lactation had clear effects on the prevalence of SCM in buffalo (Svensson et al., 2006; Compton et al., 2007; Breen et al., 2009; Bhutto et al., 2010). However, SCM prevalence has a variation with lactation stages according to previous studies. The highest prevalence of SCM was found in buffaloes in their early lactation stage (85.2%). Buffaloes in the early lactation stage (P<0.001, OR=14.5) were found at higher risk compared to late lactation (OR=3.646) of SCM (Ali et al., 2014; Asghar et al., 2019). Previous several studies have also reported a significant association between mastitis at an early stage (Breen et al., 2009; Ramírez et al., 2014). Similar findings were also reported by Beheshti et al., (2011), who discovered that the 3rd to 4th month of the lactation stage is more susceptible to SCM (37.94%), followed by the 1st to 2nd month (31.02%), the 5th to 6th month (10.34%), and the 7th month of lactation stage (6.89%). The highest incidence (43.3 %) of mastitis was found in early lactation followed by late lactation (38.6 %) and mid-lactation (18.1 %) (Ali et al., 2014). The high prevalence of SCM during the first two months of lactation may be related to the stress due to changes in the hormonal, nutritional, and metabolic condition of the animal which also happen during the peri-parturient period (Salvador et al., 2012).

Another study mentioned that, in early lactation animals had a significant incidence of CM (51.6%), while late lactation stage animals had a high incidence of SCM (12.9%)

(EL-Naker et al., 2015; de Oliveira Moura et al., 2017). Buffaloes in the 1st to 4th month of and the last part of the dry period (10th to 12th months) are found more favorable to mastitis (Moroni et al., 2006; Kavitha et al., 2009; Salvador et al., 2012). Whereas Moroni et al., (2006) specifically indicated the odds ratio has an approximate 6.3 fold difference in the probability of IMI between the first and tenth month of lactation, which means the least risk in early lactation with the highest probability of IMI infection at late lactation. However, a similar observation was also found from SCC interaction with the lactation stage. SCC decreased in the second month of lactation and increased thereafter up to the ninth month, which means elevated SCC or mastitis occurred with increasing lactation stage in buffalo milk. (Ceron-Munoz et al., 2002; Dhakal, 2006; Moroni et al., 2006; Sahİn et al., 2017). Aliul et al., (2020) also mentioned the highest prevalence of SCM at 12.82% in the late lactation period followed by 5.88 % and 10.42 % in the early and mid-lactation period in dairy buffaloes of Bangladesh (Aliul et al., 2020). Another study found the highest prevalence of SCM in mid-lactation (76.47%) followed by early (67.27%) and late (61.29%) lactation (Kashyap et al., 2019).

2.12.3.6 Milk Yield

The incidence of mastitis has a significant impact on the milk yield in buffaloes. Buffalo's milk yield of more than 5 Liters found as a risk factor for having SCM (Asghar et al., 2019). However, another study mentioned the occurrence of mastitis is a higher rate (63.7%) in lactating buffaloes whose milk output varied from 6 to 10 L/day ($P<0.05$), followed by low milk yielders (≤5L) is 27.5 %, and high milk yielders (≥11L) is 8.80% (Ali, Rahman et al. 2014). Higher SCC is associated with decreased ($P<0.05$) milk yield, possibly because of the effects of dilution (Moroni et al., 2006). High productive buffaloes (4-7L) are more prone to mastitis than low productive buffaloes (Kavitha et al., 2009). Occurrence of mastitis also high in case of high yielding dairy cattle (Slettbakk et al., 1995).

2.12.3.7 Udder shape

Udder shapes in buffalo have much more variation than in dairy cows, because most of the buffalo are not selectively bred. Buffalo udder shapes can be categorized as rounded, pendulous, bowl and cup shaped. Almost 65% of Indian Murrah buffalo have bowl shaped udders. This udder shape is more preferable compare to other shapes since this udder shape extends forwards and backwards instead of downwards (Sastry and Tripathi, 1988b). Previous reports revealed that there is association of mastitis with udder and teat traits in cattle (Chand and Behra, 1995; Shukla et al., 1997; Waage et al., 2001; Compton et al., 2007). Pendulous udders in buffaloes significantly (P=0.002) increase 6.5 times higher risk of infection compared to non-pendulous udders in buffaloes (Asghar et al., 2019). In contrast, the prevalence of mastitis in buffaloes with bowl (27.8%) or round (20%) shaped is higher compared to buffaloes with cup-shaped (7.7%) or pendulous udders (Hussain et al., 2013).

2.12.3.8 Deworming

Proper deworming at definite interval may be an important risk factor of developing SCM in buffalo. In a study it is observed that deworming practices at one year (OR=3.16) interval are 3.2 times responsible for having SCM than those are practicing at two and three year interval in buffalo of Pakistan (Asghar et al., 2019). In Bangladesh there is no published data that resembles any relation of deworming with SCM in buffalo.

2.12.3.9 Sources of animal

Source of animal have vital effect with SCM in buffalo. The buffaloes those are purchased reported by farmers (OR=7.4; P=0.008) found as a potential risk for SCM in dairy buffalo of Pakistan. May be its due to stress for moving one place to another (Asghar et al., 2019).

2.12.4 Quarter level risk factors

2.12.4.1 Quarter level prevalence

There are usually four quarters in buffaloes such as Front Right (FR), Front Left (FL), Hind Right (HR), and Hind Left (HL) quarters. Quarter level prevalence of SCM in buffalo recorded at 16.2 and 37.75% in Pakistan, whereas 42.5% found in Bangladesh. (Abid et al., 2018; Sharif and Ahmad, 2007; Singha et al., 2021). However, prevalence of SCM in buffalo differ among the all four quarters (Table 2.5).

Table 2.5: Quarter level prevalence of SCM in water buffalo milk sample

Country	Prevalence (%)				References
	FR	FL	HR	HL	
	23.8	24.5	30.5	21.2	Sharif and Ahmad, (2007)
Pakistan	6.8	4.7	8.4	5.6	Hussain et al., (2013)
	19.4	26.7	20	33.9	Ali et al., (2014)
Nepal	10	8	8	6	Dhakal, (2006)
	10.1	7.03	10.9	10.9	Kavitha et al., (2009)
India	5.5	11.1	37	46.3	Srinivasan et al., (2013)
	22.4	14.5	24	33.9	Kaur et al., (2015)
	19.2	10	8.3	30.8	Kashyap et al., (2019)

FR=Front Right; FL=Front Left; HR=Hind Right; HL=Hind Left; %=Percentage

2.12.4.2 Teat position

Mastitis is associated with teat position and establishes infection in mammary parenchyma (Nyman et al., 2007). Teat's position has a vital impact on having SCM in Buffalo. Hind quarters have more significant involvement with the prevalence of SCM than front quarters, among infected 6.4% quarters from 90 buffaloes (Naiknaware et al., 1998, Hussain et al., 2013). Ali et al., (2014) found a higher prevalence of SCM in the hind quarters of buffalo compare to the front quarters, and SCM was also higher on the left side as compared to quarters on the right side (Ali et al., 2014). Similar observations are in line with findings of other studies (Kavitha et al., 2009; Sharma et al., 2013; Srinivasan et al., 2013, Zenebe et al., 2014; Kashyap et al., 2019). This could be because of the anatomical position of hind quarters, which have a higher potential for fecal and environmental contamination (Sori et al., 2005) due to direct contact with excreta such as soiled with dung and urine (Naiknaware et al., 1998; Kavitha et al., 2009), as well as high milk yield capability (Radostits et al., 2000) and being more pendulous udder in hind than front quarters (Radostits et al., 2007). Prolonged close contact with floor, which increases the likelihood of teat injury of hind quarters than front quarter's results to IMI infection (Kashyap et al., 2019). Conversely, Dhakal et al., (2006) and Moroni et al., (2006) observed no significant (P>0.05) difference of prevalence between subclinical mastitic quarters of buffaloes. The prevalence rate of subclinical mastitis in different quarters was found to be less than 10%. However, the SCC of FR and HR quarters were significantly higher than FL and HL quarters in murrah buffaloes (Dhakal, 2006).

Single quarter infection in SCM of buffaloes is higher (51.21%) compare to double, triple and quadriple quarter infection found to be 15.85%, 14.63% and 18.29% respectively (Srinivasan et al., 2013; Kashyap et al., 2019). Similar observation recorded by a previous study (Saini et al., 1994). Hind (83.34%) and left side (57.41%) quarters are more prone to SCM than front and right side quarters (Srinivasan et al., 2013).

2.12.4.3 Teat shape

Buffalo have much longer teats than dairy cattle and known as "hard milkers". More force is needed to open the streak canal in buffalo compared to cattle, because sphincter muscle and epithelium of the streak canal are more compact and thicker (Thomas et al., 2004). Sphincter muscle in teat end have vital role for keeping the teat canal contracted between milkings and preventing the entry of microbes into the mammary gland (de Oliveira Moura et al., 2017). Therefore, this increases the epithelium's defense against bacterial invasion (Thomas et al., 2004). The teats of Murrah buffaloes can be categorized as cylindrical (44%), funnel (35%) and bottle shaped (23%) (Sastry et al., 1988a). Again, Thomas et al., (2008) also categorized diverse teat shapes as cylindrical, conical and bottle shaped: 60 to 80% have cylindrical teats, 15 to 30% have conical teats and only 3 to 8% have bottle shaped teats (Thomas, 2008). Hussain et al., (2013) observed association of mastitis with teat conformation. High prevalence of SCM (P <0.0001) was noticed in Cylindrical (29.5%) and round (21%) shape teats then pointed (5.3%) teats. Accordingly, another study also mentioned cylindrical shaped (OR=2.559, CI=0.48-13.66) teats have an association with SCM in buffalo (Asghar et al., 2019). The prevalence of mastitis was higher in buffaloes with teat/udder lesions such as teat skin abrasions (35.5%).

2.12.4.4 Teat dipping

Higher risk of SCM observed where udder preparation and dipping were mostly not performed (Ramírez et al., 2014; Asghar et al., 2019). None of the farmers in India was used to practicing teat dipping as a preventative strategy in buffalo (Kavitha et al., 2009). Asghar et al., (2019) remarked those are not performing teat dipping (OR=6.7; P=0.02) in antiseptic was found as a potential risk for SCM in buffalo (Asghar et al., 2019). Therefore, it is important to inform farmers about the risks associated with mastitis and teat dipping. In Bangladesh, there is no report that represents the relationship of teat dipping with mastitis in buffalo.

2.13 Controlling of Subclinical mastitis

2.13.1 Therapeutic management

Early detection of mastitis is thought to improve control and treatment of the condition in the developed world. However, farmers, managers, and owners in this case are not familiar with diagnostic techniques in developing countries. Mastitis affects not just the individual animal but also the entire herd, or at least a number of animals within the herd. Mastitis in buffaloes can be both clinical and subclinical form depending on the condition's severity, duration, kind of exudates, and root cause. If left untreated in the subclinical form leads to clinical form (Sharma, 2007). Vitamin (A, D3, E, and H) supplementation enhance host defense mechanism in mammary gland and helps to recover from SCM. Vitamin D turns on bovine monocytes' natural immune responses and brings the balance between oxidants and anti-oxidants back to normal (Merriman et al., 2017; Merriman et al., 2018). Intra-mammary treatment with vitamin D reduce the number of bacteria in milk. Vitamin D has been marked as therapeutic drug due to its anti-microbial activity and inflammatory response in mammary tissue (Nelson et al., 2018). Supplementation of vitamin E and selenium makes the phagocytic activity better, which decrease the risk of clinical mastitis along with reduction of SCC in mastitic animals (Heinrichs et al., 2009; Mukherjee, 2008). Patil et al., (2021) observed up to 70 and 80% therapeutic efficacy at animal and quarter level respectively in CMT positive quarters, while using herbal preparation of Aloe vera paste (Aloe vera leaves 200g, turmeric powder 10g, castor oil 10 ml and one lemon) tropically along with Vitamins, antioxidants and immune modulators.

2.13.1.1 Dry Cow therapy

Herd health management program like blanket dry-cow therapy or selectively drying off is useful to control SCM and needs fewer antimicrobials to treat mastitis cases (Stevens et al., 2016). The goal of dry cow therapy is to get rid of Intra-mammary infections (IMIs) that were already there and stop new ones from happening during the dry season (Berry & Hillerton, 2002; Derakhshani et al., 2018). When IMIs are treated during the dry season, the rate of cure is higher (Cameron et al., 2015; Halasa et al., 2009). In a study, the effects of dry cow therapy were compared to those of not treating the cows. In the treated group, there were no clinical cases of mastitis in 587 quarters during the dry period, but 14 out of 671 quarters infected with clinical mastitis and new IMIs (58 in untreated and 19 in treated group) after calving observed in the both group (Berry & Hillerton, 2002). Selective dry cow therapy is a type of dry cow therapy in which antimicrobials are chosen based on the results of the culture and sensitivity. This reduces the use of antimicrobials in dairy production that aren't needed (Cameron et al., 2015). Using internal teat sealant along with antibiotic dry-cow therapy significantly reduce the SCC and improve the prevention of SCM (Golder et al., 2016). In a study it was observed that 83% quarters kept the internal teat sealant plug till the first milking after calving, which is helpful to prevent SCM (Kabera et al., 2018).

2.13.2 Preventive measures related to Subclinical mastitis

The key to creating effective control strategies is having a detailed understanding of the epidemiology of infections in dairy animals. Contagious and environmental pathogens both are responsible for having SCM in buffaloes like other dairy animals. There are some control measures developed for controlling SCM. Implementing numerous hygienic/preventative measures can often prevent mastitis (Hussain et al., 2013). Strategical implementation of technology training package including (1) developing good husbandry practices, implementing mastitis detection and control technologies; and (2) training of technicians and farmers can reduce SCM in dairy animals. In Nepal, six months after the implementation of such a technological training package, they observed SCM prevalence reduced from 55% to 28% in dairy cows and from 78% to 18% in buffalo, respectively (Sah et al., 2020). The detection of various udder and teat features like smaller hind quarter and pendulous udder shape and absence of hyperkeratotic teat-end lesions can also help to lower the occurrence of mastitis in dairy buffalo (Carlen et al., 2004; Bhutto et al., 2010). Different good husbandry practices should be trained to the farmers to prevent SCM in buffalo such as: 1) knowledge about SCM, 2) use of a concrete and dry floor, 3) regular cutting nails of milkers, 4) washing hands with soap before milking, 5) washing udder with disinfectants like soap 6) wipe udder with a cloth before milking, 7) Strip off milk, 8) let animals stand after milking, 9) use of stainless steel milk container, 10) record keeping, 11) dry cow therapy and 12) bedding materials (Fagiolo and Lai, 2007; Adkins and Middleton, 2017; Sah et al., 2020). Similarly, frequent bacteriological screening of the herd should be done based on the SCC and CMT results in order to use an effective and prompt mastitis therapy (Fagiolo and Lai, 2007).

Awareness of potential risk factors is essential for the control of bovine mastitis. Control measures relevant to sources of mastitis pathogens are also helpful to prevent environmental mastitis. Unused or used bedding materials and cow feces are the main sources of environmental infections. For instance, sawdust is known to increase the incidence of *Klebsiella* mastitis (Munoz et al., 2007; Unnerstad et al., 2009). Straw bedding is known to increase the risk of *S. uberis* mastitis (Unnerstad et al., 2009), but in the pasture-based rearing method, *S. uberis* is also very common throughout the pasture season (Lopez-Benavides et al., 2007; Riekerink et al., 2007). Thus, priority should be given to mastitis prevention measures during the dry season, when buffalo cows are more susceptible to mastitis pathogens and environmental germs (Fagiolo and Lai, 2007). Good environmental hygiene or use of teat-sealants in the dry period and adequate hygiene or nutrition during the lactation period are helpful in case of environmental mastitis of dairy cows (Klaas and Zadoks, 2018).

2.13.3 Prevention through vaccination

Vaccination can be effective in preventing mastitis in dairy cows. Vaccination against mastitis can minimize losses due to milk yield and the severity of mastitis, which are enough to cover the cost of vaccination (Bradley et al., 2015; Schukken et al., 2011). Most of the work on making a vaccine for mastitis has been on *E. coli*, *S. uberis*, and *S. aureus*. Available vaccines against *S. aureus* mastitis are still not good enough, although it has been trying to develop since 1960s. So, vaccination is especially important to prevent environmental *S. aureus* mastitis (Landin et al., 2015). In the early 1970s, researchers started to develop vaccines against *Str. agalactiae* mastitis in dairy cows (Johnson & Norcross, 1971). According to Liu et al. (2017) it often takes a long time for mastitis vaccines to go from possibility to reality (Yancey Jr, 1999; Liu et al., 2017). However, there is no substantial data available in buffalo to vaccinate for controlling SCM.

2.14. Conclusion

SCM causes significant losses to milking buffaloes due to its effect on milk production and quality. Noakhali district is one of the most concentrated buffalo rearing riverine zone and famous for production of traditional curd. Most of the time of the year the buffalo are reared in the island where natural grasses are available. Buffalo owners and care takers are not conscious of proper milking procedures and production loss due to diseases like mastitis. Screening tests like Bulk milk somatic cell count and on-farm CMT is an important indicator of SCM diagnosis which is still unfamiliar in buffalo farmers of Noakhali. Again, according to above knowledge review it appears there are many scientific inconsistencies in assessing actual burden of SCM in dairy buffalo, determining the threshold of BMSCC in Bangladesh context, factors in association with SCM at different levels, pathogens associated with SCM in Noakhali district, Bangladesh. Thereby it’s important to conduct a cross-sectional study to identify the prevalence of SCM and potential risk factors responsible for developing SCM in water buffalo. The epidemiological and bacteriological findings through this study will lead to the development of more effective control strategies for buffalo subclinical mastitis and the implementation of the essential steps for the improvement of buffalo milk quality control in Bangladesh.

Chapter III: Materials and Methods

3.1 Study location

Bangladesh is a riverine country with a lot of rivers, low laying areas and coastal border line covering an area of 147,570 sq. km. (56,990 sq. miles). Traditionally buffaloes of Bangladesh are mostly found in the coastal and river basin areas of the country and 40% of the buffaloes are concentrated in the coastal areas (Faruque et al., 1990). The current study was conducted in Noakhali district which is a coastal area located in the south-eastern part of Bangladesh (22º07` and 23º08` north latitudes and between 90º53` and 91º27` east longitudes). The total area of the district is 3,685.87 sq. km. (1423.12 sq. miles) of which 337.13 sq. km. is under reserve forest. The average annual temperature ranges from 14.4 °C to 34.3 °C with an average rainfall of 330mm yearly (BBS, 2011). The two main rivers of the district are Meghna and Bamni. Noakhali District consists of 10 upazilas, 8 municipalities and 72 wards. Subarnachar Upazila covers 382.12 sq km area, and is located in between 22°28' and 22°44' north latitudes and in between 90°59' and 91°20' east longitudes and Hatiya upazila covers 1508.23 sq km and is located in between 22°07' and 22°35' north latitudes and in between 90°56' and 91°11' east longitudes. Hatiya upazila is bounded by Noakhali sadar and the Bay of Bengal on the south and the east (Fig. 3.1). In the south of Hatiya upazila there is an inhabited island in the Bay of Bengal named as Swarna Dweep, which is run by Bangladesh army as training camp (BBS, 2011).

3.2 Study population

In Noakhali district, buffaloes are mainly reared in bathan (free range) system and some households and semi-intensive farms are also found in this area. Farmers are solely dependent on natural grazing land in the bathan system. However, in household and semi-intensive system sometimes they provide little amount of concentrate to the buffaloes. Our reference population for the study was the buffaloes of Noakhali district and source population was Subarnachar and Hatiya upazila of Noakhali district (Fig. 3.1). In Subarnachar, some buffalo concentrated zones and in Hatiya upazila Military Buffalo Farm in Swarna Dweep, were selected for this study. The Military Farm (buffalo no =278) is a semi-intensive farm and they are supplemented with concentrate and other conventional grasses. After milking buffalos go to the nearby pasture island for grazing and comeback in the afternoon. During the study period no intensive or commercial buffalo farms were observed. According to buffalo population of each studied buffalo farm lowest 25%, 50% and 75% of herd size was Small (<15 buffalo), Medium (15-40 buffalo), and Large (>40 buffalo), respectively. The studied herds were reared in free ranging (bathan), and household (semi-intensive) system. There were 1to 3 buffaloes per household and 51 to 200 buffaloes in a bathan. During the dry season, wealthy farmers gave buffalo a small amount of concentrate feed. On the other hand, buffaloes are allowed to graze on public land in bathan. Most of the buffaloes grazed for 8 to 12 hours in daytime and take rest under the open sky at night. Average milk production of lactating buffalo was 2 to 3L in bathan. Hand milking was the only milking method and none of the milkers are concerned about udder hygiene such as udder wash, teat dipping and fore-stripping among the studied farms.

3.3 Study design and Sample size

A cross sectional study was performed during March-April, 2021 in 6 zones of Subarnachar and Hatiya upazila of Noakhali district. A total of 45 buffalo farms with 1660 animal heads and 385 lactating buffaloes were included in the study with the help of upzila veterinary hospitals and local service providers. The farms were conveniently selected according to the farmer’s willingness and ease of accessibility. The sampling population was 183 lactating buffaloes with 732 functioning udder quarters. A total of 12 household/semi-intensive (1 in Swarna Dweep island of Hatiya, 11 in Subarnachar) and 33 bathan (free ranging) type buffalo farms from Subarnachar were included in the study . Only 1 semi-intensive buffalo farm was included from Hatiya due to its large herd size (n=278). Due to same management and rearing system of the studied households and semi-intensive farms, the semi-intensive type was also considered as household farms. The average lactating buffalo was 4 and maximum was 31 in the studied 45 buffalo farms. A convenient sampling technique (2–4 lactating buffaloes from each bathan/household and all lactating buffaloes from the semi-intensive farm in Hatiya) was applied due to inaccessibility to the other buffalo farms in the other buffalo concentrated island (Urirchar) in Hatiya upazila. Due to less rain there was scarcity of grasses and thus, lactating buffalo was unavailable in several areas of Subarnachar and Hatiya upazila (Fig.3.1).

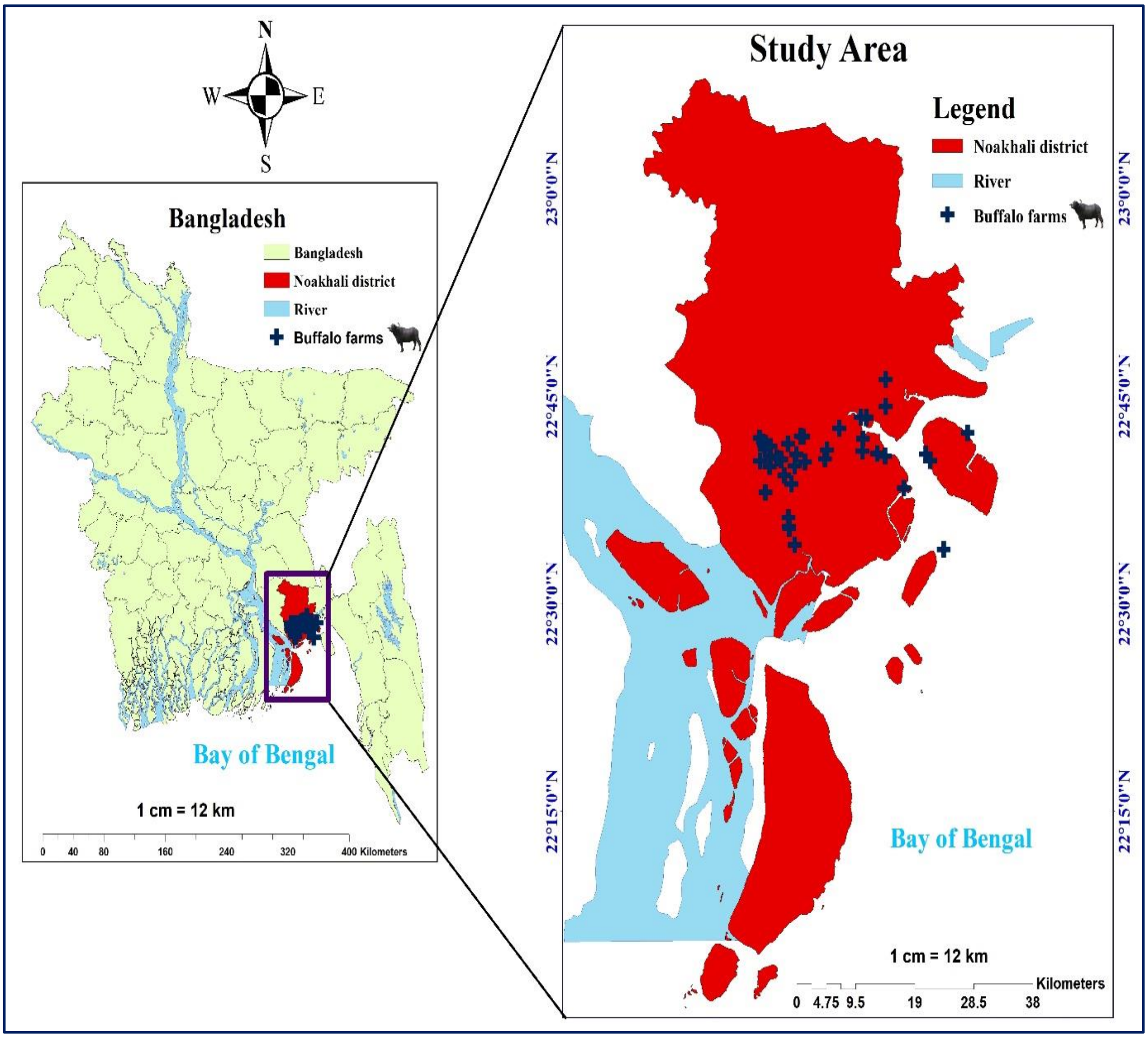


Fig. 3.1: Geographical location of buffalo herds (n=45) in studied area

3.4 Epidemiological data collection

A well-structured and pretested questionnaire was applied to collect multilevel (farm, animal, and quarter) data through face to face interview and close physical on farm observation. The questionnaire consists of multi-aspect information about water buffalo farms such as participant information, demographic features of the farm, rearing system, feeding management, record keeping, milking and information about clinical and sub-clinical mastitis, udder health and hygiene, therapeutic, and preventive management of the farm. A pilot study was conducted to find out any inconsistencies in the questionnaire in Subarnachar upazila in 14 buffalo farms. A team of 6 members collected the data through interviews. Among the variables heat stress calculated according to Tucker et al., (2008) and categorized according to Armstrong, (1994).

Before administration of the questionnaire, a written consent was taken from each of the farmers about their willingness and further collaboration in this study. All the interviews were conducted in Bengali, and the questionnaire was completed in English. The final structured questionnaire is attached in **Appendix-I.**

3.5 California Mastitis Test and Milk sample collection

Quarter milk sample (3-5ml) was collected after doing California Mastitis Test (CMT) from each clinically healthy buffaloes. Teat ends were cleaned vigorously with cotton ball soaked in 70% alcohol before doing CMT. Then, 2 ml of milk were collected from each quarter in CMT paddle and an equivalent amount of CMT reagent was mixed with them (DeLaval Group, Stockholm, Sweden; Se 67.4%; Sp 86.9%) (Fosgate et al., 2013). Quarter sample categorized as 1 to 5 score according to procedure mentioned by (Baloch et al., 2016). Both of SCM positive (CMT ≥2) and negative (CMT =1) quarter milk sample (5ml) were collected aseptically for bacteriological culture. In order to assure aseptic sample collection, the teats were cleansed once more using sterile cotton soaked in 70% ethyl alcohol before sample collection. The whole process of collecting quarter milk samples was conducted according to NMC protocol (Adkins and Middleton, 2017). The collected samples were immediately placed in an insulated icebox and two hours later, moved to a freezer (-10 to -15 °C). After completing 12 days field visit the samples were transported to the Udder Health Bangladesh Laboratory at Chattogram Veterinary and Animal Sciences University. At the laboratory, the milk samples were stored at −20 °C and bacteriological culture was performed within 24 h.

3.6 Bulk milk sample collection and BMSCC

Milk from each lactating buffalo in the farm was kept in the tank. Then, bulk milk (50 ml) samples were collected from bulk tank of each buffalo farm in 50 ml sterile falcon tubes after gently thorough mixing. Each sample was given different identification mark. Collected bulk milk samples were immediately placed in an insulated icebox at <5 °C. The whole process of collecting bulk milk sample was conducted according to NMC protocol (Adkins and Middleton, 2017). Bulk milk somatic cell count (BMSCC) was evaluated immediately using the DeLaval Somatic Cell Counter (DeLaval Group, Stockholm, Sweden; Se: 88% and Sp: 97.8%) (Zecconi et al., 2019). A cassette filled with 60 µl of bulk milk was placed in the chamber of automated DeLaval Somatic Cell

Counter (DSCC) device. Then milk was stained with preloaded DNA-specific fluorescent reagent in the cassette automatically. After two minutes the monitor represents the SCC result through counting of each cell nuclei present in per µl milk sample (Singha et al., 2021). BMSCC result was considered as SCM positive at ≥200 x 103 cells/ml of milk (Moroni et al., 2006; Dhakal, 2006; Tripaldi et al., 2010).

3.7 Identification of pathogens

Bacteriology of quarter milk sample completed at the Udder Health Bangladesh laboratory of Chattogram Veterinary and Animal Sciences University. A total of 320 quarter milk samples consisting of CMT positive quarter (n= 164) and randomly selected CMT negative milk samples (n=156) from each 183 lactating buffalo were collected. A brief flow chart of identification of pathogens is attached at **Appendix-II**

3.7.1 Pure culture attainment

Blood (5% bovine blood) agar plate prepared and evaluated for any contamination before streaking of milk sample. Each frozen (-20°C) milk sample (10 µl) was cultured in blood agar plate after thawing in room temperature and transferred for incubation for 24 h at 37 °C following the standard bacteriological protocol (Adkins & Middleton, 2017; Singha et al., 2021). Inoculated blood agar plate was checked after incubation of 24h. Colony growth in blood agar was characterized according to morphology. Samples in blood agar plate were taken in consideration of positive if at least one bacterial colony grow. The contaminated (>2 bacterial colony yield) milk samples on blood agar excluded from the analysis of the study. If two bacterial colonies yield with similar morphology in each agar plate then it was re-cultured again in bovine blood agar plate. After 24 h of incubation at 37 °C obtained pure culture was added in enriched non-selective media (Brain Heart Infusion Broth) and again incubated in 37 °C for multiplication. Then, overnight cultures (700µl) were transferred in sterilized cryovial with 50% glycerol (300µl) and stocked at the temperature of -80°C for longer time preservation (Petti & Carroll, 2011).

3.7.2 Identification of genus in conventional bacteriology

Pure culture was separately inoculated into Mannitol Salt Agar (MSA) and MacConkey Agar (MAC) for the identification of Gram-positive and Gram-negative bacteria, respectively. Those samples had positive growth in MSA, indicated as the

Staphylococcus spp. (De Visscher et al., 2013). Then, those MSA positive samples were also positive in catalase and coagulase test were identified as *Staphylococcus aureus*. Samples positive in the MSA and catalase tests but negative in the coagulase test were suspected as coagulase negative *Staphylococcus* spp. (NAS) (Persson et al., 2011). Catalase test is used to identify catalase negative bacteria like *Streptococcus* spp. from catalase positive bacteria such as *Staphyococcus* spp., *Micrococcus* spp. as well as *Bacillus* spp. Positive samples in MAC agar contained both lactose fermentative (pink color) and non-lactose fermentative (colorless) bacteria (Persson et al., 2011). Positive growth with the pink colored colony was inoculated in the EMB agar. The colonies that gave off a greenish metallic sheen on EMB agar and passed the Indole test were confirmed to be *E. coli.* (McVey et al., 2013). Colorless colonies in MAC agar and Indole negative samples selected for the motility and oxidase tests and suspected as *Enterobacter* spp., *Pseudomonas* spp., and *Klebsheila* spp. (Murray et al., 2015; Shields and Cathcart, 2010). All of the culture agar media and chemical reagents used from Oxoid® and instructions were followed as mentioned (Oxoid Ltd, Basingstoke, UK).

3.7.3 Identification of species through MALDI-TOF

Isolates in transport media were shifted to the laboratory of Microbiology, Department of Veterinary and Animal Science, Università degli Studi di Milano, Italy from the Laboratory of udder Health Bangladesh, CVASU. A total of 292 sample isolates from pure culture were evaluated by Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF). Prior to MALDI-TOF, isolates were re-cultivated on blood agar for 48 h at 37 °C. Following the manufacturer's instructions (Hijazin et al., 2012), the MALDI-TOF sample preparation, analysis, and data processing were completed. Spectra were taken with the help of the software flex Control 3.0's automatic function (Bruker Daltonik). By comparing the unknown Main spectrum profile spectra (MSPs) to MSPs from reference strains, MALDI-TOF main spectrum was used to identify the microbes. MALDI score represented by the log values in a range from 0 (no homology) to 3 (absolute identity). The MALDI definition score ≥2 considered as the threshold value for species level (Staph. aureus), and the score ≥1.7 but <2.0 for identification of all other pathogens at genus level (Barreiro et al., 2017; Du et al., 2002; Moussaoui et al., 2010).

3.8 Data management

Multilevel (farm, animal and quarter) field data and final outputs from laboratory results were entered into Microsoft Excel 2013. The whole data was rechecked for validity and consistency, and then exported to STATA-14 (StataCrop, 4905, Lakeway Drive, College Station, Texas 77845, USA) for epidemiological analysis. Continuous data in different variable were categorized with definition for the analysis purpose. Descriptive, univariable, and multivariable analysis were conducted on multilevel data sets.

3.9 Statistical analysis

3.9.1 Descriptive analysis

Proportion, percent, and summary of different variables were calculated to define the status of the diversity in farm management, feeding and udder health practices in the water buffalo farm. Multi-level (Farm, Animal, and Quarter) prevalence of SCM in water buffalo, proportionate prevalence of the organisms responsible for SCM from the quarter milk sample and summary statistics of BMSCC were computed. Proportionate prevalence was calculated by dividing the number of positive cases (either by CMT or bacteriological evaluation) by the total number of milk samples investigated. Results were expressed as frequency, percent, mean, median, standard deviation (SD) or standard error (SE), minimum, maximum and a 95% confidence interval (CI).

3.9.2 Farm level risk factor analysis for sub-clinical mastitis based on BMSCC

3.9.2.1 Farm level univariable analysis

The outcome variable bulk milk somatic cell count (BMSCC) was not normally distributed in the performed histogram of BMSCC. To correct abnormal distribution of BMSCC values were transformed into $\log_{10}$ values of the number of cells per milliliter and the geometric means of BMSCC against each farm level variable represented as using the antilog to back-transform of the parameters. As the outcome variable was continuous, so univariate analysis using one way ANOVA was conducted to assess the log-mean difference of BMSCC between categories of each selected 122 farm level factors of 45 buffalo farms. A factor with a $P<0.20$ was considered statistically significant under the investigation or according to a subjective decision to include biologically interesting factors. Farm management, udder health practices, and therapeutic management related total 13 variables with a P<0.20 in one-way ANOVA results were forwarded for multivariable linear regression

3.9.2.2 Farm level multivariable linear regression analysis

Only two factors (feeding type and stimulation in udder) were found statistically significant in the final multivariate linear model among 13 factors of univariate analysis. The model manually constructed with forward selection applying the maximum likelihood estimation procedure and the statistical significance of the factors was determined using Wald's test and the likelihood ratio test (LRT) (Dohoo et al., 2003). We looked for confounding factors by taking out one of the variables and looking at how the beta coefficients of the other variables changed. A change in the coefficient of more than 30% was thought to be a sign of confounding. No confounding was detected during the final model makeup. The factors' variance inflation factors (VIF) were looked at to find out if they were collinear. A VIF value of more than 10 indicates serious collinearity (Dohoo et al., 2003). The VIF value of the factors remained below 5, indicating the factors were not collinear. The goodness of fit test like Cameron & Trivedi's decomposition of IM-test and The Cook–Weisberg test for heteroskedasticity was applied to examine the homogeneity of variance and whether the overall data fitted the model. The results were presented for each adjusted factor as a beta-coefficient, p value and 95% CI.

3.9.3 Animal level risk factor analysis for subclinical mastitis (CMT ≥2=Yes)

3.9.3.1 Animal level univariable analysis

A total of 40 different factors were assessed individually for univariable risk factor analysis using the chi-square test. The continuous predictors such as age, lactation stage, body condition score, average milk yield, highest milk yield, and milker's experience were re-categorized before analysis. A p value of 0.2 or less was considered statistically significant and forwarded to logistic regression model.

3.9.3.2 Animal level multivariable logistic regression analysis

Ranging area, highest milk yield, teat position of the buffalo were determined to be significant at p value of 0.05 level using the chi-square test. However, all of the eight variables $P<0.20$ were subjected to the random effect (RE) logistic regression model with Buffalo ID. A forward addition procedure of variables was used to fit and eliminated the model based on the significance of individual variable. Adding or taking out a variable from the model was used to check for confounding. The Chi-square test was used to find out if the independent variables were co-linear. Variables that were significant ($p \leq 0.05$) based on a Wald test were considered as risk factors for SCM. The results were expressed as an odds ratio (OR), standard error (SE), 95% confidence interval, and p-value.

Chapter IV: Results

4.1 Descriptive statistics

4.1.1 Diversification of Buffalo farming

In Subarnachar and Hatiya upazila of Noakhali district, I evaluated 45 buffalo farms (n=1660): 8.9% coastal (n=4), 66.7% semi-coastal (n=30), and 24.4% inland (n=11) farms. Nearly 73% farmers (n=33) raise buffaloes in free ranging area (bathan type farm) at island, in contrast around 27% farmers (n=12) prefer to rear their buffaloes in semi-intensive system in household. Small (33%), Medium (38%) and Large (29%) sized herds were observed with <15 buffaloes per farm, 15-40 buffaloes per farm and >40 buffaloes per farm, respectively in the study area. The studied buffalo farms (n=45) in household and bathan had minimum 2 to maximum 35 lactating buffaloes (n=385). The study also revealed that 58% of the buffaloes are indigenous (95% CI: 0.51-0.65) and 42% are cross-bred (95% CI: 0.35-0.49) buffalo among the sampled lactating buffalo (n=183) in Noakhali district. However, 68% of the cross-bred buffaloes were indigenous with Murrah cross-bred, whereas 32% were indigenous with Nili-Ravi cross-bred buffaloes.

4.1.2 Feeding diversity of buffalo farms

Nearly 70% (95%CI: 0.55-0.82) of buffalo farmers believe that wallowing is mandatory for water buffalo farming. The source of the wallowing water is deep tube-well (24%), pond water (35%), river water (35%), and rain water (6%). Most of the buffalo farmers prefer both stall feeding and grazing system (58%) rather than only stall feeding (2%) or grazing (40%). However, farmers used to graze their buffaloes together (84%) or split into groups (8%) or single (8%) buffalo for the whole day time (63%) than a half day (37%) in the natural grazing land. During the winter season, farmers (15%) keep their buffaloes in bathan, 85% farmers move to the nearby inland with milking buffalo to use conventional feed like Kheshari (*Lathyrus sativus*) (61%), Maskolai (*Vigna mungo*) (14%) and both (25%) types of feed.

4.1.3 Udder health practices

Majority of the buffalo farmers are not concerned about udder health practices like washing udder before milking (98%), fore-stripping (95%), milker's hygiene (95%) and last milking of clinical mastitic buffalo (76%). Methods of hand milking such as stripping (64%) is more common than full hand (27%) and knuckling (9%) (Table 4.1).

Table 4.1: Udder health and hygiene practices observed in water buffalo farms in Noakhali district, Bangladesh.

Variables	Category	N	%
Washing buffalo before milking	No	42	95
	Yes	2	5
Washing udder before milking	No	44	98
	Yes	1	2
Stimulate udder	calf suckling	33	73
	Hand	2	5
	calf suckling and by hand	10	22
Fore-stripping	No	40	95
	Yes	2	5
Method of hand milking	Full hand	12	27
	Stripping	29	64
	Knuckling	4	9
Score of milker's hygiene	Good (Only hand wash)	2	5
	Poor (Never wash hands)	41	95
Last milking of CM buffalo	No	32	76
	Yes	4	10
	Don't milk	6	14
Isolation shed for animals	No	41	91
	Yes	4	9

N= number of observation in different factors varied as they were recorded based on respondents reply

4.1.4 Status of bulk milk somatic cell count among studied buffalo farms (n=45)

Bulk milk somatic cell count ranged from 124×10^3 to $1,213 \times 10^3$ cells per mL of milk among the surveyed buffalo farms (n=45) in winter season. The geometric (G) mean BMSCC was 413×10^3 (95% CI: 349-488) cells per mL of milk. The median BMSCC of the 45 buffalo farms in this study is described below along with its quartiles (Table 4.2) (Fig. 4.1).

Table 4.2: Mean bulk milk somatic cell count obtained from buffalo farms (n=45)

Unit of measurement	G Mean BMSCC ($\times 10^3$ cells/mL) (95%CI)	BMSCC[1] (min-max) ($\times 10^3$ cells/mL)
Lowest 25% farms	413	124-280
Lowest 50% farms	(349- 488)	280-437
Lowest 75% farms		437-653

[1]BMSCC = Bulk milk somatic cell count/mL of milk; the threshold used for this study was 200×10^3 cells/mL of milk

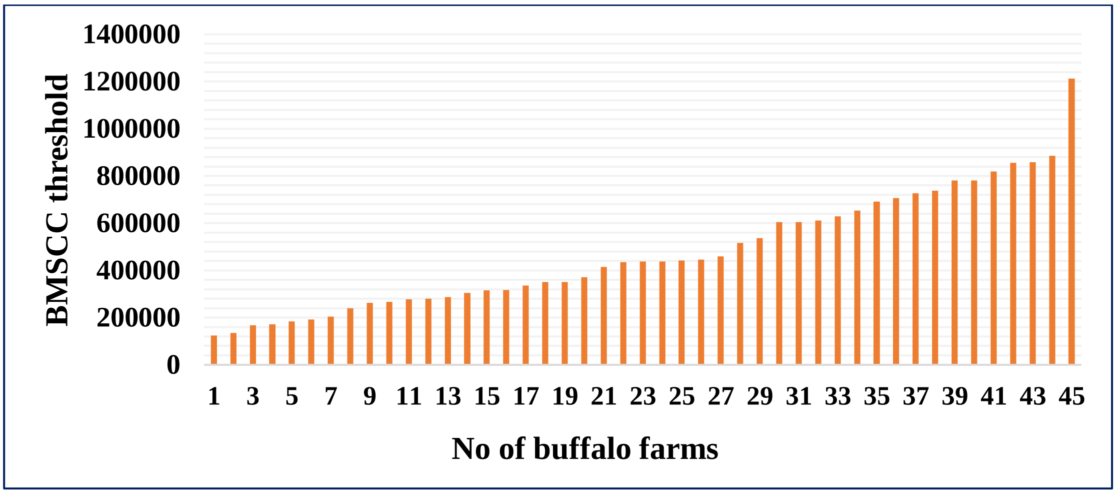


Fig. 4.1: Distribution of BMSCC in studied buffalo farms (n=45)

4.2. Prevalence of subclinical mastitis

Overall farm level prevalence of subclinical mastitis (SCM) found 86.7% at water buffalo at Noakhali district in Bangladesh. The G mean BMSCC observed lower in bathan type (408×10^3 cells/mL) rearing farm than the household farms (424×10^3 cells/mL). However, G mean BMSCC was two times higher in case of both farm type than the threshold level (200×10^3 cells/mL) (Table 4.3). Based on CMT score (≥ 2

=positive), prevalence of SCM is 47% and 27% at animal and quarter level, respectively. Higher prevalence of SCM found in buffaloes those were rearing in bathan or free ranging area at both animal (71%) (P=0.6) and quarter (75%) (P=0.02) level (Table 4.4). Among the quarters prevalence of SCM found high in front quarters than hind quarters. However, Higher significant (P=0.05) prevalence of SCM found in hind quarter of the buffaloes those were rearing in bathan (Table 4.5). According to culture results prevalence of SCM observed at 47% (n=137) (95% CI: 41-53) (Table 4.12).

Table 4.3: Prevalence of farm-level subclinical mastitis at the in water buffalo of Noakhali district, Bangladesh

Farm type	N (%)	95% C.I	G Mean BMSCC (×10^3 cells/mL)	95% C.I	Low BMSCC (< 200×10^3 cells/mL) (%)	High BMSCC (≥200×10^3 cells/mL)	P[1]
Bathan	33 (73)	0.58-0.85	408	331-504	6 (18)	27 (82)	0.1
House-hold	12 (27)	0.15-0.42	424	311-577		12 (100)	
Overall	45 (100)	0.73-0.94	413	349-488	6 (13.3)	39 (86.7)	

[1]P values were calculated based on the Chi-squared test performed between farm type and BMSCC

Table 4.4: Prevalence of subclinical mastitis based on CMT at animal and quarter level in water buffalo of Noakhali district, Bangladesh

Level	Overall ≥ 2 CMT			Bathan ≥ 2 CMT		Household ≥ 2 CMT		P
	N	N_1 (%)	95% CI	N	N_1 (%)	N	N_1 (%)	
Animal	183	86 (47)	0.40-0.54	126	61 (71)	57	25 (29)	0.6
Quarter	730	197 (27)	0.24-0.30	502	148 (75)	228	49 (25)	**0.02**

N= Number of actual observation at each category; N_1= Number of Positive observation at each category

Table 4.5: Quarter-wise prevalence of subclinical mastitis based on CMT in water buffalo of Noakhali district, Bangladesh

Level	Overall				Bathan		Household		P
	≥ 2 CMT			p	≥ 2 CMT		≥ 2 CMT		
	N	N_1 (%)	95% CI		N	N_1 (%)	N	N_1 (%)	
Front Left	183	53 (29)	0.23-0.36		126	39 (74)	57	14 (26)	0.3
Front Right	181	50 (28)	0.22-0.35	0.7	124	38 (76)	57	12 (24)	0.2
Hind Left	183	42 (23)	0.17-0.30		126	32 (76)	57	10 (24)	0.2
Hind right	183	49 (27)	0.21-0.34		126	39 (80)	57	10 (20)	**0.05**

N= Number of actual observation at each category; N_1= Number of Positive observation at each category

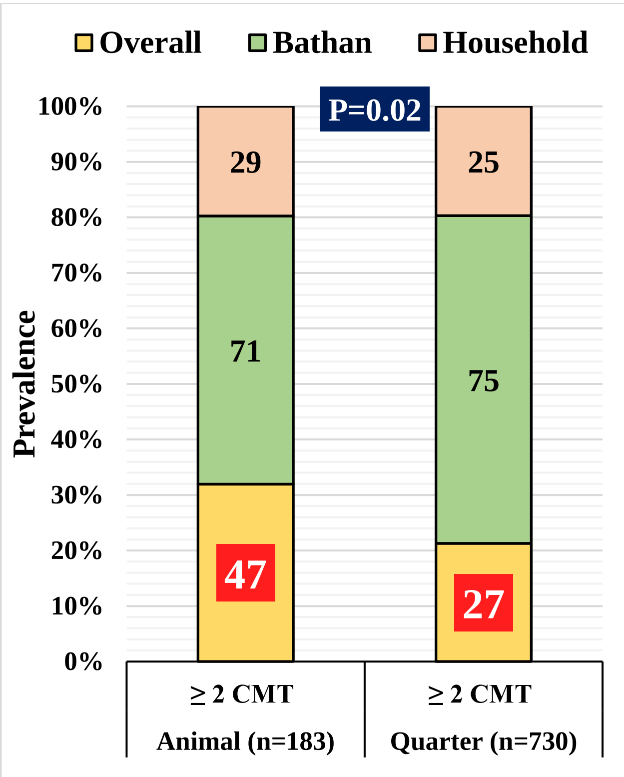


Fig. 4.2: Prevalence of SCM at animal and quarter level

4.3 Risk factors of subclinical mastitis

there was significant association mong the category of the feeding behaviors of buffalo in grazing land observed significant (P=0.02) association for having SCM during univariable analysis. However, final multivariable linear regression model revealed that buffaloes those were feeding in group at grazing land had significant (P=0.005) association with increasing bulk milk somatic cell count. Bulk milk somatic cell had significant (P=0.02) decreasing trend with udder health practices like stimulation in teat before milking by only hand than both of calf sucking and hand together at a time. According to CMT results, buffalo those had a record of high yielding (4.1 to 6 L) milk production was 3.2 times more prone to having SCM than the low (<4L) milk production buffaloes. However, asymmetrical teat positions had 2.2 times higher odds (P=0.02) for having SCM rather than symmetrical teats.

4.3.1 Risk factor analysis of bulk milk somatic cell count at the farm level

4.3.1.1 Univariable analysis (One - way ANOVA) between bulk milk somatic cell count and farm level management factors

Significant (P ≤0.05) association of Log BMSCC was found among the categories of five farm-level risk factors: (i) frequency of the anthelminthic in buffalo (P=0.01) (Table 4.6), (ii) anthelminthic usage (P=0.03) (Table 4.6), (iii) Feeding type (P=0.02) (Table 4.7), (iv) Buffaloes need to control during milking (P=0.01) (Table 4.8) and (v) stimulation in udder prior milking (P=0.04) (Table 4.8).

Table 4.6: Univariable analysis (One way ANOVA) between mean $\log_{10}$ bulk milk somatic cell count and preventive / Therapeutic related management factors

Variables	Categories	N	G mean BMSCC ($\times 10^3$cells/mL)	Log10 *P* (1-ANOVA) *P* ≤.20
Veterinary service	No	15	495	0.12
	Yes	30	376	
Veterinary service provider	Veterinarian	8	270	0.09
	VFA	14	460	
	Farmer/ Quack	7	522	
	VFA/ Farmer /Quack	16	417	
Anthelmintic to the buffaloes	No	15	531	**0.03**
	Yes	30	363	
Frequency of deworming in buffaloes	3- 4 months	11	280	**0.01**
	6 months	8	294	
	12 months	11	548	
Name of anthelmintic	L	7	494	0.13
	L+T	19	329	

N= number of observation in different factors varied as they were recorded based on respondents reply.
VFA: Veterinary field assistant; L= Levamisole; L+T= Levamisole + Trichlabendazole

Table 4.7: Univariable analysis (One way ANOVA) between mean log_{10} bulk milk somatic cell count and farm related management factors (n=45 farms)

Variables	**Categories**	**N**	**G mean BMSCC ($\times 10^3$cells/mL)**	**Log10 *P* (1-ANOVA) *P* ≤.20**
Temperature and humidity index	Comfort (<72)	37	436	0.14
	Mild stress (72-78)	7	309	
Available wallowing water	Ad-libitum	15	359	0.13
	Scarcity of water	18	485	
Feeding buffaloes	Single buffalo	3	254	**0.02**
	Split up	3	854	
	All together	31	414	

N= number of observation in different factors varied as they were recorded based on respondents reply.

Table 4.8: Univariable analysis (One way ANOVA) between mean log_{10} bulk milk somatic cell count and udder health related management factors

Variables	**Categories**	**N**	**G mean BMSCC ($\times 10^3$cells/mL)**	**Log10 *P* (1-ANOVA) *P* ≤.20**
Wash buffaloes before milking?	No	42	398	0.08
	Yes	2	818	
Control buffalo during milking?	No	13	291	**0.01**
	Yes	32	423	
Stimulate udder	calf sucking	33	455	**0.04**
	hand	2	181	
	Both	10	350	
Method of hand milking	Full hand	12	489	0.09
	Stripping	29	413	
	Knuckling	4	243	
Number of milker	Same person	14	494	0.14
	Multiple milker	28	380	

N= number of observation in different factors varied as they were recorded based on respondents reply.

4.3.1.2 Multivariable linear regression model between bulk milk somatic cell count and farm level management factors

Buffaloes grazed or were stall-fed in together or in group were less associated (P=0.005; CI=0.17-0.88) for increasing BMSCC level compare to those buffalo used to feeding in single. Stimulating udder only by calf suckling associated (P=0.02; CI=0.71-0.07) with higher BMSCC than either only hand or by both of them (Table 4.9).

Table 4.9: Multivariable linear regression analysis between $\log_{10}$ BMSCC and farm level factors in 45 buffalo farms in Noakhali district, Bangladesh (N=37)

Variables	Category		β	95% CI	*p*
Intercept			5.45	5.13-5.76	0.00
Feeding buffaloes	Single buffalo	*Ref*			
	Split up in groups		0.53	0.17-0.88	**0.005**
	All together		0.20	-0.12- 0.52	0.22
Stimulate udder	By calf sucking	*Ref*			
	By only hand		-0.39	-0.71-0.07	**0.02**
	By both		-0.06	-0.35-0.22	0.65

N= number of observation in different factors varied as they were recorded based on respondents reply. SE=Standard error mean; β= Coefficient; 95% CI: 95% confidence interval

4.3.2. Risk factor for California Mastitis Test at animal level

4.3.2.1. Univariable analysis between the results of California mastitis test and animal level factors (Chi-square test) in water buffalo

The results of California Mastitis Test (CMT) (CMT score ≤2=Yes, SCM) in water buffalo significantly varied by ranging area (P=0.06), position of teats (P=0.006), type of breed (P=0.06), pregnancy status (P=0.08), highest milk yield of the buffalo (P=0.05) (≤0.2, Chi-sqaure test) (Table 4.10). These significant risk factors were then moved to construct the logistic regression model with random effect.

Table 4.10: Univariable association (Chi-square test) between the California Mastitis Test status (CMT score ≤2=Yes, SCM) and the buffalo level factors.

Variable name	Categories	+ (%)	-	*P* ≤ 0.20
Ranging area	Muddy field	3 (4)	3	0.06
	Wallowing in water	28 (33)	17	
	Both	53 (63)	74	
Teat Position	Symmetrical	45 (52)	70	**0.006**
	Asymmetrical	41 (48)	27	
Asymmetrical position of teats	Directed outward	64 (75)	82	0.14
	Directed backward	7 (8)	5	
	Directed inward	13 (16)	6	
	long and short teats	1(1)	3	
Is the buffalo have any block teat	No	84 (98)	97	0.13
	Yes	2 (2)	0	
Teat disorders	No	82 (95)	96	0.13
	Yes	4 (5)	1	
Type of breed	Indigenous	44 (51)	63	0.06
	Cross-bred	42 (49)	34	
Pregnancy status	Pregnant	33 (38)	25	0.08
	Non-pregnant	53 (62)	71	
Highest milk yield of the buffalo	Fewer than 2 litter	13 (15)	27	**0.05**
	2.1 to 4 litter	59 (70)	61	
	4.1 to 6 litter	13 (15)	7	
Udder shape	Cup/Pendulous	1 (1)	3	0.11
	Round/Globular	-	4	
	Bowl	85 (99)	90	

+= Number of CMT positive observation at each category of each factor; %=Percentage
- = Number of CMT negative observation at each category of each factor

4.3.2.2. Multivariable logistic regression model with random effect analysis between SCM status (CMT ≤2= SCM) and animal level factors in water buffalo

The relative difference of LRT (Likelihood Ratio Test) logistic regression with random effect model was well fitted. Asymmetrical teats (60%) had 2.2 times (P=0.02, CI=1.15 - 4.05) higher association with the prevalence of SCM than symmetrical teats. The about 3.2 times higher odds of SCM in high milk yield (4.1-6 L) (65%) (P=0.05, CI=1.02 -10.31) buffaloes indicate high productive buffaloes had 3.2 times more association for developing SCM than low productive buffaloes (Table 4.11).

Table 4.11: Multivariable logistic regression with random effect model between the status of CMT result (CMT score ≤ 2=Yes, SCM) and the buffalo level factors significant factors ($P \leq 0.1$).

Variables	Category	OR	SE	95% CI	*P*
Intercept		0.38	0.14	0.19-0.77	0.007
Teat Position	Symmetrical	*Ref*			
	Asymmetrical	2.2	0.70	1.15 - 4.05	**0.02**
Milk yield of the buffalo	≤2 litter	*Ref*			
	2.1 to 4 litter	1.9	0.73	0.88 - 4.05	0.1
	4.1 to 6 litter	3.2	1.92	1.02 -10.31	**0.05**

OR= Odds Ratio; SE=Standard error mean; 95% CI: 95% confidence interval

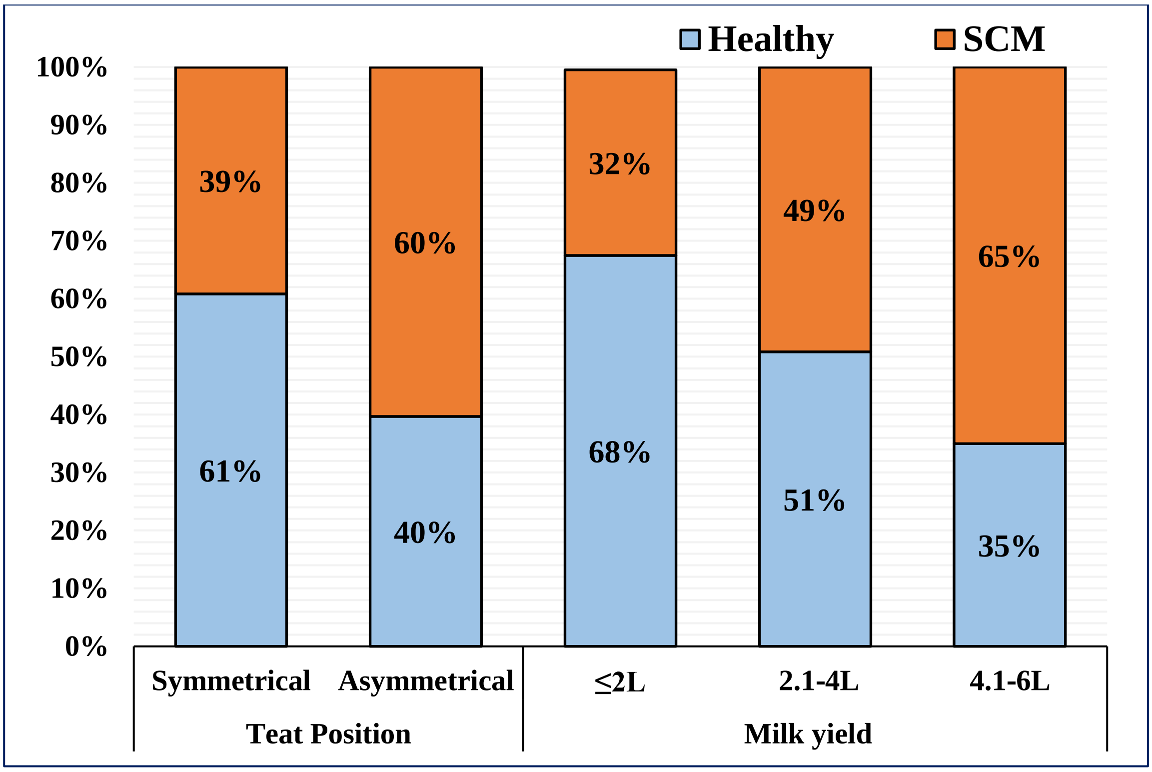


Fig. 4.3: Distribution of teat position and milk yield between Healthy and SCM quarters

4.4. Distribution of pathogens isolated from dairy milk samples

A total of the 292 culture samples obtained from 320 quarter milk samples of 183 lactating buffaloes were used for pathogen identification in MALDI-TOF. Pathogen growth was observed at 47% (n = 137) (95% CI: 41-53) of the sample (Table 4.12). Non aureus *Staphylococci* (NAS) were the most dominant organisms with the prevalence of 39.4% followed by *Staphylococcus aureus* (3.8%), Gram negative bacteria (1.4%), and other bacteria (2.4%) (Table 4.13; Figure 4.5). Among the CMT score (CMT negative=1; CMT positive ≥2) quarter sample high prevalence of *Staph. aureus* observed in CMT negative (45.45%) quarter samples followed by CMT 3 (27.27%), CMT 4 (18.18%) and CMT 2 (9.09%) (Table 4.14). Detail list of pathogens added in the table 4.15-4.16; Figure 4.4. Among the pathogens majority of the NAS (65%), *Staph. aureus* (91%), Gram negative (75%) and other bacteria (86%) observed in the buffaloes reared in bathan or free ranging area (Table 4.13) (Figure 4.5-4.6).

Table 4.12: Prevalence of SCM according to culture results from MALDI-TOF

Trait	**%**	N	SE	95% CI
Culture positive	47	137	0.03	0.41-0.53
Culture negative	51.3	150	0.03	0.46 – 0.57
Not identified	1.7	5	0.01	0.01 – 0.04

%=Percentage; SE=Standard error mean; 95% CI: 95% confidence interval

Table 4.13: Distribution of pathogens confirmed by MALDI-TOF (n=292)

Pathogens	**%**	N	95% CI
Non-aureus *staphylococci*	39.4	115	0.34 to 0.45
Staphylococcus aureus	3.8	11	0.02 to 0.07
Gram negative bacteria	1.4	4	0.005 to 0.04
Other bacteria	2.4	7	0.01 to 0.05
Culture negative sample	51.3	150	0.46 to 0.57
Not identified	1.7	5	0.007 to 0.04
Total	100	292	

%=Percentage; N=Number of observation at each category; 95% CI: 95% confidence interval

Table 4.14: Distribution of pathogens in pure culture according to CMT score (positive ≥2 and negative =1) of quarter milk samples in water buffalo

Isolated pathogens	CMT Score					Total
	1 N (%)	2 N (%)	3 N (%)	4 N (%)	5 N (%)	N%
NAS	61(53.04)	36 (31.3)	12 (10.43)	6(5.22)	0 (0)	115 (100)
Staph.aureus	5 (45.5)	1(9.1)	3 (27.3)	2 (18.2)	0 (0)	11 (100)
Gram (–ve)	1 (25)	2 (50)	0 (0)	1 (25)	0 (0)	4 (100)
Other bacteria	3(42.9)	3 (42.9)	1 (14.3)	0 (0)	0 (0)	7 (100)
Culture (–ve)	74 (49.3)	48 (32)	14 (9.3)	13 (8.7)	1 (0.7)	150 (100)
Not-identified	1 (20)	3 (60)	1 (20)	0 (0)	0 (0)	5 (100)
Total	**145 (49.7)**	**93 (31.9)**	**31(10.6)**	**22 (7.5)**	**1 (0.3)**	**292 (100)**

%=Percentage; N=Number of observation at each group; NAS= Non-aureus *Staphylococci*

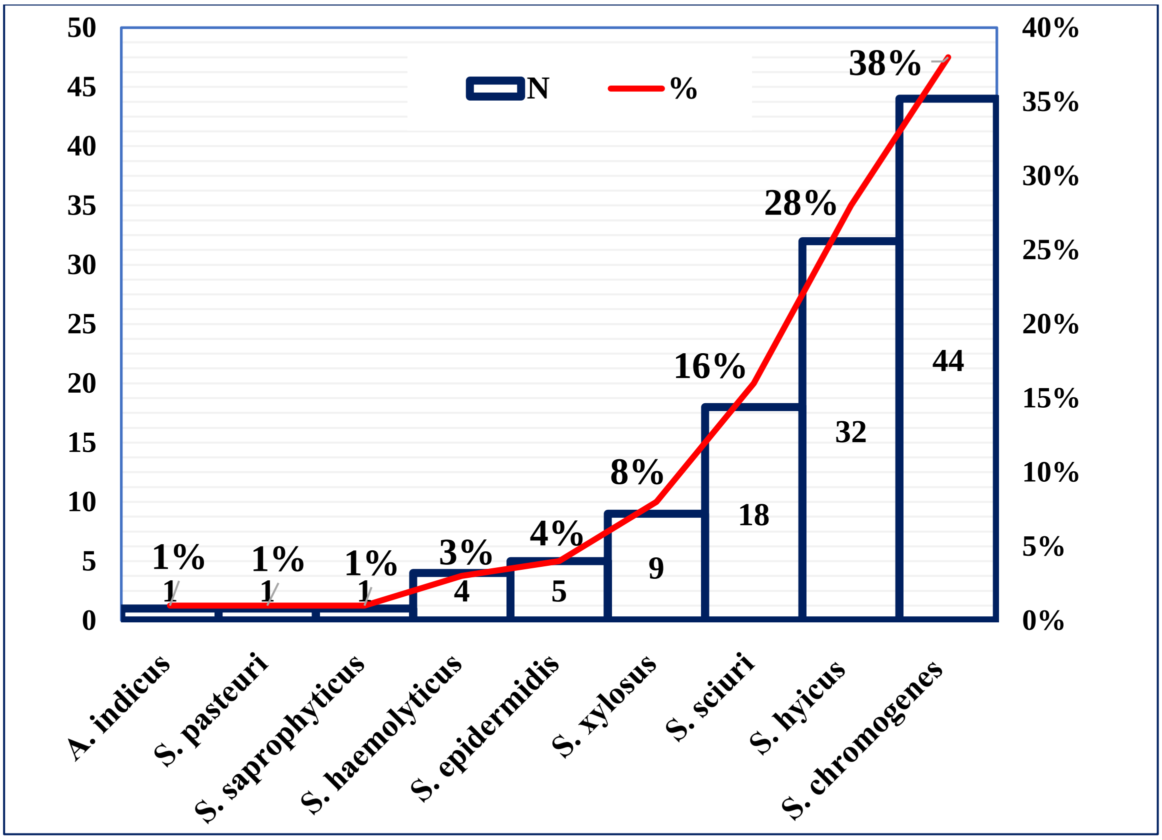


Figure 4.4: Details of NAS (39.4%) (n=115) confirmed by MALDI-TOF

Table: 4.15: Details of Gram (-ve) bacteria (1.4%) (n=4) confirmed by MALDI-TOF

Pathogens	No
Pseudomonas putida	1
Pseudomonas stutzeri	1
Ochrobactrum anthropi	1
Empedobacter falsenii	1

Table: 4.16: Details of other bacteria (2.4%) (n=7) confirmed by MALDI-TOF

Pathogens	No
Kocuria rhizophila	1
Bacillus cereus	1
Corynebacterium efficiens	1
Micrococcus endophyticus	1
Micrococcus luteus	1
Rothia amarae	1
Rhodococcus rhodochrous	1
Corynebacterium efficiens	1

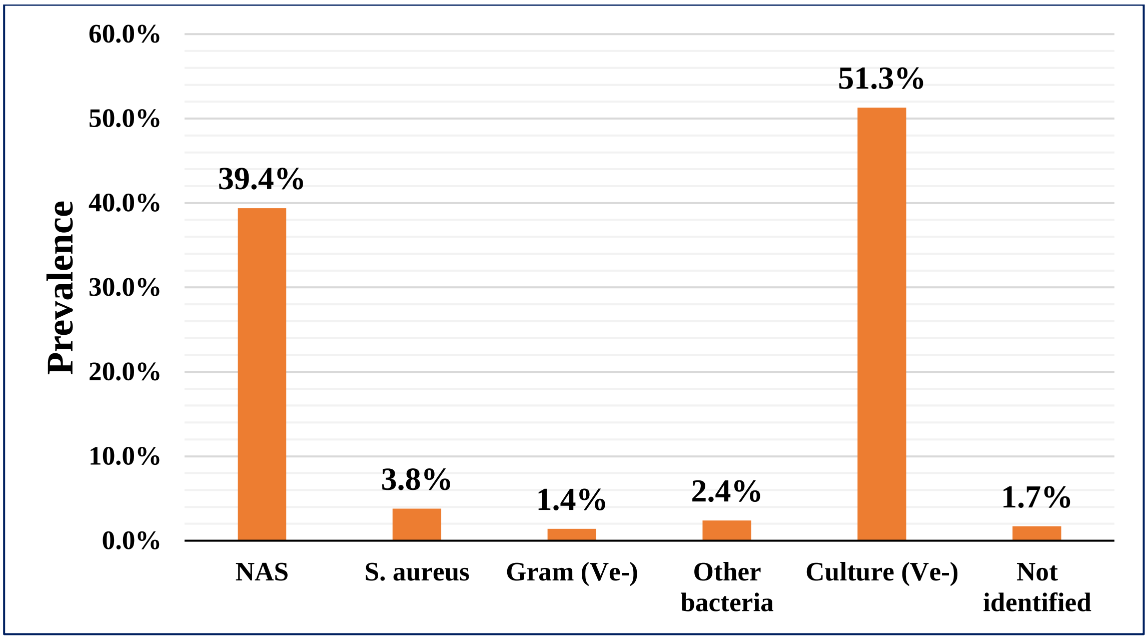


Figure 4.5: Overall distribution of pathogens according to MALDI-TOF

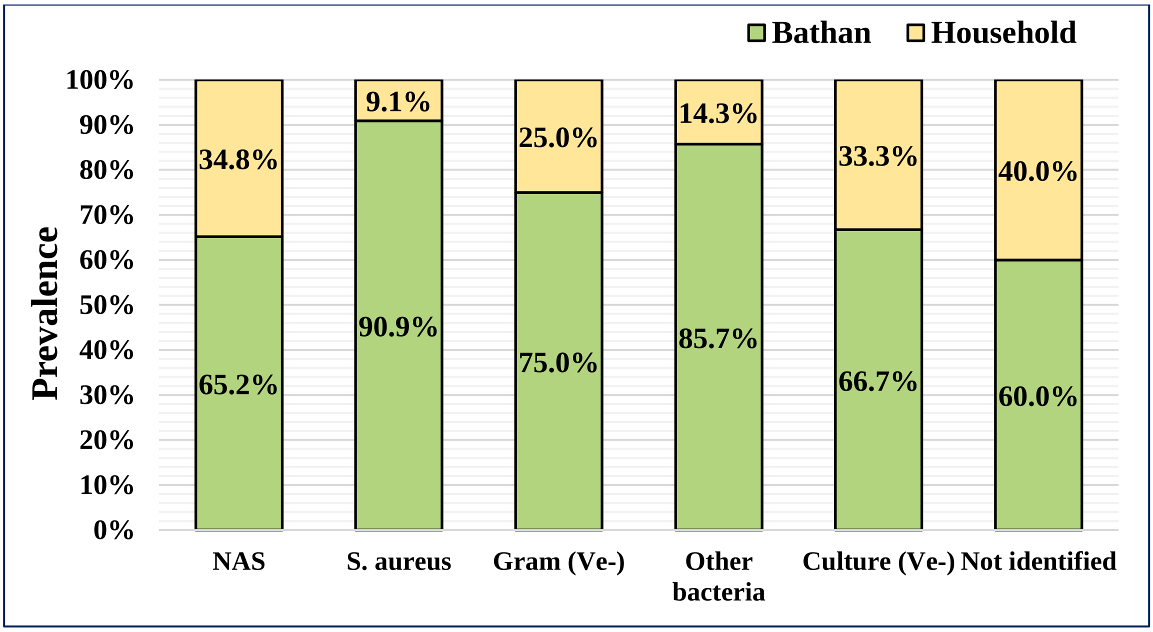


Figure 4.6: Distribution of pathogens from MALDI-TOF results according to buffalo farm rearing type in Noakhali district Bangladesh

Chapter V: Discussion

Bovine mastitis is one of the most prevalent and economically significant diseases affecting dairy herds globally. The objectives of the present study were to estimate the prevalence of subclinical mastitis (SCM) in farm, animal and quarter level along with associated risk factors and distribution of pathogens causing SCM in water buffaloes of Noakhali district in Bangladesh. The study successfully reported the prevalence of SCM and potential risk factors responsible for having SCM and the presence of pathogens in milk samples of Subarnachar and Hatiya upazila of Noakhali district.

5.1 Bulk milk somatic cell count

Bulk milk somatic cell count (BMSCC) level of the top 25% buffalo farm had ≤280 x 103 cells/ml among the studied buffalo farms in Noakhali. Although, there is no large-scale study on the threshold level of BMSCC in buffalo milk in Bangladesh, a small-scale study estimated the mean BMSCC 195 x 103 cells/ml of milk in the Noakhali and Bagerhat district of Bangladesh (Singha et al., 2021). A study on bulk milk quality of Mediterranean buffalo reported mean BMSCC at 175.57 x 103 cells/ml of milk (Pasquini et al., 2018). However, there is a lack of studies currently on established BMSCC threshold value for quality requirements for buffalo milk (Esposito et al., 1997; Dhakal, 2004; Moroni et al., 2006; Tripaldi et al., 2003). Few other studies in neighboring countries (India, Nepal) and Europe (Italy) recommended the somatic cell count (SCC) threshold level is 200 x 103 cells/ml of milk as a benchmark in dairy buffalo to diagnose SCM (Dhakal, 2006; Moroni et al., 2006; Tripaldi et al., 2010; Guha et al., 2012). Another recent study reported that within the first parity more than 28% of lactations had an average SCC ≥200 x 103 cells/ml of milk in Italy (Costa et al., 2020). According to BMSCC threshold level at 200 x 103 cells/ml of milk, 86.7% farm level SCM prevalence represented in the present study, which is consistent with the farm level prevalence (84%) obtained by Javed et al. (2022). High prevalence of SCM indicate a serious udder health concern in water buffalo farms of Noakhali district of Bangladesh. Again, dairy product such as curd from buffalo milk is a popular regional food in Noakhali district (Alam & Naser, 2020). Thus, BMSCC may help to set benchmark for drinkable buffalo milk, dairy products, create awareness among farmers and consumers, and set goals for the control and prevention of SCM in buffalo farms.

5.2 Prevalence of subclinical mastitis at the animal level

The prevalence of SCM estimated in the present study was 47% at an animal level in winter season. The present study's findings are substantially lower than Singha et al., (2021) (81.6%) in Noakhali and Bagerhat district during the monsoon season. However, estimated prevalence of the present study is higher than findings (11 and 32%) of two small scale studies in Bangladesh (Aliul et al., 2020; Islam et al., 2016). The lower prevalence of SCM in the present study at Noakhali district than the result of Singha et al., (2021) may be due to season and farm management system of studied herd. The highest prevalence of SCM was observed in rainy seasons than the summer, winter or spring seasons in water buffalo of Bangladesh, Pakistan and Turkey (Aliul et al., 2020; Baloch et al., 2016; Özenç et al., 2008; Sahİn et al., 2017). However, the current prevalence (47%) at the animal level of the present study is supported by the findings (42-51%) of other studies in neighboring tropical and subtropical climate zone such as India, Pakistan, Philippine and Egypt (Ali et al., 2014; Ali et al., 2011; Elhaig & Selim, 2015; Maalik et al., 2019; Preethirani et al., 2015; Salvador et al., 2012; Sharif and Ahmad, 2007). The variation of SCM prevalence at animal level might be also occur due to sample size, rearing, feeding and udder health management in semi-intensive and bathan (free ranging) systems at different coastal and inland zones.

5.3 Prevalence of subclinical mastitis at quarter level

The prevalence of SCM estimated in the present study was 27% at quarter level based on CMT results (Positive ≥ score 2). The present study's findings were lower compared to the findings of Singha et al., (2021) (42.5%) in Noakhali and Bagerhat district. However, present findings are consistent with the findings of Sadoon, (2022) (27.4%) in Iraq on 800 quarter milk sample and Khan, (2001) (27%) in Pakistan on 200 quarter milk samples. The present quarter level prevalence of SCM was low compared to Naiknaware et al. (1998) (28.63%) in India and Sharif and Ahmad, (2007) (37.75%) in Pakistan. In contrast, the present finding was higher compared to other tropical climate zone studies recorded prevalence such as Abid et al., (2018) (16.2%) in Pakistan, Elhaig & Selim, (2015) (21.7%) in Egypt; Kaur et al., (2015) (22.2%) and Hardenberg (2016) (10·6%) in India. However, the criteria used to identify mastitis, varied husbandry practices, diagnostic methods, environmental factors, and animal immunological status may all contribute to the variation in SCM prevalence (Kaur et al., 2018). A large scale study on prevalence of SCM in buffalo considering more buffalo concentrated zones in Bangladesh is vital to assess precise estimation.

The prevalence of SCM among the quarters in this study were not significant. These findings are consistent with Dhakal et al., (2006) and Moroni et al., (2006). Conversely, other studies observed a higher prevalence of SCM in the hind quarters of buffalo compare to the front quarters, but SCM was also higher on the left side as compared to quarters on the right side (Ali et al., 2014; Abid et al., 2018; Kaur et al., 2015; Sharma et al., 2013; Zenebe et al., 2014;). However, in the present study significant ($P<0.05$) high prevalence was observed in hind right quarters in free ranging buffaloes (80%) compare to buffaloes reared in semi-intensive/household (20%). Similarly, Sharif and Ahmad, (2007) also observed a high prevalence in hind right quarters. This could be because of the anatomical position of hind quarters, which have a higher potential for fecal and environmental contamination (Sori et al., 2005) due to direct contact with excreta such as soiled with dung and urine (Naiknaware et al., 1998; Kavitha et al., 2009). The milker's usually sit on the left side of the buffalo and milking starts from left quarters at first due to compatibility. Whereas, they milk the hind right quarter at last due to more distance from left side, which may lead to inappropriate milking and contagious Intra-mammary infection (IMI) from another quarter to hind right quarter.

5.4 Intra-mammary infection

According to culture results in MALDI-TOF IMI was 47% among 292 culture samples of studied herd, which is higher than the findings of the CMT results ($\geq$ 2 CMT positive) (27%) at quarter level. Similarly, several earlier studies revealed that CMT tests show lower SCM positive than bacteriological culture in milk samples (Dhakal, 2006; Karimuribo et al., 2006; Özenç et al., 2008; Beheshti et al., 2011). The high prevalence of IMI infection in the present study is consistent with the culture results in previous studies (40-51%) by Singha et al., (2021) in Bangladesh, Jhambh et al., (2017) and Sharma & Sindhu, (2007) in India.

Staphylococcus spp. (43%) was the most common pathogen among the culture positive samples, whereas non aureus *Staphylococci* (NAS) was the most dominant organism with a prevalence of 39.4% followed by *Staph. aureus* (3.8 %). The prevalence of *Staphylococcus* spp. (43%) in culture, results are consistent with the findings (37-46%) of several previous studies in Brazil, Italy, India and Pakistan (De Oliveira Moura et al., 2017; Fagiolo, 2007; Guha et al., 2012; Kaur at al., 2015; Srinivasan et al., 2013). In another small- scale study of udder health in buffalo by Talukder et al., (2013) in Bangladesh have been documented *Staphylococcus* spp. (50%) was the most dominant of all the isolates, which is in line with our findings. In the present study buffalo herds NAS (39%) was the most prevalent species among the *Staphylococcus* spp. Higher prevalence (36.18%- 47.72%) of NAS in SCM quarter milk sample of buffalo also mentioned in the earlier studies earlier similar studies (Behesti et al., 2011., Pankaj et al., 2013). However, the longitudinal study of SCM of water buffalo by Moroni et al., (2006) and Locatelli et al., (2013) observed NAS (66% and 76%) was the highly predominant species among all culture positive samples. Among the NAS pathogens, *Staphylococcus chromogens* (38%) was the most prevalent followed by *Staphylococcus hyicus* (28%), *Staphylococcus sciuri* (16%), and *Staphylococcus xylosus* (8%). Similarly, another investigation on SCM in buffalo in Iraq Sadoon, (2022) mentioned *Staphylococcus chromogens* (14.61%) was mostly prevalent followed by *Staphylococcus xylosus* (12.78%). *S. aureus* is the most notorious pathogen of IMI confirmed at 3.8% in the quarter milk samples. Culture results also revealed that *S. aureus* is most common in the quarter positive with CMT score 3. Similarly a recent study observed 5.8% (102 of 1760 quarters) infection rate in water buffalo of Iraq (Javed et al., 2022). IMI rate with Gram negative bacteria was 1.4% comprised of *Micrococcus* spp., *Bacillus* spp., *Kocuria* spp., *Rothia* spp., *Rhodococcus* spp., and other bacteria was 2.4% among the isolates in pure culture. *Bacillus* spp. (6-14%) was also commonly observed in the subclinical milk samples of the buffalo (Ali et al., 2011; Beheshti et al., 2011; Dhakal et al., 2008; Khan and Muhammad, 2005; Srinivasan et al., 2013). Surprisingly all types of pathogens including *S. aureus* was highly prevalent in quarter samples from bathan, that is also consistent with CMT results. Majority (75%) of CMT positive samples observed in quarter milk samples from bathan. It might be due to unhygienic milking procedure in bathan rearing system. Majority of the buffalo farmers are not concerned about udder health practices like washing udder before milking (98%), fore-stripping (95%), milker's hygiene (95%) and last milking of clinical mastitic buffalo (76%) (Vasavada, 1988; Bonfoh et al., 2003).

5.5 Risk factors associated with subclinical mastitis in water buffalo

Buffaloes grazed or stall-fed in together or in group were less associated for increasing BMSCC level compare to those buffalo used to feeding in separately. The result of the present study represent odds of BMSCC threshold were increased at those farms used to feed in together or in group than in single. It was observed that most of the farmers reared buffaloes with natural grazing at bathan farming system (73%) whereas few of them (27%) were fed in single at household or semi-intensive rearing system. All the herds with low BMSCC (< 200×103 cells/mL) value of the studied herd practices bathan farming system (18%), in contrast to high value of BMSCC (≥200 x 103cells/ml of milk) (100%) observed at all the farm practice household rearing system. Accordingly SCM incidence increase with high milk production (Schukken et al., 1990; Grohn et al., 1995). Similarly, incidence of clinical mastitis reported high in case of conventional farming than free range (organic) farming in Canadian dairy herd (Levison et al., 2016). For instance, free range rearing systems have been associated with less veterinary practice compared to intensive systems in dairy cattle (Richert et al., 2013). Additionally, Asghar et al., (2019) observed a significant (P<0.006) prevalence of SCM in buffaloes involved with feed sharing together in commercial settings. In contrast to our findings, few studies reported mastitis prevalence is higher in outdoor feeding buffaloes than indoor feeding. This could be due to injury or abrasions in teat and udder tissue during grazing (Sadoon, 2022). Aliul et al. (2020) also mentioned water buffaloes raised in free-range grazing areas had a prevalence of SCM of 10.99% compared to 5.56% in stall feeding areas.

Stimulating udder only by calf suckling associated with higher BMSCC than either only hand or both of them. Stimulating udder for milk let down by calf suckling increase prevalence of SCM or BMSCC may be due to incomplete milking in the quarters, which favors to pathogens invitation. Buffaloes in an island or coastal regions (bathan farming system) of Bangladesh are mostly used for the production of meat, while whole milk is fed to the calf or slightly milked for sale (Hamid et al., 2016). Farmers let down milk by calf suckling, but milk from 2-3 quarters to keep the rest quarters for the calf. Residual milk in teat may be responsible for further pathogens invitation. But, most of the buffalo seldom allow their calf to suck once the milker's completed milking. Similarly, another study in Pakistan observed 1.4 times (P<0.005) of higher odds of SCM with calf suckling (Hussain et al., 2013). However, Residual suckling also act as substantial risk factor in smallholder dairy farms (Kivaria et al., 2004).

Asymmetrical teats had 2.2 times higher association with the prevalence of SCM than symmetrical teats. Asymmetrical teats such as teat apex/teat base diameters have higher chances of developing SCM in buffalo, whereas longer teat length, teat to floor distance have a lower chance of developing SCM in buffalo and cow (Hussain et al., 2013; Sharma et al., 2017). Conversely, Kaur et al., (2018) postulated a significant association of IMI in buffalo with teat parameters such as length, diameter, and distance from the floor. Accordingly, teat height from the field has been associated with clinical mastitis in pasture grazed dairy heifer (Compton et al., 2007). Similarly, another study on dairy cow concluded that larger teat length, teat diameter, and teats close to the floor significantly involved with SCM prevalence (Singh et al., 2014).

Lactating buffaloes with low milk yield had less association with having SCM than the high milk yield buffaloes. The about 3.2 times higher odds of SCM in high milk yield (4.1-6 L) buffaloes indicate high productive buffaloes had 3.2 times more association for developing SCM than low productive buffaloes. Accordingly medium milk yield (2.1-4 L) buffaloes had 1.9 times association with increasing SCM than low productive buffaloes. So, this study revealed that there was more chances of developing SCM in high milk yield buffaloes in the studied herd. Similarly, Asghar et al., (2019) reported buffalo's milk yield of more than 5 liters is a risk factor for having SCM. Present findings of the study is in line with the other previous studies on buffaloes (Ali et al., 2014; Hussain et al., 2013; Kavitha et al., 2009).

5.6. Limitations of the study

Sampling area and selection bias

Noakhali consists of 10 upazillas and 8 municipalities. Buffaloes usually reared in island namely Golden Island, Urirchar, Vashanchar, Thangharchar, Ismailerchar etc by the bank of Meghna and Bamni River. It was really hard to include all the buffalo concentrated zones from all islands. A number of farms were selected according to number of lactating buffalo at that time from the six most buffalo concentrated zone by taking help from Upazila Veterinary Hospital, Subarnachar. Farmers were expecting more rainfall during the field visit. But due to less rainfall milk amount of each buffalo decreased. Additionally, due to the pandemic situation of Covid-19 Government enforced countrywide strict lock down in Bangladesh from the second week of April, 2021. So, convenient sampling was applied at the six buffalo concentrated zone. Therefore, there may be under representation of some buffalo zone in our sampling. These buffalo zone may had smaller unorganized farms at the island. The selection of farms may have caused a bias due to failure to include of all buffalo farms from other zones in the list.

Diagnostic error

All the CMT evaluation was done by a single person of the six membered team individually at each buffalo farm. Buffalo farms were located in the different zones of different villages. So, it was hard to monitor all the members at the same sampling day at the same farm. However, all the team members were trained up before starting the field visit with a pilot study. As it is a visual and therefore subjective estimation, there might have few random and systematic errors, which are misclassification bias. It would be better if more than one person could be engaged in CMT evaluation and this would also allow for assessing the between-rater agreement. The CMT has a Sensitivity (Se) of 67.4% and a Specificity (Sp) of 86.9%, that's why there might be false positive as well as false negative errors. Also, there might be some contamination during the collection of quarter milk samples for bacteriology although strict aseptic procedures have been applied. The bacteriological culture was used for the isolation of bacteria from all the quarter milk samples and all the isolates were retested using MALDI-TOF MS at the Laboratory of Microbiology, Department of Veterinary and Animal Science, Università degli Studi di Milano. The BMSCC testing has a "Se" and "Sp" of 88% and 97.8%, respectively. So, this may also result in false positive and false negative errors.

Sampling time

I collected samples from 45 buffalo farms from Noakhali district. Each day i visited buffalo farms at morning 5:00 am and closed the farm visit at 11:00 am. Each day farmers were waiting for us before milking. I have done the sampling at regular milking time of the farmers in most farms and at two to three hours delayed milking time of the farmers in rest of the farms. So, it would be less bias if I could visit all farms at the same time, although it is expected that the sampling time to have substantially affected the CMT scores or the proportion of positive culture results.

Confounding bias

There may be some more extraneous factors at various levels (farm, cow and quarter levels) which may have confounded the association of other potential risk factors with occurrence of SCM. For the variables that were measured and confounding were corrected in the multivariable models.

Chapter-VI: Conclusions

Conclusions

1. The study included six buffalo concentrated zone of two upazila in Noakhali district to get a clear picture of SCM. It was easy to motivate the amicable farmers and their co-workers who can easily accept the laps and gaps in their farm management and can adopt the change to improve the situation.
2. Bulk milk somatic cell count of the buffalo farms in Noakhali district represent that bulk milk quality in most of the farms is below the threshold level (≥200 x 103cells/ml of milk).
3. Overall prevalence of SCM was found higher in farm level followed by animal and quarter level, respectively in winter season. Culture results of MALDI-TOF represented similar prevalence as like CMT at animal level in the studied water buffalo herd.
4. Both Gram positive and negative type bacteria was present in milk sample. *Staphylococcus* spp. was the most prevalent pathogen along with *Staph. aureus.*
5. SCM is associated with buffaloes reared in conventional farming system, stimulating udder only by calf suckling, asymmetrical teats, and high milk yield lactating water buffaloes of Noakhali district in Bangladesh

Chapter-VII: Recommendations and Future Directions

7.1 Recommendations

1. Bulk milk somatic cell count (BMSCC) estimates varied from low to very high among farms. But in 25% of farms, this value is 200,000 or less cell/mL of milk. The farmers should be trained and motivated to practice hygienic farm management to keep the BMSCC low. Bulk milk somatic cell count should be continued to improve farm management factors.
2. In every dairy zone of Bangladesh, there should be facilities of estimation of BMSCC. Farmers should be advised to do BMSCC and CMT regularly once in a month on their farms.
3. Farmers should be trained on significant management related factors identified those are associated with the prevalence of SCM along with overall management to improve the situation and be motivated to adopt the management for financial benefit.
4. Several udder health related management factors like udder cleanliness, washing udder before milking, drying udder, pre and post dipping, fore-stripping, stimulating udder by both hand and calf suckling, last milking of clinical mastitic buffalo should be given priority in dairy buffalo farming. Milkers‟ hands should be cleaned between milking or gloves could be used for milking.
5. Presence of high *Staph. aureus* indicates that priority should be given to dry cow management. So, control measures such as dry cow therapy and SCM diagnostic protocols like CMT, SCC, and bacteriological culture should be adopted for the transmission of both contagious and environmental pathogens.
6. Department of livestock services should allow the import of dry cow products for efficient dry cow management or encourage pharmaceutical companies to manufacture dry cow products.
7. Farmer should maintain a proper record keeping system and strong biosecurity (like culling of cows with penicillin resistant *S. aureus*, not buying animals with penicillin resistant *Staphylococci*, milking order, grouping etc.) on the farm.

7.2 Future directions

1. Under the udder health control program this small scale cross-sectional study was performed in the Noakhali district, but a longitudinal study should be conducted considering more buffalo farms and more buffalo concentrated zones in Noakhali district. More buffalo farms with a wide range of farm level factors would be helpful to estimate a more precise BMSCC threshold with the association of factors than the current status of BMSCC for lactating buffalo of Bangladesh.

2. Further studies should be conducted to confirm the identification of all isolated organisms at the species level together with advanced testing like MALDI-TOF MS and their antibiogram.

3. Further studies on the isolation of zoonotic bacteria regarding buffalo milk, local milk products and mastitis and their route of transmission along with their impact on public health should be considered.

References

Abd El-Salam MH, El-Shibiny S. 2011. A comprehensive review on the composition and properties of buffalo milk. Dairy Science & Technology. 91 (6): 663.

Abdel-Shafy H, Deng T, Zhou Y, Low WY, Hua G. 2022. Editorial: Buffalo Genetics and Genomics. Frontiers in Genetics. p12.

Abdul G, Khan M, Mirza M, Pirzada W. 1991. Effect of year and calving season on some traits of economic importance in Nili-Ravi buffaloes. Pakistan Journal of Agricultural Research. 12 (3): 217-221.

Abid H, Mansur-ud-Din A, Mushtaq MH, Mamoona C, Khan MS, Reichel M, Tanveer H, Amjad K, Muhammad N, Khan IA. 2018. Prevalence of overall and teatwise mastitis and effect of herd size in dairy buffaloes. Pakistan Journal of Zoology. 50 (3): 1107-1112.

Adkins PR, Middleton JR. 2017. Laboratory handbook on bovine mastitis.

Alam SN, Naser MN. 2020. Role of traditional foods of Bangladesh in reaching-out of nutrition. Elsevier. pp. 217-235.

Ali MA, Ahmad MD, Muhammad K, Anjum AA. 2011. Prevalence of sub clinical mastitis in dairy buffaloes of Punjab, Pakistan. Journal of Animal and Plant Sciences. 21 (3): 477-480.

Ali T, Rahman A, Qureshi MS, Hussain MT, Khan MS, Uddin S, Iqbal M, Han B. 2014. Effect of management practices and animal age on incidence of mastitis in Nili Ravi buffaloes. Tropical Animal Health Production. 46 (7): 1279-1285.

Aliul H, Kumar PA, Mahmood RM, Mizanur R, Selim AM. 2020. Investigation of prevalence and risk factors of subclinical mastitis of dairy buffaloes at Bhola district of Bangladesh. Asian Journal of Medical and Biological Research. 6 (4): 697-704.

Allore H. 1993. A review of the incidence of mastitis in buffaloes and cattle. Pakistan Veterinary Journal. 13: 1-1.

Amin M, Siddiki M, Kabir A, Faruque M, Khandaker Z. 2015. Status of buffalo farmers and buffaloes at Subornochar upozila of Noakhali district in Bangladesh. Progressive Agriculture. 26 (1): 71-78.

Andrew S, Moyes K, Borm A, Fox L, Leslie K, Hogan JS, Oliver S, Schukken Y, Owens W, Norman C. 2009. Factors associated with the risk of antibiotic residues and intramammary pathogen presence in milk from heifers administered prepartum intramammary antibiotic therapy. Veterinary Microbiology. 134 (1-2): 150-156.

Armstrong DV. 1994. Heat stress interaction with shade and cooling. Journal of Dairy Science. 77 (7): 2044-2450.

Asghar K, Durrani A, Arfan Y, Khan J, Mamoona C, Khan M, Amjad K. 2019. Epidemiology of bovine sub-clinical mastitis in Pothohar Region, Punjab, Pakistan in 2018. Pakistan Journal of Zoology. 51 (5): 1667-1674.

Bachaya H, Raza M, Murtaza S, Akbar I. 2011. Subclinical bovine mastitis in Muzaffar Garh district of Punjab (Pakistan). Journal of Animal and Plant Sciences. 21 (1): 16-19.

Badran A. 1985. Genetic and environmental effects on mastitis disease in Egyptian cow and buffalo. Indian Journal of Dairy Science. 38: 230-234.

Baloch H, Rind R, Umerani AP, Bhutto AL, Abro SH, Rind MR, Abro R, Rizwana H, Kamboh AA, Baloch AK. 2016. Prevalence and risk factors associated with sub-clinical mastitis in Kundhi buffaloes. Journal of Basic Applied Sciences. 12: 301-305.

Bansal B, Singh K, Mohan R, Joshi D, Nauriyal D. 1995. Incidence of subclinical mastitis in some cow and buffalo herds in Punjab. Journal of Research: Punjab Agricultural University. 32 (1): 79-81.

Barbosa JD, da Silva JB, Rangel CP, da Fonseca AH, Silva NS, Bomjardim HA, Freitas NF. 2014. Tuberculosis prevalence and risk factors for water buffalo in Pará, Brazil. Tropical Animal Health Production. 46 (3): 513-517.

Barreiro JR, Gonçalves JL, Braga PAC, Dibbern AG, Eberlin MN, Veiga dos Santos M. 2017. Non-culture-based identification of mastitis-causing bacteria by MALDI-TOF mass spectrometry. Journal of Dairy Science. 100 (4): 2928-2934.

Bartlett PC, Miller GY, Lance SE, Heider LE. 1992. Managerial determinants of intramammary coliform and environmental streptococci infections in Ohio dairy herds. Journal of Dairy Science. 75 (5): 1241-1252.

BBS. 2011. Bangladesh Bureau of Statistics. District Statistics 2011: Noakhali. Government of the People's Republic of Bangladesh, Dhaka-1207. Accessed on 08/09/2022.

Beede D, Collier R, Wilcox C, Thatcher W. Year. Effects of warm climates on milk yield and composition (short-term effects). Milk production in developing countries: proceedings of the conference held in Edinburgh from the 2nd to 6th April 1984/organised by the Centre for Tropical Veterinary Medicine; edited by AJ Smith, p. 214-220.

Beheshti R, Eshratkhah B, Shayegh J, Ghalehkandi JG, Dianat V, Valiei K. 2011. Prevalence and etiology of subclinical mastitis in Buffalo of the Tabriz region, Iran. Journal of American Science. 7 (5): 642-645.

Beheshti R, Shaieghi J, Eshratkhah B, Ghalehkandi JG, Maheri-Sis N. 2010. Prevalence and etiology of subclinical mastitis in ewes of the Tabriz region, Iran. Global Veterinaria. 4 (3): 299-302.

Berry E, Hillerton J. 2002. The effect of selective dry cow treatment on new intramammary infections. Journal of Dairy Science. 85 (1): 112-121.

Bertoni A, Álvarez-Macias A, Mota-Rojas D. 2019. Productive performance of buffaloes and their development options in tropical regions. Socio Rural Production Medio Ambient. 19: 59-80.

Bertoni A, Napolitano F, Mota-Rojas D, Sabia E, Álvarez-Macías A, Mora-Medina P, Morales-Canela A, Berdugo-Gutiérrez J, Guerrero-Legarreta I. 2020. Similarities and differences between river buffaloes and cattle: Health, physiological, behavioral and productivity aspects. Journal of Buffalo Science. 9: 92-109.

Bharti P, Bhakat C, Pankaj PK, Bhat SA, Prakash MA, Thul MR, Japheth KP. 2015. Relationship of udder and teat conformation with intra-mammary infection in crossbred cows under hot-humid climate. Veterinary World. 8 (7): 898-901.

Bhutto AL, Murray RD, Woldehiwet Z. 2010. Udder shape and teat-end lesions as potential risk factors for high somatic cell counts and intra-mammary infections in dairy cows. The Veterinary Journal. 183 (1): 63-67.

Biggadike HJ, Ohnstad I, Laven RA, Hillerton JE. 2002. Evaluation of measurements of the conductivity of quarter milk samples for the early diagnosis of mastitis. Veterinary Record. 150 (21): 655-658.

BILAL M. 2004. Factors affecting the prevalence of clinical mastitis in buffaloes around Faisalabad district (Pakistan). International Journal of Agricultural Biology. 6 185-187.

Blowey RW, Edmondson P. 2010. Mastitis control in dairy herds. p.266.

Bonfoh B, Wasem A, Traoré AN. 2003. Microbiological quality of cows' milk taken at different intervals from the udder to the selling point in Bamako (Mali). Food Control.14:495-500.

Bonini Pardo R, Mendoza-Sànchez G, Nader Filho A, Santos T, Langoni H, Tonhati H, Ferreira EBS, Ravena DL, Oliveira MEA, Sturion DJ. 2007. Microbiological evaluation of milk samples positive to California Mastitis Test in dairy buffalo cows (Buballus bubalis). Italian Journal of Animal Science. 6 (sup2): 884-887.

Borghese A. 2011. Situation and perspectives of buffalo in the world, Europe and Macedonia. Macedonian Journal of Animal Science. 1 (2): 281-296.

Borghese A. 2013. Buffalo livestock and products in Europe. Buffalo Bulletin. 32 (Special Issue 1): 50-74.

Borghese A, Moioli B. 2016. Buffalo: Mediterranean Region. Reference Module in Food Science. B978-0-08-100596-5. P21232-8.

Bradley A. 2002. Bovine mastitis: an evolving disease. The Veterinary Journal. 164 (2): 116-128.

Bradley A, Breen J, Payne B, White V, Green M. 2015. An investigation of the efficacy of a polyvalent mastitis vaccine using different vaccination regimens under field conditions in the United Kingdom. Journal of Dairy Science. 98 (3): 1706-1720.

Breen JE, Bradley AJ, Green MJ. 2009. Quarter and cow risk factors associated with a somatic cell count greater than 199,000 cells per milliliter in United Kingdom dairy cows. Journal of Dairy Science. 92 (7): 3106-3115.

Bruford MW, Bradley DG, Luikart G. 2003. DNA markers reveal the complexity of livestock domestication. Nature Reviews Genetics. 4 (11): 900-910.

Caizares MAR, Suaz EC, Padrid JC, Lapitan JE, Molina MCM, Lantican JAG, Lantican FA, Carandang LAM. 2017. Buffalo Meat Value Chain Analysis in Luzon, Philippines. Books on Agricultural Research Development. Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA). p.384

Cameron M, Keefe G, Roy J-P, Stryhn H, Dohoo I, McKenna S. 2015. Evaluation of selective dry cow treatment following on-farm culture: Milk yield and somatic cell count in the subsequent lactation. Journal of Dairy Science. 98 (4): 2427-2436.

Carlen E, Strandberg E, Roth A. 2004. Genetic parameters for clinical mastitis, somatic cell score, and production in the first three lactations of Swedish Holstein cows. Journal of Dairy Science. 87 (9): 3062-3070.

Ceron-Munoz M, Tonhati H, Duarte J, Oliveira J, Munoz-Berrocal M, Jurado-Gamez H. 2002. Factors affecting somatic cell counts and their relations with milk and milk constituent yield in buffaloes. Journal of Dairy Science. 85 (11): 2885-2889.

Chakravarty A. 2013. Strategies for genetic improvement of buffaloes through production of quality male germplasm in SAARC countries. Seminar paper presentation in "High Yielding Dairy Buffalo Breed Development in SAARC Countries.

Chand P, Behra G. 1995. Factors influencing occurrence of mastitis genetic and environmental factors. Indian Journal of Dairy Science. 48 (4): 271-273.

Chunxi Z, Zhongquan L. 2001. Buffalo genetic resources in China. Buffalo Newsletter. 16: 1-7.

Clerc O, Prod'hom G, Vogne C, Bizzini A, Calandra T, Greub G. 2013. Impact of matrix-assisted laser desorption ionization time-of-flight mass spectrometry on the clinical management of patients with Gram-negative bacteremia: a prospective observational study. Clinical Infectious Diseases. 56 (8): 1101-1107.

Cockrill WR. 1974. The husbandry and health of the domestic buffalo.

Compton C, Heuer C, Parker K, McDougall S. 2007. Risk factors for peripartum mastitis in pasture-grazed dairy heifers. Journal of Dairy Science. 90 (9): 4171-4180.

Costa A, Neglia G, Campanile G, De Marchi M. 2020. Milk somatic cell count and its relationship with milk yield and quality traits in Italian water buffaloes. Journal of Dairy Science. 103 (6): 5485-5494.

Costa Eod, Garino Junior F, Watanabe ET, Ribeiro A, Vezon P, Baruselli P, Paske A. 1997a . Study of mastitis among ten dairy buffaloes herds (Bubalus bubalis) in the vale do Ribeira (Ribeira river valley) São Paulo, Brazil. Proceedings 5th World Buffalo Congress, 13-16 October, 1997. Caserta, Italy. P 635-638.

Costa Eod, Garino Junior F, Watanabe ET, Ribeiro A, Silva J, Vezon P, Gabaldi S, Benites NR, Baruselli P, Paske A. 1997b. Evaluation of the CMT positivity and the microbiologic status of the mammary gland over the different lactation phases in buffalo cows (Bubalus bubalis). Proceedings 5th World Buffalo Congress, 13-16 October, 1997. Royal Palace, Caserta, Italy. P 631-634

Costa EOd, Watanabe ET, Ribeiro A, Garino Júnior F, Horiuti A, Baruselli PS. 2000. Mastite bubalina: etiologia, índices de mastite clínica e subclínica. Napgama. 3 (1): 12-15.

Cruz-Monterrosa R, Mota-Rojas D, Ramirez-Bribiesca E, Mora-Medina P, Guerrero-Legarreta I. 2020. Scientific findings on the quality of river buffalo meat and prospects for future studies. Journal of Buffalo Science. 9: 170-80.

Cunha A, da Silva L, Pinheiro Júnior J, da Silva D, Oliveira AdF, da Silva K, Mota R. 2022. Perfil de sensibilidade antimicrobiana de agentes contagiosos e ambientais isolados de mastite clínica e subclínica de búfalas. Arquivos do Instituto Biológico. 73: 17-21.

Das GK, Khan FA. 2010. Summer anoestrus in buffalo--a review. Reproduction in Domestic Animals. 45 (6): e483-94.

De Graaf T, Dwinger R. 1996. Estimation of milk production losses due to sub-clinical mastitis in dairy cattle in Costa Rica. Preventive Veterinary Medicine. 26 (3-4): 215-222.

De Oliveira Moura E, do Nascimento Rangel AH, de Melo MCN, Borba LHF, de Lima Júnior DM, Novaes LP, Urbano SA, de Andrade Neto JC. 2017. Evaluation of microbiological, cellular and risk factors associated with subclinical mastitis in female buffaloes. Asian-australasian Journal of Animal Sciences. 30 (9): 1340-1349.

De Visscher A, Haesebrouck F, Piepers S, Vanderhaeghen W, Supré K, Leroy F, Van Coillie E, De Vliegher S. 2013. Assessment of the suitability of mannitol salt agar for growing bovine-associated coagulase-negative staphylococci and its use under field conditions. Research in Veterinary Science. 95 (2): 347-351.

Degrandi TM, Marques JRF, Gunski RJ, Travassos da Costa MR, Marques LC, Figueiró MR, Vinadé L, Garnero ADV, Degrandi TM, Marques JRF, Gunski RJ, Costa MRT, Figueiró MR, Vinadé L, Marques LC, Garnero ADV. 2014. Identificação citogenética de quatro gerações de búfalos mestiços mantidos em um programa de conservação na ilha de Marajó/Brazil. Journal of Biotechnology and Biodiversity. 5 (2): 162-171.

Denis M, Wedlock DN, Lacy-Hulbert SJ, Hillerton JE, Buddle BM. 2009. Vaccines against bovine mastitis in the New Zealand context: What is the best way forward? New Zealand Veterinary Journal. 57 (3): 132-140.

Derakhshani H, Fehr KB, Sepehri S, Francoz D, De Buck J, Barkema HW, Plaizier JC, Khafipour EJJoDS. 2018. Invited review: Microbiota of the bovine udder: Contributing factors and potential implications for udder health and mastitis susceptibility. Journal of Dairy Science. 101 (12): 10605-10625.

Dhakal I. 1997. Drug selection and use on clinical mastitis in buffaloes at Chitwan Valley of Nepal. Bubalus bubalis. 11 56-70.

Dhakal I. 2002. Economic impact of clinical mastitis in the buffaloes in Nepal. Buffalo Journal. 2 225-234.

Dhakal I. 2004. Normal somatic cell count and subclinical mastitis in Murrah buffaloes. Buffalo Journal. 20 (3): 261.

Dhakal IP. 2006. Normal somatic cell count and subclinical mastitis in Murrah buffaloes. Journal of Veterinary Medicine. 53 (2): 81-86.

Dhakal IP, Dhakal P, Koshihara T, Nagahata H. 2007. Epidemiological and bacteriological survey of buffalo mastitis in Nepal. Journal of Veterinary Medical Science. 69 (12): 1241-1245.

Dhakal IP, Neupane M, Nagahata H. 2008. Evaluation of direct and indirect measures of quarter milk from crossbred buffaloes. Animal Science Journal. 79 (5): 628-633.

Di Luccia A, Satriani A, Barone CMA, Colatruglio P, Gigli S, Occidente M, Trivellone E, Zullo A, Matassino D. 2003. Effect of dietary energy content on the intramuscular fat depots and triglyceride composition of river buffalo meat. Meat Science. 65 (4): 1379-1389.

DLS. 2018. Union based statistics of domestic animals. Livestock Dairy Development Project, Department of Livestock Services. Accessed on 14/07/2021.

DLS. 2022. Livestock Economy at a Glance. Department of Livestock Services. Accessed on 14/09/2022.

Dohoo I, Martin W, Stryhn H. 2003. Veterinary Epidemiologic Research.

Donovan DM, Kerr DE, Wall R. 2005. Engineering disease resistant cattle. Journal of Transgenic research. 14 (5): 563-567.

Du Z, Yang R, Guo Z, Song Y, Wang J. 2002. Identification of *Staphylococcus aureus* and determination of its methicillin resistance by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry. Analytical Chemistry. 74 (21): 5487-5491.

Dubey P, Suman C, Sanyal M, Pandey H, Saxena M, Yadav P. 2013. Factors affecting composition of milk of buffaloes. Directorate of Knowledge Management in Agriculture.

EL-Naker Y, Sayed-Ahmed M, Saad Z, Reiad E, Younis E. 2015. Prevalence of buffalo mastitis in Dakahyllia Governorates Assiut Veterinary Medical Journal. 61 (145): 32-43.

Elahi E, Abid M, Zhang H, Cui W, Ul Hasson S. 2018. Domestic water buffaloes: Access to surface water, disease prevalence and associated economic losses. Preventive Veterinary Medicine. 154: 102-112.

Elahi E, Abid M, Zhang L, Alugongo GM. 2017a. The use of wastewater in livestock production and its socioeconomic and welfare implications. Environmental Science and Pollution Research. 24 (21): 17255-17266.

Elahi E, Zhang L, Abid M, Javed MT, Xinru H. 2017b. Direct and indirect effects of wastewater use and herd environment on the occurrence of animal diseases and animal health in Pakistan. Environmental Science and Pollution Research. 24 (7): 6819-6832.

Elhaig MM, Selim A. 2015. Molecular and bacteriological investigation of subclinical mastitis caused by Staphylococcus aureus and *Streptococcus agalactiae* in domestic bovids from Ismailia, Egypt. Tropical Animal Health Production. 47 (2): 271-276.

Esposito L, Palo Rd, De Barros Pinto H, Ricci G, Zicarelli L. 1997. Variations in lactodynamometric characteristics of mediterranean buffalo milk from individual animals. Proceedings 5th World Buffalo Congress, 13-16 October, 1997. Royal Palace, Caserta, Italy. p 225-230.

Everley RA, Mott TM, Wyatt SA, Toney DM, Croley TR. 2008. Liquid chromatography/mass spectrometry characterization of Escherichia coli and Shigella species. Journal of the American Society for Mass Spectrometry. 19 (11): 1621-1628.

Fagiolo A, Lai O. 2007. Mastitis in buffalo. Italian Journal of Animal Science. 6 (sup2): 200-206.

Falvey L, Chantalakhana C. 1999. Smallholder dairying in the tropics.

FAO. 2000. World Watch List for Domestic Animal Diversity.

FAO. 2015. The Second Report on the State of the World's Animal Genetic Resources for Food and Agriculture. p.606.

FAOSTAT. 2018. About live animals, data on buffaloes. FAO. Accessed on 25/05/2020.

Faruque M, Hasnath M, Mostafa K, Okada I, Amano T, Kurosawa Y, Ota K, Namikawa T. 1987. Conservation of livestock genetic resources in Bangladesh–Past, present and future. Genetic studies on breed differentiation of native domestic animals in Bangladesh. Hiroshima University. 129-142.

Faruque M, Hasnath M, Siddique N. 1990. Present status of buffaloes and their productivity in Bangladesh. Asian-australasian journal of animal sciences. 3 (4): 287-92.

Faruque MO, Aziz SA, Banu LA, Trisha AA, Islam F. 2019. Breeding practices and milk production performances of Buffalo at rural villages in Bangladesh. International Journal of Business, Social and Scientific Research. 7 (3): 25-32.

Faull WB, Hughes J. 1985. Mastitis notes for the dairy practitioner.

Ferroni A, Suarez S, Beretti J-L, Dauphin B, Bille E, Meyer J, Bougnoux M-E, Alanio A, Berche P, Nassif X. 2010. Real-time identification of bacteria and Candida species in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Journal of Clinical Microbiology. 48 (5): 1542-1548.

Fosgate GT, Petzer IM, Karzis J. 2013. Sensitivity and specificity of a hand-held milk electrical conductivity meter compared to the California mastitis test for mastitis in dairy cattle. The Veterinary Journal. 196 (1): 98-102.

Fregonesi JA, Leaver JD. 2001. Behaviour, performance and health indicators of welfare for dairy cows housed in strawyard or cubicle systems. Livestock Production Science. 68 (2-3): 205-216.

Galiero G, Lai O, Fenizia D, Palladino, M. e Cuoco E. 1996a. Stato sanitario delle aziende bufaline nella provincia di Salerno: indagini chimiche e batteriologiche sul latte destinato alla trasformazione. Veterinaria Italiana. 20: 29-34.

Galiero G, Morena C. 2000. The meaning of the somatic cell count in buffalo milk. Bubalus bubalis. 6 (4): 26-27.

Galiero G, Palladino M, Lai O, Goffredi C. 1996b. Buffaloe mastitis: bacteria identification and susceptibility to antimicrobial drugs. Bubalus bubalis. 2: 61-66.

Gay J. 2009. Epidemiology concepts for disease in animal groups. .

Giordano G, Guarini P, Ferrari P, Biondi-Zoccai G, Schiavone B, Giordano A. 2010. Beneficial impact on cardiovascular risk profile of water buffalo meat consumption. European Journal of Clinical Nutrition. 64 (9): 1000-1006.

Giuffrida-Mendoza M, de Moreno LA, Huerta-Leidenz N, Uzcátegui-Bracho S, Valero-Leal K, Romero S, Rodas-González A. 2015. Cholesterol and fatty acid composition of longissimus thoracis from water buffalo (Bubalus bubalis) and Brahman-influenced cattle raised under savannah conditions. Meat Science. 106: 44-49.

Golder H, Hodge A, Lean I. 2016. Effects of antibiotic dry-cow therapy and internal teat sealant on milk somatic cell counts and clinical and subclinical mastitis in early lactation. Journal of Dairy Science. 99 (9): 7370-7380.

Gröhn YT, Eicker SW, Hertl JA. 1995. The Association between Previous 305-day Milk Yield and Disease in New York State Dairy Cows. Journal of Dairy Science. 78 (8): 1693-1702.

Guccione J, Cosandey A, Pesce A, Di Loria A, Pascale M, Piantedosi D, Steiner A, Graber HU, Ciaramella P. 2014. Clinical outcomes and molecular genotyping of Staphylococcus aureus isolated from milk samples of dairy primiparous Mediterranean buffaloes (Bubalus bubalis). Journal of Dairy Science. 97 (12): 7606-7613.

Guerrero-Legarreta I, Napolitano F, Cruz-Monterrosa R, Mota-Rojas D, Mora-Medina P, Ramírez-Bribiesca E, Bertoni A, Berdugo-Gutiérrez J, Braghieri A. 2020.

River buffalo meat production and quality: Sustainability, productivity, nutritional and sensory properties. Journal of Buffalo Science. 9: 159-69.

Guha A, Gera S, Sharma A. 2012. Evaluation of Milk Trace Elements, Lactate Dehydrogenase, Alkaline Phosphatase and Aspartate Aminotransferase Activity of Subclinical Mastitis as an Indicator of Subclinical Mastitis in Riverine Buffalo (Bubalus bubalis). Asian-Australasian Journal of Animal Sciences. 25 (3): 353-360.

Halasa T, Nielen M, Whist A, Østerås O. 2009. Meta-analysis of dry cow management for dairy cattle. Part 2. Cure of existing intramammary infections. Journal of Dairy Science. 92 (7): 3150-3157.

Hamid M, Ahmed S, Rahman M, Hossain K. 2016. Status of buffalo production in Bangladesh compared to SAARC countries. Asian Journal of Animal Sciences. 10 (6): 313-329.

Hamid M, Siddiky M, Rahman M, Hossain K. 2017a. Scopes and opportunities of buffalo farming in Bangladesh: A review. SAARC Journal of Agriculture. 14 (2): 63-77.

Hamid M, Zaman M, Rahman A, Hossain K. 2017b. Buffalo genetic resources and their conservation in Bangladesh. Research Journal of Veterinary Sciences. 10: 1-13.

Han B-Z, Meng Y, Li M, Yang Y-X, Ren F-Z, Zeng Q-K, Robert Nout MJ. 2007. A survey on the microbiological and chemical composition of buffalo milk in China. Food Control. 18 (6): 742-746.

Hardenberg F, 2016. Clinical and subclinical mastitis in dairy cattle and buffaloes in Bihar, India: prevalence, major pathogens and risk factors. Swedish University of Agricultural Sciences: Veterinary Medicine. Sweden.

Harmon RJ. 1994. Physiology of mastitis and factors affecting somatic cell counts. Journal of Dairy Science. 77 (7): 2103-12.

Harwood VJ, Staley C, Badgley BD, Borges K, Korajkic A. 2014. Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. FEMS Microbiology Reviews. 38 (1): 1-40.

Hasina B, Rahmatullah R, Rind M, Viram K, Nazia B, Oad R. 2018. Effect of diverse factors on the frequency of clinical and subclinical mastitis in Kundhi buffaloes of Sindh, Pakistan. Pakistan Journal of Zoology. 50 (5): 1619-1628.

Heinrichs A, Costello S, Jones C. 2009. Control of heifer mastitis by nutrition. Veterinary Microbiology. 134 (1-2): 172-176.

Hijazin M, Ulbegi-Mohyla H, Alber J, Lämmler C, Hassan AA, Timke M, Kostrzewa M, Prenger-Berninghoff E, Weiss R, Zschöck M. 2012. Identification of Arcanobacterium (Trueperella) abortisuis, a novel species of veterinary importance, by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Berliner und Munchener tierarztliche Wochenschrift. 125 (1-2): 32-7.

Hoare R, EA R. 1972. Investigations in mastitis problem herds. II. Effect of herd size, shed type, hygiene and management practices. Australian Veterinary Journal. 48 (12): 661-663.

Huque Q, Borghese A. 2012. Production potentiality and perspective of buffalo in Bangladesh. Procedings of the 15th AAAP Animal Science Congress, p 26-30.

Hussain R, Javed MT, Khan A, Muhammad G. 2013. Risks factors associated with subclinical mastitis in water buffaloes in Pakistan. Tropical Animal Health Production. 45 (8): 1723-1729.

Islam KBM, bin Kabir H, Rahman M, Kabir M. 2016. Status of buffalo diseases in Bangladesh in relation to casual agents and predisposing factors. International Journal of Life Sciences and Technology. 9: 44-50.

Javed S, McClure J, Syed MA, Obasuyi O, Ali S, Tabassum S, Ejaz M, Zhang K. 2022. Epidemiology and molecular characterization of Staphylococcus aureus causing bovine mastitis in water buffaloes from the Hazara division of Khyber Pakhtunkhwa, Pakistan. PloS One. 17 (5): e0268152.

Jhambh R, Dimri U, Gopalakrishnan A, Singh M, Chhabra R. 2017. Prevalence and risk factors of subclinical mastitis in buffaloes at an organized dairy farm in western Haryana. Haryana Veterinarian. 56 (2): 189-193.

Johnson SD, Norcross NL. 1971. An experimental method of vaccination for a *Streptococcus agalactiae* infected herd. Cornell Veterinarian. 61 (2): 258-264.

Kabera F, Dufour S, Keefe G, Roy J-P. 2018. An observational cohort study on persistency of internal teat sealant residues in milk after calving in dairy cows. Journal of Dairy Science. 101 (7): 6399-6412.

Kalra D, Dhanda M. 1964. Incidence of mastitis in cows and buffaloes in North West India. Veterinary Record. 76 (2): 9-222.

Kandeepan G, Biswas S, Rajkumar R. 2009. Buffalo as a potential food animal. International Journal of Livestock Production. 1 (1): 1-5.

Kandeepan G, Mendiratta S, Shukla V, Vishnuraj M. 2013. Processing characteristics of buffalo meat-a review. Journal of Meat Science and Technology. 1 (1): 1-11.

Karimuribo ED, Fitzpatrick JL, Bell CE, Swai ES, Kambarage DM, Ogden NH, Bryant MJ, French NP. 2006. Clinical and subclinical mastitis in smallholder dairy farms in Tanzania: Risk, intervention and knowledge transfer. Preventive Veterinary Medicine. 74 (1): 84-98.

Kashyap DK, Giri DK, Govina D. 2019. Prevalence of sub clinical mastitis (SCM) in she buffaloes at Surajpur district of Chhattishgarh, India. Buffalo Bulletin. 38 (2): 373-381.

Kaur G, Bansal B, Singh R, Kashyap N, Sharma S. 2018. Associations of teat morphometric parameters and subclinical mastitis in riverine buffaloes. The Journal of Dairy Research. 85 (3): 303.

Kaur M, Verma R, Bansal BK, Mukhopadhyay CS, Arora JS. 2015. Status of sub-clinical mastitis and associated risk factors in Indian water buffalo in Doaba region of Punjab, India. Indian Journal of Dairy Science. 8 483-7.

Kavitha KL, Rajesh K, Suresh K, Satheesh K, Sundar NS. 2009. Buffalo mastitis - risk factors. Buffalo Bulletin. 28 (3): 135-137.

Khademi P, Ownagh A, Mardani K, Khalili M. 2019. Prevalence of Coxiella burnetii in milk collected from buffalo (water buffalo) and cattle dairy farms in Northwest of Iran. Comparative Immunology, Microbiology & Infectious Diseases. 67: 101368.

Khan A, Muhammad G. 2005. Quarter-wise comparative prevalence of mastitis in buffaloes and crossbred cows. Pakistan Veterinary Journal. 25 (1): 9-12.

Khan MK, Sajid MS, Khan MN, Iqbal Z, Iqbal MU. 2009. Bovine fasciolosis: Prevalence, effects of treatment on productivity and cost benefit analysis in five districts of Punjab, Pakistan. Research in veterinary science. 87 (1): 70-75.

Khan S. 2001. Water buffaloes for food security and sustainable rural development in Pakistan. Regional Workshop on Water Buffalo Development, 8-10 Feb 2001. Bangkok (Thailand).

Kitchen BJ. 1981. Bovine mastitis: milk compositional changes and related diagnostic tests. Journal of Dairy Research. 48 (1): 167-188.

Kivaria F, Noordhuizen J, Msami H. 2007. Risk factors associated with the incidence rate of clinical mastitis in smallholder dairy cows in the Dar es Salaam region of Tanzania. The Veterinary Journal. 173 (3): 623-629.

Kivaria FM, Noordhuizen JPTM, Kapaga AM. 2004. Risk Indicators Associated with Subclinical Mastitis in Smallholder Dairy Cows in Tanzania. Tropical Animal Health Production. 36 (6): 581-592.

Klaas IC, Zadoks RN. 2018. An update on environmental mastitis: Challenging perceptions. Transboundary Emerging Disease. 65 (Suppl.1): 166-185.

Kohlmann R, Hoffmann A, Geis G, Gatermann S. 2015. MALDI-TOF mass spectrometry following short incubation on a solid medium is a valuable tool for rapid pathogen identification from positive blood cultures. International Journal of Medical Microbiology. 305 (4-5): 469-479.

Krishnaswamy S, Vedanayaham ARa, Varma K. 1965. Studies on Mastitis in cattle. Indian Veterinary Journal. (42): 92-103.

Kumar S, Nagarajan M, Sandhu JS, Kumar N, Behl V. 2007. Phylogeography and domestication of Indian River buffalo. BMC Evolutionary Biology. 7 (1): 186.

Landi N, di Giuseppe AMA, Ragucci S, Dil Maro A. 2016. Free amino acid profile of Bubalus bubalis L. meat from the Campania region. Revista Brasileira de Zootecnia. 45 (10): 627-631.

Landin H, Mörk MJ, Larsson M, Waller KP. 2015. Vaccination against Staphylococcus aureus mastitis in two Swedish dairy herds. Acta Veterinaria Scandinavica. 57 (1): 1-6.

Lescourret F, Coulon JB, Faye B. 1995. Predictive Model of Mastitis Occurrence in the Dairy Cow. Journal of Dairy Science. 78 (10): 2167-2177.

Levison LJ, Miller-Cushon EK, Tucker AL, Bergeron R, Leslie KE, Barkema HW, DeVries TJ. 2016. Incidence rate of pathogen-specific clinical mastitis on conventional and organic Canadian dairy farms. Journal of Dairy Science. 99 (2): 1341-1350.

Li WC, Huang JM, Fang Z, Ren Q, Tang L, Kan ZZ, Liu XC, Gu YF. 2020. Prevalence of Tetratrichomonas buttreyi and Pentatrichomonas hominis in yellow cattle, dairy cattle, and water buffalo in China. Parasitology Research. 119 (2): 637-647.

Liu G, Yin J, Barkema HW, Chen L, Shahid M, Szenci O, De Buck J, Kastelic JP, Han B. 2017. Development of a single-dose recombinant CAMP factor entrapping poly (lactide-co-glycolide) microspheres-based vaccine against Streptococcus agalactiae. Vaccine. 35 (9): 1246-1253.

Locatelli C, Piepers S, De Vliegher S, Barberio A, Supre K, Scaccabarozzi L, Pisoni G, Bronzo V, Haesebrouck F, Moroni P. 2013. Effect on quarter milk somatic cell count and antimicrobial susceptibility of Staphylococcus rostri causing intramammary infection in dairy water buffaloes. Journal of Dairy Science. 96 (6): 3799-3805.

Lopez-Benavides M, Williamson J, Pullinger G, Lacy-Hulbert S, Cursons R, Leigh J. 2007. Field observations on the variation of Streptococcus uberis populations in a pasture-based dairy farm. Journal of Dairy Science. 90 (12): 5558-5566.

Ma Y, Ryan C, Barbano D, Galton D, Rudan M, Boor K. 2000. Effects of somatic cell count on quality and shelf-life of pasteurized fluid milk. Journal of Dairy Science. 83 (2): 264-274.

Maalik A, Ali S, Iftikhar A, Rizwan M, Ahmad H, Khan L. 2019. Prevalence and Antibiotic Resistance of Staphylococcus aureus and Risk Factors for Bovine Subclinical Mastitis in District Kasur, Punjab, Pakistan. Pakistan Journal of Zoology. 51 (3): 1123-1130.

MacGregor R. 1941. The domestic buffalo. Veterinary Record. 53 (31): 443-450.

Maheswarappa NB, Muthupalani M, Mohan K, Banerjee R, Sen AR, Barbuddhe SB. 2022. Water Buffalo: Origin, Emergence, and Domestication. Springer Nature Singapore. pp. 1-10.

Marai I, Habeeb A, Daader A, Yousef H. 1995. Effects of Egyptian subtropical summer conditions and the heat-stress alleviation technique of water spray and a diaphoretic on the growth and physiological functions of Friesian calves. Journal of Arid Environments. 30 (2): 219-225.

Marai I, Habeeb A, Gad A. 2002. Rabbits' productive, reproductive and physiological performance traits as affected by heat stress: a review. Livestock Production Science. 78 (2): 71-90.

Marai IF, El-Darawany AHA, Ismail ESAF, Abdel-Hafez MA. 2006. Tunica dartos index as a parameter for measurement of adaptability of rams to subtropical conditions of Egypt. Animal Science Journal. 77 (5): 487-494.

Marai IFM, Haeeb AAM. 2010. Buffalo's biological functions as affected by heat stress — A review. Livestock Science. 127 (2): 89-109.

Martini M, Spinelli S, Scolozzi C, Cecchi F. 2003. Studio delle caratteristiche lipidiche del latte di bufale allevate in Toscana: nota II. 2° Congresso nazionale sull'allevamento del buffalo. p.147-151.

McVey DS, Kennedy M, Chengappa M. 2013. Veterinary microbiology: Bacteria and Fungi. John Wiley & Sons. 59.

Mekibib B, Furgasa M, Abunna F, Megersa B, Regassa A. 2010. Bovine mastitis: Prevalence, risk factors and major pathogens in dairy farms of Holeta Town, Central Ethiopia. Veterinary World. 3 (9): 397-403.

Ménard O, Ahmad S, Rousseau F, Briard-Bion V, Gaucheron F, Lopez C. 2010. Buffalo vs. cow milk fat globules: Size distribution, zeta-potential, compositions in total fatty acids and in polar lipids from the milk fat globule membrane. Food Chemistry. 120 (2): 544-51.

Merriman KE, Poindexter MB, Kweh MF, Santos JE, Nelson CD. 2017. Intramammary 1, 25-dihydroxyvitamin D3 treatment increases expression of host-defense genes in mammary immune cells of lactating dairy cattle. The Journal of Steroid Biochemistry Molecular Biology. 173: 33-41.

Merriman KE, Powell JL, Santos JE, Nelson CD. 2018. Intramammary 25-hydroxyvitamin D3 treatment modulates innate immune responses to endotoxin-induced mastitis. Journal of Dairy Science. 101 (8): 7593-7607.

Milner P, Page KL, Walton AW, Hillerton JE. 1996. Detection of Clinical Mastitis by Changes in Electrical Conductivity of Foremilk before Visible Changes in Milk. Journal of Dairy Science. 79 (1): 83-86.

Minervino AHH, Zava M, Vecchio D, Borghese A. 2020. Bubalus bubalis: A Short Story. Frontiers in Veterinary Science. 7.

Moioli B, Borghese A. 2005. Buffalo breeds and management systems. A Borghese.Food and Agriculture Organization of the United Nations. pp. 51-76.

Moroni P, Sgoifo Rossi C, Pisoni G, Bronzo V, Castiglioni B, Boettcher PJ. 2006. Relationships between somatic cell count and intramammary infection in buffaloes. Journal of Dairy Science. 89 (3): 998-1003.

Moussaoui W, Jaulhac B, Hoffmann AM, Ludes B, Kostrzewa M, Riegel P, Prévost G. 2010. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry identifies 90% of bacteria directly from blood culture vials. Clinical Microbiology and Infection. 16 (11): 1631-1638.

Muehlhoff E, Bennett A, McMahon D. 2013. Milk and dairy products in human nutrition. Food and Agriculture Organization of the United Nations (FAO). p.376

Mukherjee R. 2008. Selenium and vitamin E increases polymorphonuclear cell phagocytosis and antioxidant levels during acute mastitis in riverine buffaloes. Veterinary Research Communications. 32 (4): 305-313.

Munoz MA, Welcome FL, Schukken YH, Zadoks RN. 2007. Molecular epidemiology of two Klebsiella pneumoniae mastitis outbreaks on a dairy farm in New York State. Journal of Clinical Microbiology. 45 (12): 3964-3971.

Murray P, Rosenthal K, Pfaller M. 2015. Medical microbiology: Elsevier health sciences.

Musser JM, Anderson KL, Caballero M, Amaya D, Maroto-Puga J. 1998. Evaluation of a hand-held electrical conductivity meter for detection of subclinical mastitis in cattle. American Journal of Veterinary Research. 59 (9): 1087-1091.

Mustafa YS, Awan FN, Zaman T, Chaudhry SR, Zoyfro V. 2011. Prevalence and antibacterial susceptibility in mastitis in buffalo and cow in and around the district Lahore, Pakistan. Pakistan Journal of Pharmacy. 24 (2): 29-33.

Nagel JL, Huang AM, Kunapuli A, Gandhi TN, Washer LL, Lassiter J, Patel T, Newton DW. 2014. Impact of antimicrobial stewardship intervention on coagulase-negative Staphylococcus blood cultures in conjunction with rapid diagnostic testing. Journal of Clinical Microbiology. 52 (8): 2849-2854.

Naiknaware H, Shelke D, Bhalerao D, Keskar D, Jagadesh S, Sharma L. 1998. Prevalence of subclinical mastitis in buffaloes in and around Mumbai. Indian Veterinary Journal. 75 (4): 291-292.

Naveena BM, Kiran M. 2014. Buffalo meat quality, composition, and processing characteristics: Contribution to the global economy and nutritional security. Animal Frontiers. 4 (4): 18-24.

Nayak S, Mishra M. 1984. Dairy temperament of Red Sindhi, Crossbred cows and Murrah buffaloes in relation to their milking ability and composition. Indian Journal of Dairy Science.

Nazifi S, Haghkhah M, Asadi Z, Ansari-Lari M, Tabandeh M, Esmailnezhad Z, Aghamiri M. 2011. Evaluation of sialic acid and acute phase proteins (haptoglobin and serum amyloid A) in clinical and subclinical bovine mastitis. Pakistan Veterinary Journal. 31 (1): 55-59.

Ndahetuye JB, Persson Y, Nyman A-K, Tukei M, Ongol MP, Båge R. 2019. Aetiology and prevalence of subclinical mastitis in dairy herds in peri-urban areas of Kigali in Rwanda. Tropical Animal Health Production. 51 (7): 2037-2044.

Neath KE, Del Barrio AN, Lapitan RM, Herrera JRV, Cruz LC, Fujihara T, Muroya S, Chikuni K, Hirabayashi M, Kanai Y. 2007a. Difference in tenderness and pH decline between water buffalo meat and beef during postmortem aging. Meat Science. 75 (3): 499-505.

Neath KE, Del Barrio AN, Lapitan RM, Herrera JRV, Cruz LC, Fujihara T, Muroya S, Chikuni K, Hirabayashi M, Kanai Y. 2007b. Protease activity higher in postmortem water buffalo meat than Brahman beef. Meat Science. 77 (3): 389-396.

Neave FK, Dodd FH, Kingwill RG, Westgarth DR. 1969. Control of mastitis in the dairy herd by hygiene and management. Journal of Dairy Science. 52 (5): 696-707.

Neijenhuis F, Klungel GH, Hogeveen H. 2001. Recovery of Cow Teats after Milking as Determined by Ultrasonographic Scanning. Journal of Dairy Science. 84 (12): 2599-2606.

Nelson CD, Merriman KE, Poindexter MB, Kweh MF, Blakely LP. 2018. Symposium review: Targeting antimicrobial defenses of the udder through an intrinsic cellular pathway. Journal of Dairy Science. 101 (3): 2753-2761.

Nickerson S, 1990. Production of quality milk and control of mastitis in Mexico, in Diary Research Report. Hill Farm Research Station Route-1, Box 10. Homer, LA.

Nonnemann B, Tvede M, Bjarnsholt T. 2013. Identification of pathogenic microorganisms directly from positive blood vials by matrix-assisted laser desorption/ionization time of flight mass spectrometry. Journal of Pathology, Microbiology and Immunology. 121 (9): 871-877.

Nyman A-K, Ekman T, Emanuelson U, Gustafsson A, Holtenius K, Waller KP, Sandgren CH. 2007. Risk factors associated with the incidence of veterinary-treated clinical mastitis in Swedish dairy herds with a high milk yield and a low prevalence of subclinical mastitis. Preventive Veterinary Medicine. 78 (2): 142-160.

Nyman A-K, Emanuelson U, Gustafsson A, Waller KP. 2009. Management practices associated with udder health of first-parity dairy cows in early lactation. Preventive Veterinary Medicine. 88 (2): 138-149.

Oliveira M, 1997. Exame microbiológico do leite de búfalas e avaliação dos testes de Whiteside e Califórnia Mastitis Test no diagnóstico da mastite bacteriana subclínica em bubalinos. 1997, Brazil: Dissertação (Mestrado)-Universidade Federal Rural de Pernambuco, Recife.

Ordovas JM, Ferguson LR, Tai ES, Mathers JC. 2018. Personalised nutrition and health. British Medical Journal. 361.

Özenç E, Vural MR, ŞEKER E, Uçar M. 2008. An evaluation of subclinical mastitis during lactation in Anatolian buffaloes. Turkish Journal of Veterinary & Animal Sciences. 32 (5): 359-368.

Pandey N, Hopker A, Prajapati G, Rahangdale N, Gore K, Sargison N. 2022. Observations on presumptive lumpy skin disease in native cattle and Asian water buffaloes around the tiger reserves of the central Indian highlands. New Zealand Veterinary Journal. 70 (2): 101-108.

Pankaj, Anshu S, Rajesh C, Neelesh S. 2013. Sub-clinical mastitis in Murrah buffaloes with special reference to prevalence, etiology and antibiogram. Buffalo Bulletin. 32 (2): 107-115.

Parker K, Compton C, Anniss F, Weir A, Heuer C, McDougall S. 2007. Subclinical and clinical mastitis in heifers following the use of a teat sealant pre-calving. Journal of Dairy Science. 90 (1): 207-218.

Pasha T, Hayat Z. 2012. Present situation and future perspective of buffalo production in Asia. The Journal of Animal and Plant Sciences. 22: 250-256.

Pasquini M, Osimani A, Tavoletti S, Moreno I, Clementi F, Trombetta MF. 2018. Trends in the quality and hygiene parameters of bulk Italian Mediterranean buffalo (Bubalus bubalis) milk: A three year study. Animal Science Journal. 89 (1): 176-185.

Patil NA, Satbige AS, Awati B, Halmandge S. 2021. Therapeutic management of subclinical mastitis in buffaloes. Buffalo Bulletin. 40 (1): 157-160.

Paul OB, Chowdhury S, Nath SC, Singha S, Eima FE, Akter S, Bari S, Debnath N, Biswas PK, Hoque MA, Rahman MM, Hossain D, Khatun M, Islam MT, Alam MGS, Ahmed SSU, Hossain MK, Biswas D, Juli SB, Karim MR, Sayeed MA, Hossain MF, Amin MN, Huq MR, Rahman M, Kamaruddin K, Mansor R, S. S, Ahmed A, Pathirana I, Fernando P, White E, Derks M, Koop G, Persson Y. Year. Clinical mastitis decision tree: A guideline for field veterinarians, and dairy farmers to improve udder health of dairy cows in Bangladesh. 26th BSVER Annual International Scientific Conference, Bangladesh.

Payne W, Tech. 1990. Cattle and buffalo meat production in the tropic. Intermediate Tropical Agriculture Series. Longman Science. 210.

Persson Y, Nyman A-KJ, Grönlund-Andersson U. 2011. Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden. Acta Veterinaria Scandinavica. 53 (1): 1-8.

Petti CA, Carroll KC. 2011. Procedures for the Storage of Microorganisms. 10th. American Society of Microbiology. pp. 124-131.

Preethirani PL, Isloor S, Sundareshan S, Nuthanalakshmi V, Deepthikiran K, Sinha AY, Rathnamma D, Nithin Prabhu K, Sharada R, Mukkur TK, Hegde NR. 2015. Isolation, Biochemical and Molecular Identification, and In-Vitro Antimicrobial Resistance Patterns of Bacteria Isolated from Bubaline Subclinical Mastitis in South India. PloS One. 10 (11): e0142717-e.

Pyörälä S. 2003. Indicators of inflammation in the diagnosis of mastitis. Vet Res. 34 (5): 565-578.

Radostits OM, Gay CC, Blood DC, Hincheliff KW. 2000. Mastitis. In: VeterinaryMedicine: A text book of diseases of cattle, sheep, pig, goat and horses. pp. 603–700.

Radostits OM, Gray CC, Hinchcliff KW, Constable PD. 2007. A text book of the disease of Cattle, Horse, Sheep, Pigs and Goats. p.673–749.

Ramírez NF, Keefe G, Dohoo I, Sánchez J, Arroyave O, Cerón J, Jaramillo M, Palacio LG. 2014. Herd- and cow-level risk factors associated with subclinical mastitis in dairy farms from the High Plains of the northern Antioquia, Colombia. Journal of Dairy Science. 97 (7): 4141-4150.

Reichmuth. 1975. Somatic cell counting interpretation of results. International Dairy Federation.

Ren D-x, Zou C-x, Lin B, Chen Y-l, Liang X-w, Liu J-x. 2015. A Comparison of Milk Protein, Amino Acid and Fatty Acid Profiles of River Buffalo and Their F1 and F2 Hybrids with Swamp Buffalo in China. Pakistan Journal of Zoology. 47 (5): 1459-1465

Richert RM, Cicconi KM, Gamroth MJ, Schukken YH, Stiglbauer KE, Ruegg PL. 2013. Management factors associated with veterinary usage by organic and conventional dairy farms. Journal of the American Veterinary Medical Association. 242 (12): 1732-1743.

Riekerink RO, Barkema H, Stryhn H. 2007. The effect of season on somatic cell count and the incidence of clinical mastitis. Journal of Dairy Science. 90 (4): 1704-1715.

Robertson J, Bouton PE, Harris PV, Shorthose WR, Ratcliff D. 1983. A Comparison of Some Properties of Beef and Buffalo (Bubalus bubalis) Meat. Journal of Food Science. 48 (3): 686-690.

Rollin E, Dhuyvetter K, Overton M. 2015. The cost of clinical mastitis in the first 30 days of lactation: An economic modeling tool. Preventive Veterinary Medicine. 122 (3): 257-264.

Roy S, Vishwakarma P, Roy M, Sharma M. 2009. Prevalence and control of bubaline mastitis in Chhattisgarh State in India. Pakistan Journal of Zoology. 9: 281-287.

Saadullah M. 1998. Buffalo Production in the Tropics. Journal of Bangladesh Agricultural University.

Saadullah M. 2012. Buffalo production and constrains in Bangladesh. Journal of Animal Plant Sciences. 22: 221-224.

Sadoon AS. 2022. Clinical and subclinical mastitis in buffalue in Mosul area, Iraq Iraqi Journal of Veterinary Sciences. 36 (1): 177-186.

Sah K, Karki P, Shrestha RD, Sigdel A, Adesogan AT, Dahl GE. 2020. MILK Symposium review: Improving control of mastitis in dairy animals in Nepal. Journal of Dairy Science. 103 (11): 9740-9747.

Sahİn A, Yİldİrİm A, Ulutas Z, Ugurlutepe E. 2017. The effects of stage of lactation, parity and calving season on somatic cell counts in Anatolian water buffaloes. Indian Journal of Animal Research. 51 (1): 35-39.

Saini S, Sharma J, Kwatra M. 1994. Prevalence and etiology of subclinical mastitis among crossbred cows and buffaloes in Punjab. Indian Journal of Dairy Science. 47 (2): 103-106.

Salvador RT, Beltran JM, Abes NS, Gutierrez CA, Mingala CN. 2012. Short communication: Prevalence and risk factors of subclinical mastitis as determined by the California Mastitis Test in water buffaloes (Bubalis bubalis) in Nueva Ecija, Philippines. Journal of Dairy Science. 95 (3): 1363-6.

Samad M. 2020. A systematic review of research findings on buffalo health and production published during the last six decades in Bangladesh. Journal of Veterinary Medical and One Health Research. 2: 1-62.

Sastry N, Bhagat S, Bharadwaj A. 1988a. Aspects to be considered in milking management of buffaloes. Indian Journal of Animal Production Management. 4: 4378-4393.

Sastry N, Tripathi V. 1988b. Modern management innovations for optimizing buffalo production. Buffalo production and health, A compendium of latest research information based on Indian studies. 2nd World Buffalo congress, New Delhi, p. 38-62.

Sauer S, Freiwald A, Maier T, Kube M, Reinhardt R, Kostrzewa M, Geider K. 2008. Classification and identification of bacteria by mass spectrometry and computational analysis. PloS One. 3 (7): e2843.

Saxena H. 1973. Variation in shape and size of teats in Murrah buffaloes. Indian Veterinary Journal.

Schalm O. 1957. Experiments and observations leading to development of the California Mastitis Test. Journal of the American Veterinary Medical Association. 130: 199-204.

Schukken YH, Grommers FJ, Van De Geer D, Erb HN, Brand A. 1990. Risk Factors for Clinical Mastitis in Herds with a Low Bulk Milk Somatic Cell Count. 1. Data and Risk Factors for All Cases. Journal of Dairy Science. 73 (12): 3463-3471.

Schukken YH, Günther J, Fitzpatrick J, Fontaine MC, Goetze L, Holst O, Leigh J, Petzl W, Schuberth H-J, Sipka A. 2011. Host-response patterns of intramammary infections in dairy cows. Veterinary Immunology and Immunopathology. 144 (3-4): 270-289.

Schwarz DGG, de Sousa Júnior PF, Saraiva da Silva L, Polveiro RC, de Oliveira JF, Faria MPO, Marinho G, de Oliveira RP, Moreira MAS. 2021. Spatiotemporal distribution and temporal trends of brucellosis and tuberculosis in water buffalo (Bubalus bubalis) in Brazil. Preventive Veterinary Medicine. 193: 105417.

Scott JS, Sterling SA, To H, Seals SR, Jones AE. 2016. Diagnostic performance of matrix-assisted laser desorption ionisation time-of-flight mass spectrometry in blood bacterial infections: a systematic review and meta-analysis. Infectious Diseases. 48 (7): 530-536.

Seegers H, Fourichon C, Beaudeau F. 2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. Veterinary Research. 34 (5): 475-91.

Sharif A, Ahmad T. 2007. Prevalence of severity of mastitis in buffaloes in district Faisalabad (Pakistan). Journal of agriculture and social sciences. 03 (1): 34-36.

Sharma A, Chhabra R, Singh M, Charaya G. 2018. Prevalence, etiology and antibiogram of bacterial isolates recovered from mastitis of buffaloes. Buffalo Bulletin. 37 (3): 313-320.

Sharma A, Sindhu N. 2007. Occurrence of clinical and subclinical mastitis in buffaloes in the State of Haryana (India). Italian Journal of Animal Science. 6 (sup2): 965-967.

Sharma N. 2007. Alternative approach to control intramammary infection in dairy cows-A review. Asian Journal of Animal and Veterinary Advances. 2 (2): 50-62.

Sharma N, Gandemer G, Goutefongea R, Kowale BN. 1986. Fatty acid composition of water buffalo meat. Meat Science. 16 (3): 237-243.

Sharma N, Kang TY, Lee S-J, Kim JN, Hur CH, Ha JC, Vohra V, Jeong DK. 2013. Status of bovine mastitis and associated risk factors in subtropical Jeju Island, South Korea. Tropical Animal Health Production. 45 (8): 1829-1832.

Sharma T, Das PK, Ghosh PR, Banerjee D, Mukherjee J. 2017. Association between udder morphology and in vitro activity of milk leukocytes in high yielding crossbred cows. Veterinary World. 10 (3): 342-347.

Shields P, Cathcart L. 2010. Oxidase test protocol. American Society for Microbiology. 1-9.

Shukla S, Dixit V, Thapliyal D, Garg S, Kumar A. 1997. A note on the incidence of bovine mastitis in relation to teat shape, size and quarters affected. Indian Veterinary Journal. 74 (11).

Silva I. 1994. Total and differential cell counts in buffalo milk. Buffalo Journal. 2 133-237.

Singh I. 2013. High yielding dairy buffalo breed development in South Asia: Constraints and opportunities. Seminar Paper Presentation in High Yielding Dairy Buffalo Breed Development in SAARC Countries, SAARC Agriculture Centre, BARC Complex, Farm Gate, Dhaka.

Singh R. 2004. Variation in selected components of milk among different milk fractions and it's relevance to diagnosis of mastitis in buffaloes. Buffalo Journal. 3 213-24.

Singh RS, Bansal BK, Gupta DK. 2014. Udder health in relation to udder and teat morphometry in Holstein Friesian × Sahiwal crossbred dairy cows. Tropical Animal Health Production. 46 (1): 93-98.

Singha S, Ericsson CD, Chowdhury S, Nath SC, Paul OB, Hoque MA, Boqvist S, Persson Y, Rahman MM. 2021. Occurrence and aetiology of subclinical mastitis in water buffalo in Bangladesh. Journal of Dairy Research. 88 (3): 314-320.

Slettbakk T, Jørstad A, Farver TB, Holmes JC. 1995. Impact of milking characteristics and morphology of udder and teats on clinical mastitis in first-and second-lactation Norwegian cattle. Preventive Veterinary Medicine. 24 (4): 235-244.

Smith K. 2002. A discussion of normal and abnormal milk based on somatic cell count and clinical mastitis. Bulletin-International Dairy Federation. (372): 43-45.

Sohel MMH, Amin MR. 2015. Identification of types of buffaloes available in Kanihari buffalo pocket of Mymensingh district. Research in Agriculture Livestock and Fisheries. 2 (1): 109-115.

Sori H, Zerihun A, Abdicho S. 2005. Dairy cattle mastitis in and around Sebeta, Ethiopia. Journal of Applied Research in Veterinary Medicine. 3 (4): 332.

Srinivasan P, Jagadeswaran D, Manoharan R, Giri T, Balasubramaniam GA, Balachandran P. 2013. Prevalence and etiology of subclinical mastitis among buffaloes (Bubalus bubalus) in Namakkal, India. Pakistan Journal of Biological Sciences. 16 (23): 1776-1780.

Ståhl Högberg M, O. Lind. 2003. Buffalo Milk Production. Milk Production. Accessed on 22/11/2006.

Steeneveld W, Hogeveen H, Barkema HW, van den Broek J, Huirne RBJJods. 2008a. The influence of cow factors on the incidence of clinical mastitis in dairy cows. Journal of Dairy Science. 91 (4): 1391-1402.

Steeneveld W, Hogeveen H, Barkema HW, van den Broek J, Huirne RBM. 2008b. The Influence of Cow Factors on the Incidence of Clinical Mastitis in Dairy Cows. Journal of Dairy Science. 91 (4): 1391-1402.

Stevens M, Piepers S, De Vliegher S. 2016. Mastitis prevention and control practices and mastitis treatment strategies associated with the consumption of (critically important) antimicrobials on dairy herds in Flanders, Belgium. Journal of Dairy Science. 99 (4): 2896-2903.

Svensson C, Nyman A-K, Waller KP, Emanuelson U. 2006. Effects of housing, management, and health of dairy heifers on first-lactation udder health in southwest Sweden. Journal of Dairy Science. 89 (6): 1990-1999.

Talukder AA, Rahman HH, Mahmud SJ, Alam F, Dey SK. 2013. Isolation, identification and resistance pattern of microorganisms associated with mastitis in Buffalo. Bangladesh Journal of Microbiology. 30 (1-2): 1-5.

Tamburrano A, Tavazzi B, Callà CAM, Amorini AM, Lazzarino G, Vincenti S, Zottola T, Campagna MC, Moscato U, Laurenti P. 2019. Biochemical and nutritional characteristics of buffalo meat and potential implications on human health for a personalized nutrition. Italian Journal of Food Safety. 8 (3): 8317.

Terramoccia S, Bartocci S, Amici A, Martillotti F. 2000. Protein and protein-free dry matter rumen degradability in buffalo, cattle and sheep fed diets with different forage to concentrate ratios. Livestock Production Science. 65 (1-2): 185-195.

Thiers FdO, Benites N, Ribeiro A, COSTA Ed. 1999. Correlação entre contagem direta de células somáticas e o teste de “California Mastitis Test” (CMT) no leite de vacas. Napgama. 2 (4): 9-12.

Thomas C. 2008. Efficient dairy buffalo production. DeLaval International AB, Tumba, Sweden. p. 6-62.

Thomas CS, 2004. Milking management of dairy buffaloes, in Department of Animal Nutrition and Management. Uppsala: Swedish University of Agricultural Sciences.

Thomas CS, Svennersten-Sjaunja K, Bhosrekar MR, Bruckmaier RM. 2004. Mammary cisternal size, cisternal milk and milk ejection in Murrah buffaloes. Journal of Dairy Research. 71 (2): 162-168.

Thrustfield M. 2007. Veterinary Epidemiology. Blackwell publishing. Oxford. p.182-193.

Tripaldi C. 2005. Buffalo milk quality. A Borghese. FAO. pp. 173-183.

Tripaldi C, Palocci G, Miarelli M, Catta M, Orlandini S, Amatiste S, Bernardini RD, Catillo G. 2010. Effects of Mastitis on Buffalo Milk Quality. Asian-Australasian Journal of Animal Sciences. 23 (10): 1319-1324.

Tripaldi C, Terramoccia S, Bartocci S, Angelucci M, Danese V. 2003. The effects of the somatic cell count on yield, composition and coagulating properties of Mediterranean buffalo milk. Asian-Australasian journal of animal sciences. 16 (5): 738-742.

Tucker CB, Rogers AR, Schütz KE. 2008. Effect of solar radiation on dairy cattle behaviour, use of shade and body temperature in a pasture-based system. Applied Animal Behaviour Science. 109 (2): 141-154.

Uddin M, Mintoo A, Awal T, Kondo M, Kabir A. 2016. Characterization of buffalo milk production system in Bangladesh. Bangladesh Journal of Animal Science. 45 (1): 69-77.

Udvardy MD. 1975. A classification of the biogeographical provinces of the world.

Unnerstad HE, Lindberg A, Waller KP, Ekman T, Artursson K, Nilsson-Öst M, Bengtsson B. 2009. Microbial aetiology of acute clinical mastitis and agent-specific risk factors. Veterinary Microbiology. 137 (1-2): 90-97.

Valde J, Lawson L, Lindberg A, Agger J, Saloniemi H, Østerås O. 2004. Cumulative risk of bovine mastitis treatments in Denmark, Finland, Norway and Sweden. Acta Veterinaria Scandinavica. 45 (4): 201-210.

Vasavada PC. Pathogenic Bacteria in Milk — A Review. 1988. Journal of Dairy Science.71:2809-2816.

Villanueva MA, Mingala CN, Tubalinal GAS, Gaban PBV, Nakajima C, Suzuki Y. 2018. Emerging infectious diseases in water buffalo: An economic and public health concern. p. 62.

Vink D, 1995. Subclinical mastitis in the Nile Delta—A cross-sectional study, in Faculty of Veterinary Medicinethe Netherlands. University of Utrecht.

Waage S, Ødegaard S, Lund A, Brattgjerd S, Røthe T. 2001. Case-control study of risk factors for clinical mastitis in postpartum dairy heifers. Journal of Dairy Science. 84 (2): 392-399.

Wanapat M, Chanthakhoun V. Recent advances in rumen ecology, digestion and feeding strategies of swamp buffaloes. Simpósio de Búfalos das Américas. Europe and Americas Buffalo Symposium. Brazil. p. 27-36.

Wanapat M, Chanthakhoun V. 2015. Buffalo production for emerging market as a potential animal protein source for global population. Buffalo Bulletin. 34 (2): 169-180.

Wanasinghe D. Year. Mastitis among buffaloes in Sri Lanka. First World Buffalo Congress, Cairo, Egypt. p. 1331-1333.

Xia S, 2006. The rheology of gel formed during the California Mastitis Test, The University of Waikato.

Yancey Jr RJ. 1999. Vaccines and diagnostic methods for bovine mastitis: fact and fiction. Advances in Veterinary Medicine. 41: 257-273.

Yindee M, Vlamings BH, Wajjwalku W, Techakumphu M, Lohachit C, Sirivaidyapong S, Thitaram C, Amarasinghe AAAWK, Alexander PABDA, Colenbrander B, Lenstra JA. 2010. Y-chromosomal variation confirms independent domestications of swamp and river buffalo. Animal Genetics. 41 (4): 433-435.

Yong Z, Fang J, Mei Y, Narisu S, Binzhong L. 2009. Isolation and identification of pathogens from mastitis cow and drug sensitivity test. China Animal Husbandry & Veterinary Medicine. 36: 136-140.

Zecconi A, Vairani D, Cipolla M, Rizzi N, Zanini L. 2019. Assessment of subclinical mastitis diagnostic accuracy by differential cell count in individual cow milk. Italian Journal of Animal Science. 18 (1): 460-465.

Zenebe N, Habtamu T, Endale B. 2014. Study on bovine mastitis and associated risk factors in Adigrat, Northern Ethiopia. African Journal of Microbiology Research. 8 (4): 327-331.

Zhang Y, Colli L, Barker JSF. 2020. Asian water buffalo: domestication, history and genetics. Animal Genetics. 51 (2): 177-191.

Zicarelli L. 2004. Buffalo Milk: Its Properties, Dairy Yield and Mozzarella Production. Veterinary Research Communications. 28 (1): 127-135.

Zotos A, Bampidis VA. 2014. Milk fat quality of Greek buffalo (Bubalus bubalis). Journal of Food Composition and Analysis. 33 (2): 181-186.

Appendix

Appendix I

Survey questionnaire for assessing risk factors of subclinical mastitis in buffaloes in intensive and free ranging system in Noakhali district of Bangladesh.

SVA | UHB | SLU | Utrecht University

Survey questionnaire for assessing risk factors of SCM in buffaloes in intensive and free ranging system Bangladesh

Objective:

Identification of risk factors associated with subclinical mastitis in Buffalo

Questionnaire sheet ID [ ]

Declaration: I have answered all the questions in the interview sheet and I have full consent about the information given. Best of my Knowledge, the information given by me is correct and can be used in research. If necessary the researcher can contact me for further information or vice versa in future.

==============

Interviewee signature

Name of the Interviewer: ....

Farm/Bathan ID:............ BMSCC....................................../mL **Date:** ...../...../ 20.....

1. Participant information

1.1 Name of the Interviewee:			1.2 Mobile no:	
1.3 Gender: ☐ Male	☐ Female		1.3.1 Age:	
1.4 Title of Interviewee:	☐ Owner	☐ Manager	☐ Worker	☐ Other ....
1.5 Education:	☐ Illiterate	☐ Primary	☐ Secondary	☐ Graduation
1.6 Farm owner's communication with staffs	☐ Good contact with the staffs ☐ Irregularly come to see the farm ☐ Scarcely come to the farm ☐ Others *(Please specify)*:...			

2. Demographic features of farm/Bathan

2.1 Farm / Bathan name:	
2.2 Farm zone: ☐ Coastal ☐ Semi-coastal ☐ River basin ☐ Inland ☐ Others *(Please specify)*: .... Coastal: Permanently stay in the coastal area Semi-costal: Partly the animals stay in coastal area and partly in home River basin: The animals remains in riverine area Inland: The animals remain in fresh water area not connected to river	
2.3 Farm Location	
2.3.1 Village/Ward:	
2.3.2 Upazila:	2.3.3 District:
2.4 GPS co-ordinates	
2.4.1. Latitude (Degree-minute):	2.4.2. Longitude (Degree-minute):
2.4.3. Elevation from sea level (meters):	
2.5 Temperature Humidity Index	**2.5.1 Time of observation:**
2.5.2 Temperature: ....°C	2.5.3 Relative Humidity: ....%
2.5.4 THI score for Heat stress	☐ Comfort ☐ Mild stress ☐ Severe stress ☐ Very severe stress

Page 1 of 8

2.6 Total number of Buffalo:			
2.7 Composition of population: Total number of animals in a particular category			
☐ Milk Buffalo:	☐ Dry Cow:		☐ Bull:
☐ Heifer:	☐ Calf:		☐ Pregnant:
Other animals:	Sheep/goat:	Cattle:	Poultry( )...............
2.8 Type of Buffalo:	☐ Swamp type		☐ River type ☐ Both
2.9. What are the available breeds in your farm	☐ Indigenous ☐ Murrah ☐ Nili-Ravi ☐ Albino ☐ Surti ☐ Jaffrabadi ☐ Mediterranean ☐ others......		
2.9.1. If cross breed what are the breeds	....		

3. Rearing System

3.1 Rearing type	☐ Free ranging/bathan ☐ Household ☐ Commercial/intensive ☐ Semi-intensive ☐ Semi-bathan Free ranging/bathan: They always stay out of home and permanently stay in grazing land Household/backyard: If the buffaloes are raised in home and also carried to grazing land Commercial/intensive: If the buffaloes are raised intensively and never allowed to grazing land Semi-intensive: If the buffaloes are raised intensively and have some free space to move, also have access to wallowing area outside. Semi-bathan: They are reared both in house and in free-ranging area.
3.2 Types of ranging area used	☐ Muddy field ☐ Wallowing in water ☐ Both ☐ Never
3.3 If wallowing kind of wallowing water	☐ Pond water ☐ River water ☐ Sea water ☐ Others ....
3.3.1 Please specify duration of wallowing:	☐ <6hr ☐ 6-12hr ☐ 12-18hr ☐ >18hr
3.3.2 Does the frequency of wallowing change during the year depending on the season?	☐ Yes ☐ No
3.3.3 If yes, please specify duration of season	☐ Summer......... ☐ Winter.......... ☐ Rainy........... ☐ Others ....
3.4 Is wallowing mandatory for buffalo	☐ Yes ☐ No
3.5 If there is no wallowing facility, what they usually do?	☐ Spray water ☐ Others...............................
3.5.1 If yes, Source of that water facility?	☐ Tube-well ☐ Deep tube well ☐ Pond water ☐ River water ☐ Others ....
3.6.1 pH of drinking/ wallowing water (0-14)	
3.6.3 TDS (Total dissolved solids) of wallowing water	
3.6.4 Hygienic score of drinking/ wallowing water	☐ Excellent = Water is clear and water interface is excellent ☐ Good = Water is cloudy and interface is moderately dirty ☐ Poor = Water is very cloudy and interface is very dirty

4. Bathan/Semi-bathan rearing system

5.5 Availability of Water	☐ Ad-libitum ☐ Scarcity of water	
5.5.1 If water is scarce, What you do to supply drinking/ wallowing water?	....	
4.1. Do you move your herd from pasture land during winter season for long period?	☐ Yes Period:.............	☐ No
4.2 Do you have any temporary shelter for herd at bathan?	☐ Yes	☐ No
4.3 Score of floor in milking place?	☐ Excellent =Dry and clean floor (not more than 10% area covered with dung) ☐ Good = Intermediate level of cleanliness (between 10%- 50% area covered with dung) ☐ Fair = Dirty floor (more than 50% area covered with dung)	
4.3 Sources of drinking water	☐ Deep Tubewell ☐ Tube-well ☐ Pond water ☐ Sea water ☐ River water ☐ Rain water ☐ Others......................	
4.4 Parameters on drinking/ wallowing water	pH of water (0-14):.............. Temperature of water:.......... TDS of water:............	

5. Commercial/Semi-intensive/ Household farm

5.1. Does the farm have any protected boundary?	☐ Yes	☐ No
5.2. Does the farm have any bedding material?	☐ Yes ☐ No	
5.2.1. Kind of bedding material?	☐ Straw ☐ Rubber mattress ☐ Others... ☐ No bedding materials	
5.2.2. Scoring of particular bedding	☐ Excellent (Clean and dry animal lies in completely dry place) ☐ Good (Wet and 20% covered with mud) ☐ Fair (Dirty and more than 20% covered with mud)	
5.3. Sources of drinking water	☐ Deep well ☐ Tube-well ☐ Pond water ☐ Sea water ☐ River water ☐ Others...............	
5.3.1. Parameters on drinking water	pH of water (0-14):................ Temperature of water:............. TDS of water:.........	
5.3.2 How they have facility to drink water?	☐ Individual	☐ In group
5.3.3 If in group, how many buffaloes in a group drinking water? (if more than 50 buffaloes, they will be divided into groups) (at least 25) at least 2 groups will be considered	....	
5.4.1. Do you have a water trough?	☐ Yes ☐ No	

5.4.2 If yes, how often do you clean water trough?	☐ Weekly ☐ Monthly ☐ Never ☐ Others(Please specify) ………………………………………………………………………
5.4.3 Hygienic score of water trough?	☐ Excellent ☐ Good ☐ Poor Excellent (Water clear and no evidence of crusts of dirt or decay, Clean water trough) Good (Water clear but contains wastes, Partly discolored trough) Poor (Water colored, such as brown, green, red, Moldy water trough)
5.5 Availability of Water	☐ Ad-libitum ☐ Scarcity of water
5.5.1 If water is scarce, when/ how much is available per day?	………………………………………………
5.6 Are different types of animals/birds kept in same house?	☐ Yes ☐ No
5.6.1 If yes, which animals?	………………………………………………
5.7. Where do they dispose the manure?	☐ Adjacent to the farm ☐ Away from farm
5.8. Is there a drainage facility?	☐ Yes ☐ No
5.8.1 If yes, score of the drainage facility?	☐ Excellent (Cleaned daily) ☐ Good (Cleaned weekly) ☐ Poor (Cleaned monthly / more)
5.9 How is the floor made of?	☐ Soil/ Muddy floor ☐ Sand ☐ Concrete ☐ Bricks ☐ Others (Specify)…………………
5.9.1. If concrete floor, daily frequency of cleaning floor?	☐ Once ☐ Twice ☐ Thrice ☐ Others:……………
5.9.2 Score of the floor in terms of cleanliness?	☐ Excellent =Dry and clean floor (not more than 10% area covered with dung) ☐ Good = Intermediate level of cleanliness (between 10%- 50% area covered with dung) ☐ Fair = Dirty floor (more than 50% area covered with dung) **Time** **Observation time:**………………… **Time elapsed after last cleaning (minutes):** …………………
5.9.3. Score of the floor in terms of condition of walking surface?	☐ Excellent = Non-slippery and non-cracked on the floor surface ☐ Good= not more than 20% of floor is slippery and non-cracked all surfaces on which animal walk ☐ Poor = Smooth and slippery, More than 8% Cracked,
5.9.4. Do they disinfect the floor?	☐ Yes ☐ No
5.9.4.1. If yes, how often they disinfect the floor?	☐ Daily ☐ Weekly ☐ Monthly ☐ Others *(Please specify)*………………………………………………
5.9.4.2. If disinfection is applied, what kinds of disinfectants are used? (specify name pls.)	………………………………………………………………
5.10. Does the farm have free space for exercising or walking	☐ Yes ☐ No

6. Feeding management

6.1 Feeding system?	☐ Stall feeding ☐ Grazing ☐ Both
6.2 What type of feed is provided to your Buffaloes?	☐ Roughage ☐ Concentrate ☐ Both ☐ Other……………………………………
6.2.1 When you supply concentrates/ feed	☐ Round the year ☐ ……………………………
6.2.1.1 Amount of each kind of feed for lactating animals?	☐ Roughages: ……… ☐ Concentrates…… ☐ Others………
6.2.2. Frequency of feeding per day? (Grazers)	☐ Graze all day ☐……… Graze half day ☐………Others………
6.2.3. Frequency of feeding per day? (Non-grazers)	☐ Once ☐ Twice ☐ Thrice ☐ Others………
6.3 Do they provide any other feed supplement? (unconventional feed) *locally available feeds	………………………………………………………
6.4. How do you feed your buffalo?	☐ Single buffalo ☐ Split up between groups ☐ All buffaloes together ☐ Others………………………………………
6.5. Do you provide formulated ration?	☐ Yes ☐ No
a. To whom you provide that ration?	☐ Calves ☐ Heifer ☐ Cow ☐ Others
b. Who formulated that ration?	☐ Self ☐ Vet ☐ Feed company ☐ Specialist
c. Do you provide any vitamin mineral supplement in feed (pls. specify)?	☐ Yes ☐ No If yes…………………………………………

7. Sources of Animal

7.1 What are the sources of animals? ☐ Own stock ☐ Purchased ☐ Both

7.2 How many buffaloes purchased over last 12 months: ☐

7.3 If purchase milk buffalo, what are the purchase considerations?

☐ Health condition ☐ Udder conformation ☐ Breed ☐ Milk production history
☐ Parturition history ☐ Body conformation ☐ Low price ☐ Hooves shape:…………
☐ Wide hind quarter ☐ Others (*Please specify*):
…………………………………………………………………………………………

8. Record keeping

8.1 Do you keep any farm records?	☐ Yes ☐ No
8.1.1 If yes, what types of records you record?	☐ Disease ☐ Breeding ☐ Milk production ☐ Mortality ☐ Treatment ☐ Prevention ☐ Others…………………
8.2 Mention type of keeping tool.	☐ Log book ☐ Computer ☐ Others……………………

8.3 What clinical signs/ diseases did you notice in the herd in last 12 months	....

9. Mastitis related records	
9.1. How many buffalo had clinical mastitis in last 12 months?	....
9.2. Any buffalo culled due to mastitis in last 12 months?	☐ Yes ☐ No
9.3. Any buffalo died in your farm in last 12 months?	☐ Yes ☐ No
9.4 How many calves died in last 12 months?	....
9.5. How many adult buffalo died in last 12 months?	....

10. Udder Hygiene

10.1 Do you wash your buffaloes before milking?	☐ Yes ☐ No
10.1.1 If yes, which types of water do you use for bathing?	☐ River ☐ Pond ☐ Tube-well
10.2. Do you need to control the buffalo during milking	☐ Yes .... ☐ No
10.3. Does the milker wash udder before milking?	☐ Yes ☐ No
10.4. Do you let the udder be dried before milking?	☐ Yes ☐ No
10.4.1. If yes, how do you dry the udder?	☐ Towel ☐ Tissue ☐ Others
10.5 Do you practice teat dipping?	☐ Yes, Everyday ☐ Often ☐ Not usually ☐ Never
10.5.1. If yes, what are the dipping materials you use?	
10.6. What do you use to stimulate the udder before milking?	☐ Calf suckling ☐ Hand ☐ Warm water ☐ Warm cloth ☐ Others..................
10.7 Do you practice fore-striping?	☐ Yes ☐ No
10.8 What types of milking procedure do you practice in your farm?	☐ Hand milking ☐ Machine milking
10.8.1. If hand milking, which method do you follow? ***Please see in illustration**	☐ Full hand ☐ Stripping ☐ Knuckling ☐ Others....................... Full hand milking: Grasping the teat with all the five fingers and pressing it against the palm. Stripping: Firmly holding the teat between the thumb and fore finger and drawing it down the length of the teat and at the same time pressing it to cause the milk to flow down in a stream. Knuckling: Bend their thumb against the teat. The method is known as knuckling.
10.9. Frequency of milking of Buffalo/day	☐ Once ☐ Twice ☐ Others
10.10 Score of udder hygiene:	☐ Excellent (Washing udder, Pre & post dipping, use of towel) ☐ Good (Washing udder, Pre/ post dipping) ☐ Fair (Washing udder) ☐ Poor (Don't wash the udder)
10.10. Milking is done by:	☐ Same person ☐ Multiple persons
10.10.1 Score of milkers hygiene?	☐ Excellent = Milkers use antiseptics and wash hands ☐ Good = Milkers only wash hand ☐ Poor = Milkers don't wash hand
10.11. Do you use any antiseptics before and after milking?	☐ Yes ☐ No

10.11.1. If yes, which antiseptics?	....
10.12 Do you usually offer feeds after milking? (To keep the animals in standing position)	☐ Yes ☐ No
10.13. Does the milker milk the clinical mastitis affected animal at last?	☐ Yes ☐ No

11. Udder Health

11.1. Bulk milk somatic cell count:	..../mL of milk
11.2 How many clinical mastitic animals you have right now?	....
11.3 Which signs of clinical mastitis you observed?	☐ Swollen udder ☐ Redness of udder ☐ Painful udder ☐ Abnormal milk ☐ Loss of appetite ☐ Depressed ☐ Dehydration ☐ Fever ☐ Rumination ☐ Others............................
11.4 Other udder related diseases you noticed in your farm in last 12 months.	Udder edema/ Buffalo pox/ Ulcerative mammilitis/Wart/abscess/Hematoma/Udder rot/Blood in milk/Rupture of suspensory ligament of udder/Prepubic tendon rupture
11.5. What is the udder related symptoms encountered in the last 12 months in your farm?	....

12. Therapeutic & preventive management

12.1 Does the farm animal get veterinary service?	☐ Yes ☐ No
12.1.1 If yes, How often do you get veterinary service?	☐ On call ☐ weekly ☐ Monthly ☐ rarely ☐ Others (specify):..
12.1.2 If yes, who gives you the service?	☐ Private Vet. ☐ Govt. Vet. ☐ VFA ☐ Farmers himself ☐ Others....................
12.2 Kind of drugs you used to treat clinical mastitis cases?	☐ Antibiotics .... ☐ Other drugs: ....
12.3. Do you know about drug withdrawal period of antibiotics?	☐ Yes ☐ No
12.3.1. What do you do with the milk after following antibiotics treatment?	....
12.4.1. What do you do with the milk from clinical mastitis affected animals?	☐ Drink ☐ Discard ☐ Sell ☐ Others:..........
12.5. Do you provide vaccines to the animals?	☐ Yes ☐ No
12.5.1. If yes, which vaccines do you use in your farm?	☐ FMD ☐ HS ☐ BQ ☐ Anthrax ☐ Others.................................

12.6. Do you use any anthelmintics in your farm?	☐ Yes ☐ No
12.6.1. If yes, how often do you deworm buffaloes?	☐ 4 month ☐ Yearly ☐ Others.....................
12.6.2. Do you use same strategy for calf	☐ Yes ☐ No
12.6.3. How often do you deworm calves	....
12.6.2. Which anthelmintic do you use?	....
12.7. Do you use any herbal or traditional treatment for clinical mastitis?	☐ Yes ☐ No
12.7.1 If yes, name of those?	....
12.8. Are there any quarantine facilities for new animals? * If they keep the newly purchased animals separately for a period of at least 3-4 weeks to observe manifestation of any disease.	☐ Yes ☐ No
12.9. Do you have isolation shed for sick animals? * If Sick animals are separated from healthy animals and should be treated.	☐ Yes ☐ No

Information from individual mastitis affected cow: (Animal level risk factors)

Questionnaire ID:	Farm/Bathan ID:	Animal Id:	Date: ...../...../ 20.....

1. Month of the observation: ....
2. Season of the observation: ☐ Summer ☐ Autumn ☐ Winter ☐ Spring
3. Affected quarter and CMT score in mastitis affected cow?
   ☐ FL: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5
   ☐ FR: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5
   ☐ HL: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5
   ☐ HR: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5
   * FL (front left) FR(front right) HL (Hind left) HR (Hind right)
4. **Type of mastitis in the animal?** ☐ Clinical/Chronic ☐ Sub-clinical ☐ Normal
5. Position of teats? ☐ Symmetrical ☐ Directed backward ☐ Directed inward ☐ others...
6. Teat shape:
   FL ☐ Funnel shaped ☐ Bottle shaped ☐ Cylindrical shaped
   FR ☐ Funnel shaped ☐ Bottle shaped ☐ Cylindrical shaped
   HL ☐ Funnel shaped ☐ Bottle shaped ☐ Cylindrical shaped
   HR ☐ Funnel shaped ☐ Bottle shaped ☐ Cylindrical shaped
7. Visible lesions in teat (teat end callosity):
   FL ☐ Yes ☐ No
   FR ☐ Yes ☐ No
   HL ☐ Yes ☐ No
   HR ☐ Yes ☐ No
8. Blocked quarters due to previous clinical mastitis:
   ☐ FL(front left) ☐ FR (front right) ☐ HL (hind left) ☐ HR (hind right)
9. If any abnormalities in teat, what are those (**Teat cheilitis/ chaps/ warts/ bovine herpes mammalities**)? ....
   ☐ FL(front left) ☐ FR (front right) ☐ HL (hind left) ☐ HR (hind right)
10. Any congenital anomalies in teats? ☐ Yes ☐ No
    10.1. If yes, mention the anomalies ....
    ☐ FL(front left) ☐ FR (front right) ☐ HL (hind left) ☐ HR (hind right)
11. Breed: ☐ Indigenous ☐ Crossbred ☐ others.............................
    If crossbred, please specify: ....
12. Source of the buffalo? ☐ Own herd ☐ Purchased ....M ☐ Take care ....M
13. Age (Number of rings in the horn): ....years
14. Parity: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ Others: ....
15. Lactation stage in (month): .... *(Number of days in milk after calving: Equivalent to age of the recent calf)
16. Pregnancy? ☐ Pregnant ☐ Non-pregnant
17. Average milk yield (Litres):........; Highest milk yield:......; Todays milk yield...........
18. BCS: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 (See the illustration)
    1. **Definitely emaciated**: Ribs and bone structures visible; physically weak; difficulty in walking; no presence of fat in sight or pressure.
    2. **Emaciated**: Similar but not physically weak.
    3. **Very skinny**: No visible fat in ribs/ brisket; muscles in spinous process and hind quarter is evident.
    4. **Scrawny**: Ribs and bony eminences easily visible with absence of fat evident on palpation. The individual muscles on the hindquarter are still visible.
    5. **Sufficient nutrition**: The first ribs are covered, while the last 2 or 3 ribs are easily evident. The triangle formed by the iliac, ischial tuberosity and coxomedoral joint is evident and the muscular masses are concave.
    6. **Discrete state of nutrition**: Fat deposit in ribs but absent in brisket; tuberosity triangle is still evident; mascular margin is straight; spinous process is evedent; absence of fat in tail.

Page 1 of 2

7. **Good level of nutrition**: Slight deposition of fat in brisket region; 1cm fat deposit over ribs; mascular mass is convex; invisible spinous and transverse process; base of tail is full.
8. **Fat**: Brisket is full; Bony projections show fat deposits; back becomes square in shape with fat; 1-2 cm fat over last 3-4 ribs; excessive accumulation of fat in tail and no visible dimple.
9. **Very fat**: Very square rear. Particularly pronounced breast tip extended by fat. Large fat deposits on the bony projections and at the base of the tail. Big neck. At least 3-4 cm fat on the last 3 ribs. Line of demarcation very evident on the spine.

19. Udder symmetry? ☐ Symmetric ☐ Asymmetric
20. Udder shape: ☐ Cup/Pendulous ☐ Round/Globular ☐ Bowl
21. Cleanliness of the hind quarter? ☐ Excellent ☐ Good ☐ Poor
    * Overall cleanlines of udder along with hind legs
22. Did you notice any udder related diseases in this animal before?
    ☐ Yes ☐ No
    If, yes
    ☐ Udder edema ☐ Buffalo pox ☐ Wart ☐ Ulcerative mammilitis ☐ Hematoma
    ☐ Udder rot ☐ Blood in milk ☐ Rupture of suspensory ligament of udder
    ☐ Prepubic tendon rupture ☐ Abscess
23. Did the animal have history of any other previous diseases? ☐ Yes ☐ No
    If yes, ...
24. Previous history of lameness? ☐ Yes ☐ No
25. Previous history of clinical mastitis in last 12 months? ☐ Yes ☐ No
26. History of reproductive disease in last 12 months? ☐ Yes ☐ No
27. History of abortion in last 12 months? ☐ Yes ☐ No
28. History of calf mortality in last 12 months? ☐ Yes ☐ No
29. Did you use any antibiotics (Trade name) in previous disease treatment: ....
30. Did you vaccinate this buffalo? ☐ Yes; last vaccination (months ago):......... ☐ No
31. If yes, which vaccine you used? ☐ FMD ☐ HS ☐ BQ ☐ Anthrax
    ☐ Others...
32. Did you deworm the buffalo? ☐ Yes; last deworming (months ago):................ ☐ No
33. Which milking system do you follow? ☐ Machine ☐ Hand
34. If Hand milking, then milking done by? ☐ Same milker ☐ Multiple milker
35. Experience of the milkers? ☐ Old; .... years ☐ New;......... months ☐ Both

Clinical mastitis

36. If clinical mastitis, duration of the illness: .... days
37. Do you practice forestripping before milking? ☐ Yes ☐ No
38. Which clinical signs did you observe?
    ☐ Swollen udder ☐ Redness of udder ☐ Painful udder ☐ Abnormal milk ☐ Loss of appetite ☐ Depressed ☐ Dehydration ☐ Fever ☐ Rumination ☐ others...
39. If treated which antibiotic you administered? (trade name)................................
40. Average daily milk yield: a) 1 week before mastitis... Litre a) 1 week after mastitis:.........Litre

Sub-Clinical mastitis

41. Have you seen reduction of milk yield during lactation? ☐ Yes ☐ No
42. Do you have any idea about sub-clinical mastitis? ☐ Yes ☐ No
43. If yes, do you practice CMT for detection of SCM? ☐ Yes ☐ No
44. If the CMT is positive what do you do?...

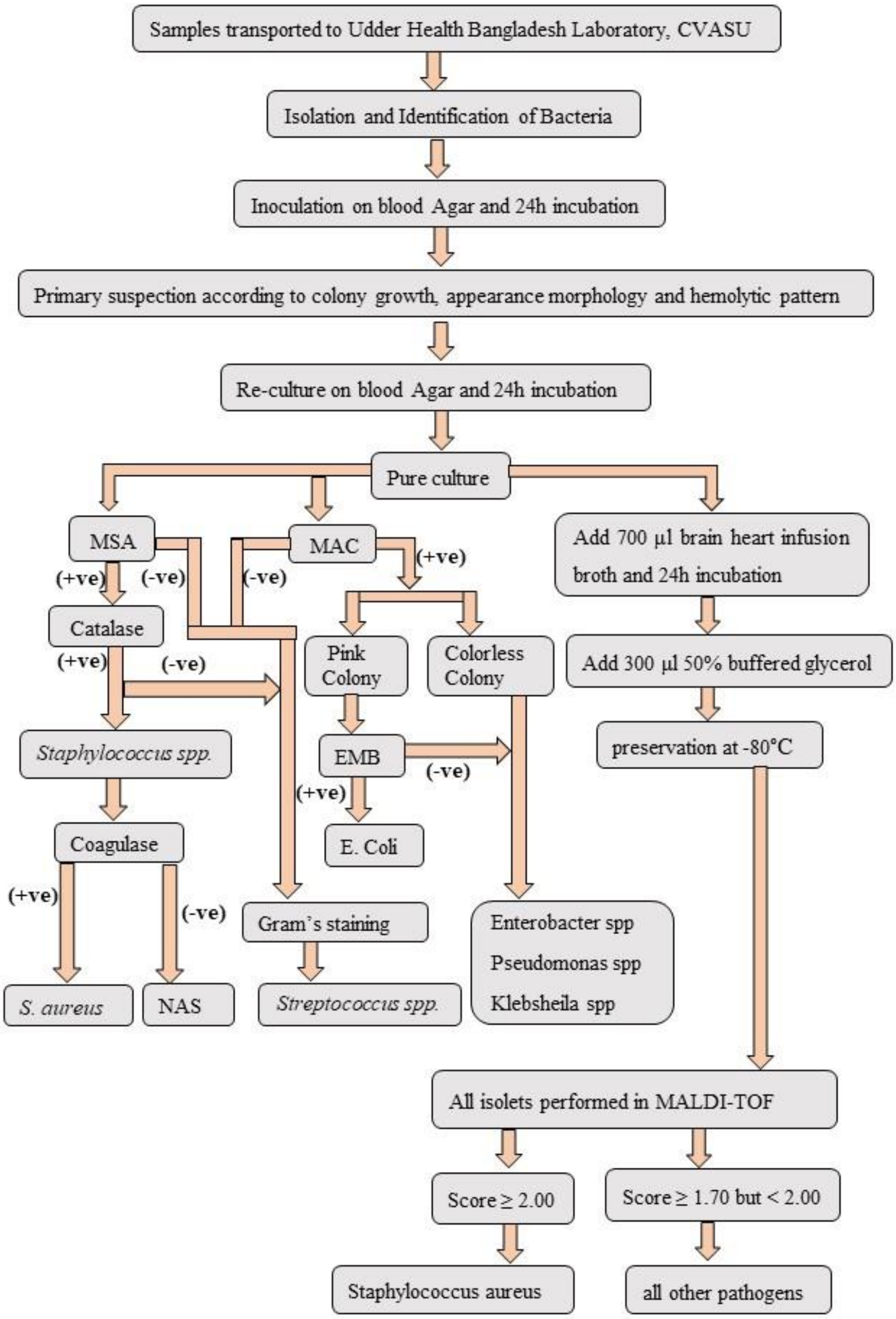
Is there anything more you would like to add?

....

THANK YOU FOR PARTICIPATING IN THE RESEARCH ☺

Apendix II

A brief flow chart on methodology of pathogen identification from milk sample.



Biography

Ovirup Bhushan Paul, working as a Scientific Officer, Buffalo Research and Development Project, Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, Bangladesh since May 2021. He has submitted his thesis for fulfillment of the MS in Epidemiology degree under the Dept. of Medicine and Surgery at Chattogram Veterinary and Animal Sciences University (CVASU). He has obtained a CGPA of 3.88 out of 4.00 so far in this MS program. He completed his Doctor of Veterinary Medicine (DVM) degree in 2018 from CVASU. He has secured an excellent academic record (CGPA of 3.35 out of 4.00) with a one-year duration internship and externship program to complete his DVM. He has an immense interest in working for animal health and welfare. He has been working as a Research Assistant (RA) in a Bangladesh-Sweden-Netherlands-Italy collaborative project "Climate change mitigation by a sustainable water buffalo dairy chain in Bangladesh" funded by the Swedish Research Council, Sweden from March 2019 to February 2021. He is now continuing research activities as a project investigator in the "Buffalo Research and Development Project" focused on "Epidemiological investigation of buffalo diseases in Bangladesh" financed by the Ministry of Fisheries and Livestock, Government of the People's Republic of Bangladesh.