MICROBIOLOGICAL AND PHYSICOCHEMICAL ANALYSES OF SUPPLIED WATER IN SELECTED DAIRY FARMS OF DHAKA CITY

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CERTIFICATE

This is to certify that the thesis entitled **"MICROBIOLOGICAL AND PHYSICOCHEMICAL ANALYSES OF SUPPLIED WATER IN SELECTED DAIRY FARMS OF DHAKA CITY"** *submitted to the Department of Medicine and Public Health, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of* **MASTER OF SCIENCE in MEDICINE**, *embodies the result of a piece of bona fide research work carried out by* **MD. ROKNUZZAMAN KHAN,** *Registration No.* **13-05512** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

Dated: December, 2020   _____
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DEDICATED TO-

*My Beloved Parents and
Respected Research Supervisor*

ACKNOWLEDGEMENTS

December, 2020   *The Author*

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LIST OF ABBREVIATIONS AND ACRONYMS

CFU	Colony forming unit	PBS	Phosphate buffered saline
DLS	Department of livestock	EDTA	Ethylene diamine tetra acetic acid
EC	Electric conductivity	Min.	Minimum
BBS	Bangladesh Bureau of Statistics	Max.	Maximun
GDP	Gross domestic product	ºC	Degree celcius
TDS	Total dissolving solid	µl	Micro litre
WHO	World health organization	ppm	Parts per million
MAR	Multiple antibiotic resistant	nm	Nano metre
TC	Total coliform	UV	Ultra violet
FC	Faecal coliform	EDT	Eriochrome black-T
FS	Faecal streptococci	no.	Number
MFT	Membrane filtration technique	e.g.	that is
NRC	National research council	rpm	Rotation per minute
cm	Centimeter	ltd.	Limited
m	Meter	%	percent
h	hour	mg	miligram
M. S.	Master of Science	dm^3	Decimeter per qube
et al.	and others (at elli)	µS	Microsecond
MC	MacConkey	L	Litre
EBM	Eosin methyl blue	etc.	Et cetera
g	Gram	TCC	Total coliform count
ml/L	Milliliter per liter	USA	United states of America
SE	Standard error	USEPA	United states environmental protection agency
m^2	Meter squares	MEPH	Medicine and public health
LSD	Least Significant Difference		
CV%	Percent of coefficient of Variation		

MICROBIOLOGICAL AND PHYSICOCHEMICAL ANALYSES OF SUPPLIED WATER IN SELECTED DAIRY FARMS OF DHAKA CITY

ABSTRACT

This study aimed to assess the quality of supplied drinking water for dairy farm in selected farms of Dhaka city by considering the microbiological and physicochemical parameters. The study areas comprised of 5 different locations in Dhaka city namely 60 feet area, Mirpur-1, Mirpur-2, Kalshi and Sher-e-Bangla Agricultural University (SAU) campus area. Samples were collected randomly from each site in four replicates. Microbiological analyses were conducted in laboratory of Medicine and Public Health (MEPH), SAU and physicochemical analyses were conducted in ACI Animal Health Diagnostic Laboratory. Load of coliform bacteria, *E. coli, Balantidia and Giardia* were taken into account under microbiological parameter. pH level, concentration of TDS, hardness, chlorides and iron were analyzed for physicochemical parameters. The study showed that the prevalence of coliform bacteria and *E. coli* were 100% and the bacterial load were higher than the maximum safety limit for cattle in all study area. Among those sites, concentration of coliform (2.63×10^8) and E. coli (7.05×10^7) were highest in the water of Mirpur-1, and lowest in SAU campus area. However, no *Giardia and Balantidia* were present in these samples of these study area. The physicochemical studies showed that water samples of 60 feet area held the highest concentration of TDS (397.00 ± 6.27) and chloride (0.95 ± 0.06), and lowest pH value (6.13 ± 0.05). Water of all study sites contained higher iron concentration than recommended safety margin where Mirpur-1 contained highest concentration of iron (0.97 ± 0.04) while Mirpur-2 contained lowest (0.78 ± 0.02). The water of Mirpur-2 contained highest value of pH (6.70 ± 0.14) and hardness (165.00 ± 2.45). The water samples of kalshi showed lowest concentration of chlorides (0.52 ± 0.02) and hardness (75.00 ± 4.08). The water of SAU cams area held lowest level of TDS (307.50 ± 6.46). Concentration of all physicochemical quality factors (pH, TDS, hardness, chlorides) lied within the expected ranges except iron. Compendium of this study disclosed that the supplied water quality of dairy farm in selected area of Dhaka city is good apart from higher bacterial load (Coliform and *E. coli*) and iron concentration which have inverse impact on health and production of cattle. Consequently, water quality of SAU campus area is comparatively better and Mirpur-1 is comparatively poorer than other study areas. This study recommended that water of Dhaka city should be treated for minimizing the iron concentration and good water management should be exerted to reduce the bacterial contamination for enhancing better health and performance of dairy farm in Dhaka city.

CHAPTER I

INTRODUCTION

Dairy farming is a raising sector in the national economy of Bangladesh. Nearly 40.6% of the population of the country are engaged in agriculture and livestock sector according to Bangladesh Economic Review 2020. In 2019-2020 fiscal year total number of cattle was about 243.91 lac. whereas total milk production was 106.80 lac metric ton and GDP growth rate of Livestock (Constant Prices) was 3.04% (DLS 2020).

Drinking water management is very important part of livestock farming as water is an essential nutrient which is second only to oxygen in importance to sustain life and optimize growth, lactation, and reproduction of cattle. Within normal physiological limits there is also a direct positive relationship between water intake and feed intake (Beede, 2006). As with feed ingredients, livestock water should meet the nutritional needs of the animal (Donald *et al*.1932). Water makes up between 50 to 80% of the animals' weight and helps the physiological processes that connected with digestive system, absorption of nutrients, mitigation of body temperature, regulation of osmotic pressure of blood, transfer of hormones and secretions of saliva, milk, etc. (Lardner *et al*., 2005).

In developing countries, there is a belief that animals and poultry can drink any quality of water but the truth is, the drinking water of poor quality only when they do not have another option. Livestock are sensitive to the taste and smell of water, which may limit their water consumption and may lead to reduced weight and productivity of animals (Deshmukh, 2013; Umar *et al*., 2014). The weight of calves increased by 23% when watered good quality water as compared to those left to drink poor quality water from ponds and swamps (Willms *et al*., 2002).

Physiochemical and biological properties of water can be a useful way of helping to determine water quality. These include pH, total dissolved solids (TDS), hardness, other substances in excess, iron, chloride, parasites and microorganisms (David K. Beede *et al*.,2008).

The livestock can support variations of water pH between 6.5-8.5 (Curran *et al*., 2007) Exceeding this threshold, the water has lye taste, and under 6.5unit pH the taste of water is acidulous-prickly that reduce the water intake (Man, 1989, 2007; Draghici, 2001;

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Popescu, 2010). Unfavorable action of low or high pH were associated with decreasing milk production, decreased average daily gain and increase the susceptibility at infection, reducing fertility (Adams *et al*., 2009). Alkaline water with pH higher than 8.5unit pH lead to heightened risk of metabolic alkalosis occurrence (Swistock, 2012), B-vitamin deficiencies (Grant, 1993), digestive disorder, diarrhea, poor feed conversion and reduced water/feed intake (Bagley *et al*., 1997; Man 1989, 2002).

TDS is a general term defining the sum of all inorganic matter dissolved in water. TDS also indicates the salinity of water that means total amount sodium, chloride, bicarbonate, sulfate, calcium, magnesium, silica, iron, nitrate, strontium, potassium, carbonate, phosphorous, boron and fluoride present in water. High amounts of TDS generally are considered an unwanted characteristic. Threshold concentration of TDS is 2500 mg/L and the limiting concentration is 5000mg/L (David K. Beede *et al*.,2008). Water with high level in salt content reduced the water and feed intake; toxic levels of sulfur ingestion; or can induce trace mineral deficiencies (Patterson *et al*.,2003). Several studies have shown that TDS between 4,000 to 5, 000 ppm negatively affect daily average gain, decrease milk production in lactating cows which cause a reduction in weights at calves at weaning (Dyer, 2012).

Chlorides are another important factor that can degrade water quality. Chlorides above 250 mg/dm^3 can imprint a salty taste to water which could result in reduced water intake and milk production. High amount of chlorides present in water should be considered when formulating diets, to prevent the excess which could be detrimental to rumen function (Swistock, 2016). High consumption of water with a high concentration of chloride as sodium chloride with low potassium diet causes toxic encephalosis: hyperexcitability (tremor, muscle cramps, colic), followed by amaurosis and paraparesis (El Mahdy *et al*., 2016).

Total hardness is given by all calcium and magnesium salts that are found in water and is considered overall indicator of water mineralization (Straus, 1981). Depending on salts concentration from water (carbonate, bicarbonate, sulfates, silica, nitrate, phosphates, by calcium and magnesium, along with potassium, sodium, iron, manganese, etc., the waters can have variable hardness like soft, semi soft & hard water (El Mahdy *et al*., 2016). Consuming the water whose hardness is too high or too low represents a permanently topic for research, controversially in terms of action and the

effect on health condition of animals (Draghici, 2001). The high hardness can cause altering of health condition by the presence of renal calculus, gastric disorders, chronic catarrh of the digestive mucosa and even methaemoglobinaemia especially when animals accustomed to a type of water are forced to consume water with high hardness (El Mahdy, 2013).

Amounts of iron is more than 0.3 ppm in drinking water, this may cause problems for cows (Weiss, 2008; 2010). That induce an unpleasant taste of water, which leads to decrease water consumption concomitantly with the milk production (Swistock, 2012), chronic iron intoxication is manifested by reducing feed intake and feed conversion efficiency (Man, 2002). Excessive intake of iron has an adverse effect due to increasing the reactivity of oxidative species (oxidative stress) that harms the cell membranes and interrupt several biochemical reactions in the body. Usually the adverse effects of iron are indirect through association with secondary deficiencies resulting from antagonistic action (Beede, 2006).

Another anti-quality factor of drinking water is the contamination of drinking water with microorganisms especially with bacteria is a great concern for human and animal health. Open sewage, drain rusted pipelines etc. are the major source Coliform contamination of water (Patoli *et al*., 2010). Coliform bacteria are defined as facultative anaerobic, Gram-negative, non-spore-forming rods that ferment <u>lactose</u> vigorously to acid and gas at 35 ± 2 °C within 24 or 48h. Coliform bacteria generally belong to four genera of the <u>Enterobacteriaceae</u>: *<u>Citrobacterfreundii</u>*, *<u>Enterobacter</u> cloacae*, *Enterobacter aerogenes*, *E. coli*, and *<u>Klebsiella pneumoniae</u>* (Halkman *et al*.,2014). Coliforms are found large amounts in the feaces of warm-blooded animals, aquatic environment, soil and on vegetation. These bacteria have a great ability to live in different organs of the body and become opportunistic pathogen (Dodds B *et al*., 1984). Contaminated drinking water with bacteria over 1,000000/100 ml can cause health problems, although Broadwater (2007) considers that over 500/100 ml total bacteria counts may indicate water quality problems and the water with over 1,000000 total bacteria counts should be avoided as a source of water for cows. Cattle are commonly hosts to *Giardia* spp., *Balantidia* spp., nematodes and others parasites that affect their health. *Giardia* is the most common intestinal parasites in livestock (Thompson *et al*., 2008) Giardia is a ubiquitous enteric protozoan of class-Mastigophora and family-Hexamitidae. Amongst the six currently accepted species of *Giardia*, G. duodenalis

(syn. intestinalis/lamblia) has the broadest host range with the greatest public and animal health significance in terms of gastrointestinal diseases. Giardiasis causes diarrhea in calves and lambs (Olson *et al*. 1995). Among the protozoan diseases balantidiasis caused by *Balantidium coli*, is a common disease of ruminants (cattle, buffaloes, sheep and goats), pig, monkey, chimpanzee, orangutan, guinea pig and man (Rahman, 1985; Samad, 1996a and Levine, 1985). The geo-climatic condition of Bangladesh is favorable for the development and survival of *B. coli* (Datta *et al*., 2004). *B. coli* also produces hyaluronidase (Tempels and Lipenko, 1957) which potentially enhancing its ability to invade the intestinal mucosa, causing enteritis where the clinical features are manifested by loose faces to watery persistent foetid diarrhea, dehydration, loss of appetite, retarded growth, loss of body condition and reduced production performance of the animals which impacts on the economy of the farmers as well as the country (Lazar *et al*., 2004).

Our capital city Dhaka is abundant with a large number of dairy farms which contributes significantly in our livestock sector. pollution of the water sources (underground water, surface water etc.) are increasing day by day through improper sewerage system, faulty drainage management, improper management of heavy industrial effluents, fecal contamination etc. As water is an important factor for physical process and productivity, anti-quality factors of water cause several physical disorders, diseases and hamper the optimum production level. Though many studies are found on assessment of the quality of drinking water in different countries, in our country very rare studies we found on quality of drinking water in dairy farming. Too often dairy producers and their advisers have insufficient understanding of water nutrition/quality of dairy cattle. Having knowledge about provision of this most important essential nutrient is crucial for normal performance of cattle and the financial success of cattle farming. So, this study has emphasized practical evaluation of drinking water quality of cattle farm in selected regions of Dhaka city by marking the water quality factors like pH, TDS, hardness, chloride, iron, Coliform, *E. coli, Giardia spp.* and *Balantidia spp.*

Objectives:

1. To know the microbiological status (Coliform, *E. coli, Giardia spp.* and *Balantidia spp.)* of drinking water of dairy farms in selected areas of Dhaka city

2. To know the physicochemical properties (pH, TDS, hardness, chloride, iron) of drinking water supplied for dairy farm in selected regions of Dhaka city

CHAPTER II

REIVEW OF LITERATURE

Drinking water is a vital nutrient to maintain good health, normal physiology and optimum production of cattle. In this study water quality factors like pH, TDS, hardness, chloride, iron, Coliform, *E. coli, Giardia spp.* and *Balantidia spp.* etc. were taken into account to study the water quality of cattle farm in selected study sites of Dhaka city. The anti- quality factors (constituents) in drinking water that are known from research reports, journals or experience to cause problems are addressed and summarized below.

2.1 Microbiological Parameters

Domestic waste water and sewage loaded with human excreta and direct human defaecations are major sources of faecal pollution in Indian sub-continent rivers and water sources. Microbiological studies on water quality of major Indian rivers have shown the presence of faecal coliform and faecal streptococci as an indication of faecal contamination (Shukla *et al*., 1992; Gaur *et al*., 1997). The high faecal load indicates the high degree of human defaecation by thick urban population on the bank of river finally which comes to the river. The most common and widespread danger associated with the drinking water is directly or indirectly contaminated by sewage, human and animal faecal matter and other wastes (Clark *et al*., 1982).

Breede (2006) showed that according to national academy of science average Total coliform/100 ml in water is 933 whereas, expected level is Less than 1 but possible problem will be occurred over 1 for calves; over 15-50 for cows.

Thelin and Gefford (1983) observed that the faecal coliform bacteria released 30 days old of faecal deposits amounts to several millions per 100 ml. The survival and multiplication of total coliform bacteria species in water depend upon several factors like temperature, light, various chemicals, which are directly proportionate to the amount of sewage and human interferences (Hiraishi, 1984). The content of Escherichia coli (E. coli) in open water bodies varies with seasons and level and sharply increases after heavy rainfall (Voznaya, 1981). The Escherichia coli and coliform

group or organisms as a whole have been recommended for the detection of water quality. The concentration of coliform bacteria is usually an index of civic pollution.

The most essential feature of a river water supply is sanitation or the prevention of infection, because the water cycle represents an obvious mode of transmission of enteric disease in the community (Rashu, 2017).

An understanding of the survival of fecal indicator organisms and the enteric pathogens in water is basic to the meaningful interpretation of sanitary water quality data. This is so because the isolation of faecal Streptococci (Geldreich and Kenner, 1969) or coliform bacteria is commonly used to signify the potential presence of intestinal pathogens. Although detection of indicator bacteria suggests occurrence of pathogenic organisms in water, the potential health hazard is expendant on retention of critical density levels and associated virulence for the pathogens in an open time frame during transmission via the cancer route. Furthermore, once these bacteria are deposited into the water they are in an environment that is not favorable to the maintenance of viability of most bacteria.

Clark, 1980 has described a presence - absence (P - A) test for detection of coliform bacteria in samples of potable water. This test uses a single fermentation bottle, and the sample volume can be either 100 or 50 ml. When a positive result is obtained, there is no information about the number of coliforms in the sample. Clark's P - A test has been used in Ontario for over 15 years, and he has compared the results of the P - A test with those of other coliform detection methods for thousands of samples.

Coliforms excreted by humans and animals eventually pollute drinking water sources. The occurrence of antibiotic resistant coliforms in drinking water is a public health problem because resistant characteristics can be transferred to sensitive recipient organisms in the gut via R - factor plasmid vectors. The antibiotic resistance pattern among coliforms isolated from drinking water sources are well documented (Grabow *et al*., 1975), Bell, 1988 found that the population of R - factor containing faecal coliforms was as high as 1400 organism ml-I from the Red River, Manitoba, Canada. Helmay found presence of multiple antibiotic resistant (MAR) strains even in chlorinated drinking water of Cairo.

(Khan *et al*., 2016) carried out an eperiment to observe the presence of *Coliform* bacterial species from drinking water samples obtained from randomly selected dairy

forms at Quetta. *Coliform* bacterial species were identified by performing the different cultural, staining and biochemical tests. Among 100 water samples obtained from different dairy farms of Quetta, 17 samples were contaminated with *Coliform* bacteria. It was observed that buffalo farms were the most contaminated (22%) than the cattle farms (12%). Of 50 water samples studied from buffalo farms, the prevalence of *Coliform* bacteria was recorded in 11 samples (22 %). While 50 water samples were obtained cattle farms showed 6 (12%) the presence of *Coliform* organisms. The contamination of water samples with Coliform bacteria was found higher in buffalo farms than cattle farms. The bacterial load in ml^{-1} water sample of buffalo farms the mean number of 197 colonies was counted while bacterial counts were recorded as 3.29X104 and the mean number of 167 colonies ml^{-1} was recorded at cattle farms. While bacterial counts was recorded as 2.93×10^4. Overall, drinking water samples collected from different dairy farms in Quetta contaminated with *Coliform* bacteria. The bacterial load/population in water samples of different dairy farms at Quetta was detected higher than standard bacterial concentration level by WHO.

(LeJeune *et al*., 2001) studied the microbial quality of livestock drinking water in 473 cattle water troughs located at 99 different cattle operations. The mean log10-transformed coliform and *Escherichia coli* concentrations per milliliter of trough water were 1.76 ± 1.25 (SD) and 0.98 ± 1.06 (SD), respectively. The degree of *E. coli* contamination was positively associated with the pr oximity of the water through to the feedbunk, protection of the trough from direct sunlight, lower concentrations of protozoa in the water, and warmer weather. *Salmonella* sp. were isolated from 2/235 (0.8%) troughs and shigatoxigenic-*E. coli* O157 was recovered from 6/473 (1.3%) troughs. Four experimental microcosms simulating cattle water troughs were used to further evaluate the effects of protozoal populations on the survival of *E. coli* O157 in cattle water troughs. *Escherichia coli* O157 of bovine fecal origin proliferated in all microcosms. Reduction of protozoal populations by treatment with cycloheximide was associated with increased persistence of *E. coli* O157 concentrations in the microcosms. Water troughs are a major source of exposure of cattle to enteric bacteria, including a number of foodborne pathogens, and this degree of bacterial contamination appeared to be associated with potentially controllable factors.

The microbiological quality of surface waters helps to determine their acceptability for both drinking and recreational purposes (Hooda *et al*., 2000). the use of raw sewage in

farming systems in some developing countries e.g. China, India Thailand, Indonesia can also contaminate water with bacterial and protozoan pathogens Adhikari *et al*., 1997; Wang, 1997 . Bacterial contamination of runoff water has traditionally been assessed using counts of selected bacterial indicators, such as total coliforms (TC), faecal coliforms (FC), faecal streptococci (FS) or enterococci. Livestock grazing activities have been found to increase bacterial counts in runoff water. For example, indicator bacterial densities in streamwater were significantly higher when at least 150 cattle were grazing, but bacterial counts dropped to levels similar to those adjacent to an ungrazed pasture following the removal of cattle or when only 40 head of cattle were grazing (Gary *et al*., 1983). Similarly, cattle grazing increased FC counts which exceeded several-fold the USEPA standard for bacterial contamination of primary contact water 200 faecal coliforms 100 ml^{-1} (Doran *et al*., 1981; Howell *et al*., 1995).

This is another highly potential protozoan parasite that can cause waterborne diarrhoreal infections to both man and animals livestock and wild populations a like. It is now considered as the leading water-borne parasitic disease in the USA, and thought to be one of the most common intestinal diseases world-wide Bemrick and Erlandsen, 1988.The organism infects the small intestine and is excreted in large numbers, as small cysts, during an infection. The infection is more prevalent in children and young animals than in adults. As with Cryptosporidium, Giardia infection is also prevalent among young farmed animals e.g. cattle, pigs, sheep, horses and very often the infections are concurrent Xiao *et al*., 1993; Quilez *et al*., 1996; Olson *et al*., 1997. *Giardia labbila* cysts have survived up to 33 days in animal waste and 47 days in water Snowdon *et al*., 1987. Runoff from infected-waste applied fields can therefore contaminate fresh waters with this organism. In a survey of sheep and cattle in Canada, Buret *et al*., 1990 found that 35.7% of suckling lambs and 22.7% of calves faecal samples showed the presence of cysts. No incidence of cysts was found in adult cattle, but 4.1% of the adult sheep did excrete cysts. Similarly, Giardia spp. has been reported in cattle in Switzerland Gasser et al., 1987. Like *C. parvum*, *Giardia* cysts are hard to destroy with conventional water disinfection treatments, and prevention of their spread is therefore essential.

(Hannan et al., 2010) determine the bacteriological status of drinking water. They evaluated 100 samples of drinking water from some areas of Lahore by the Membrane Filtration Technique (MFT) using CHROMagar. Using this technique in one step a

much large volume of water can be evaluated quantitatively in a short time. Use of CHROMagar straightaway confirms the presence of Escherichia coli which is accepted universally as the indicator of fecal contamination. A volume of 100 ml water was filtered under the vacuum pressure through Millipore membrane filters. After filtration, membrane filters were placed on CHROMagar and incubated at 35ºC for 24 hr. Escherichia coli appeared as blue coloured colonies while coliforms yielded colonies of pink colour. *Escherichia coli* were further identified by API 20E and confirmed by Eijkman test. *Escherichia coli* was grown from 42% samples (all Eijkman positive). Coliform organisms were grown from 54% specimens. It was alarming that 59% of drinking water was unsatisfactory for human consumption.

(ANWAR et al., 2017) assessed the bacteriological quality of drinking water in Lahore-Pakistan. They had performed the study in Lahore city during the months of April and May 2008. A total of 530 water samples were collected from different localities of whole of the Lahore city. These represented areas with different socio-economic conditions (SEC). The samples were collected in sterilized containers and brought to the laboratory within two hours of collection. All the samples were tested for contamination with bacteria using multiple tube method to determine most probable number of total coliforms and faecal coliforms using standard procedure. Among 530 water samples, 197 samples (37.2%) were positive for bacterial contamination. It was observed that bacterial contamination was maximum in areas with low SEC (43.6%), followed by intermediate SEC (36.5%) and high SEC (22.9%). The difference was found to be statistically significant (p<0.15) between areas with High and Low SEC while it was non-significant (p>0.5) between areas with Low and Intermediate SEC. Bacterial contamination is significant problem in Lahore. Regular monitoring and chlorination/establishment of water filtration plants can improve this situation.

(Mashiatullah et al., 2010) investigated the population of total coliform colonies as well as fecal coliform contamination in Rawal lake, which is one of major source of drinking water supply to inhabitants of Rawalpindi, and its feeding streams (mainly Kurang River and three perennial streams) flowing in the administrative jurisdiction of the capital city, Islamabad, Pakistan. Coliform bacteria in Rawal lake and feeding streams water was determined by membrane filtration technique. The results indicated that E. *Coli* population in four streams (input waters) feeding the Rawal Lake ranged from 25 - 57 (mean 36) fecal coliform per 100 ml. The Kurang River, one of the feeding

streams, hosted the largest population of fecal coliform (57 fecal coliform per 100 mL). The highest population of fecal coliform (105 fecal coliform per 100 mL) in Rawal Lake surface water was observed at the confluence of Kurang River and the Lake in the vicinity of village "New Ampler". While in the Rawal Lake water columns, it ranged from 12 - 65 (mean 25) fecal coliform/ 100mL. The measured levels of fecal coliform bacteria are much higher than the maximum permissible levels for drinking water as recommended by WHO and USEPA (No fecal coliform in drinking water). It is concluded that the indiscriminate amount of pollution from domestic sewage and poultry industry has seriously affected the biological quality of stream waters and the Rawal Lake waters. (Abdul Hussain Shar, 2012) The objective of this study was to determine whether there was an association of seasonal variation and bacterial communities of municipal water. The sampling was carried out fortnightly after a flow time of 5 min to eliminate any contaminant present in the mouth of tap in sterilized screw caped 500 ml white glass flasks (Pyrex), containing 0.1 ml of a 1.8% solution of sodium thiosulphate pentahydrate ($Na_2S_2O_3.5H_2O$) per 100 ml of sample. Samples were placed in ice boxes and brought to laboratory within 1 h of collection. Samples were analyzed for bacterial communities using standard microbiological method (membrane filtration technique). The suspected colonies were then further purified and identified using API 20E (BioMerieux) commercial identification kit. Twelve pathogenic bacterial species were isolated and identified from municipal water on conventional and selective media. Their prevalence was higher in summer season. The average isolation rate was as follows: Escherichia coli 69.4%, Proteus mirabilis 65.2%, Providencia rettgeri 65.2%, Providencia stuarti 61%, Klebsiella oxytoca 54.1%, Citrobacter youngae 60%, Non fermenter species 57%, Chryseobacterium meningosepticum 51.3%, Vibro mimicus 39%, V. cholerae 38%, Aeromons hydrphilia 65.2% and Pseudomonas aeruginosa 78%. It is important to mention that water samples were positive for the above pathogens throughout the study period (2005 to 2007). The temperature of water samples was reported highest in July to September and the pH of water samples ranged 7 and 7.8. The bacteriological quality of drinking water under study was very poor. In summer, the isolation rate of bacterial communities was higher than in winter.

(Willms et al., 2002) conducted an experiment to examine the effects of water source on cattle production and behavior, to determine the relationship of selected chemical

and biological constituents on the observed response and to test the effect of fecal contamination on water consumption. Four dugouts or ponds were selected at 4 sites: 2 in the Fescue Prairie near Stavely in southwestern Alberta, 1 in the Mixed Prairie at Onefour in southeastern Alberta, and 1 in the Palouse Prairie near Kamloops, British Columbia. Yearling Herefords were tested at 3 sites and Hereford cow-calf pairs at 1 Stavely site. At each site, three paddocks radiated from the pond that were stocked with 10 yearlings or cow-calf pairs randomly assigned to either clean water (water delivered to a trough from a well, river, or pond), pond water pumped to a trough (pond$_{trough}$), or direct access into the pond (pond$_{direct}$) The trials were repeated at each site for 3 to 6 years. Clean water sample from Stavely contained number of coliform (108 ± 83 no./100ml) whereas pond water contained (233 ± 39 no./100 ml). In case of Oneford number of coliform in clean water (7 ± 4 no./100ml) and pond water (58 ± 25 no./100ml). On the third site Kamloops contained number of coliform in clean water was (24 no./100ml) whereas pond water contained (182 ± 62 no./100ml). Canadian water quality Guidelines (1999) represented that number of coliform in water were 1000 per 100 ml water.

2.2 Physicochemical parameters

2.2.1 Total dissolved solids (TSD)

Willms et al. (2002) conducted an experiment to examine the effects of water source on cattle production and behavior, to determine the relationship of selected chemical and biological constituents on the observed response and to test the effect of fecal contamination on water consumption. Four dugouts or ponds were selected at 4 sites: 2 in the Fescue Prairie near Stavely in southwestern Alberta, 1 in the Mixed Prairie at Onefour in southeastern Alberta, and 1 in the Palouse Prairie near Kamloops, British Columbia. Yearling Herefords were tested at 3 sites and Hereford cow-calf pairs at 1 Stavely site. At each site, three paddocks radiated from the pond that were stocked with 10 yearlings or cow-calf pairs randomly assigned to either clean water (water delivered to a trough from a well, river, or pond), pond water pumped to a trough (pond trough), or direct access into the pond (pond direct) The trials were repeated at each site for 3 to 6 years. Clean water sample from Stavely contained total TDS (675 ± 32 mg/liter) whereas pond water contained (177 ± 7 mg/liter). In case of One Ford Total TDS in

clean water (675 ± 32 mg/liter) and pond water (233 ± 39 mg/liter). On the third site Kamloops contained total TDS (783 mg/liter) whereas pond water contained (22 mg/liter). Canadian water quality Guidelines (1999) represented that Total TDS in water were 3000 mg/liter

High levels of specific ions in water can cause animal health problems and death. The National Academy of Sciences, 1972 offers upper limits for toxic substances in water. Recommendations for levels of toxic substances such as total TDS in drinking water for livestock was 10,000mg/L respectively (Soltanpour et al., 1999).

Salinity of water depends by quantity of salts: sodium, chloride, bicarbonate, sulfate, calcium, magnesium and smaller quantity of silica, iron, nitrate, strontium, potassium, carbonate, phosphorus, boron and fluoride (El Mahdy *et al*., 2016). TSD represents in the same time a guide of waters quality (Broadwater, 2007), a pre-indicator of poor quality water (Adams *et al*., 2009). Two water sources may have similar salinity levels but different effects, depending on the salts present (Higgins *et al*., 2008). Sodium chloride it is first parameter taken into account when TSD values are high but the action is less harmful on heath than the sulfates combined with magnesium and/or sodium (Linn, 2008; Lardy *et al*., 2008) and of these magnesium chloride has action much damaging than calcium salts or sodium (Griffith, 1998).

After Patterson *et al*., (2003) water with high level in salt content can compromise performance and health of cattle by: reducing the water and feed intake; toxic levels of sulfur ingestion; or can induce trace mineral deficiencies.

In National Research Council (N.R.C.), (2001) it is specified that, the water with salinity under 1000 ppm is consider safe, higher values having negative effects on health status and animal products as fallow: values between 1000-2999 ppm have moderate action through installation of temporary diarrhea at animals who are not accustomed with quality of the water source. TDS between 3000-4999 ppm/dm^3 reduce water consumption and sometime can install moderate diarrhea. Several studies have shown that TDS between 4000 to 5000 ppm negatively affect daily average gain, decrease milk production in lactating cows which cause a reduction in weights at calves at weaning (Dyer, 2012). If the salinity is high (around EC 4000 μS/cm) but chloride levels are normal, shall be analyzed other salts that contribute to raising TSD values (Curran, 2014). There is a warning in particular for lactating cows as well as gestating,

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to avoid the water whose content in TDS is between 5000-6000 ppm, because cause diarrhea and in this case is compulsory determination of sulfates from water (Tennis, 2007). When TSD value is between 4400- 6000 ppm cattle has lower weight gains than cattle drinking normal water (TDS = 1300 ppm), only in conditions where, content of energy from feeds is low during heat stress. In other circumstances: intake of feeds with high energy even in cold environmental it does not have negative repercussions (Looper *et al*., 2002). Over 7000 ppm/dm^3, the water is saline, which affect the intake of water with repercussions on milk production, who decrease and in the same time affect the health of animals. (El Mahdy *et al*., 2016) specify the fact that, the consumption of water with value of TSD between 7000 and 10000 ppm is sure for dry beef cows in condition of minimal environmental stress. Waldner *et al*., (2012) considers that the water with a content higher than 10000 ppm is unsafe and should not be administrate to animals.

Breede (2006) showed that according to national academy of science average Total dissolved solids in water is 368 ppm whereas, expected level is 500 ppm or less but possible problem will be occurred over 3,000ppm.

2.2.2 Water P^H

Willms *et al*. (2002) were conducted two experiments to determine the effects of water source on performance of Hereford cattle: One examined the effects on yearling cattle (Yearling Experiment) while the second examined the effects on cow-calf pairs (Cow-Calf Experiment). Four dugouts or ponds were selected at 4 sites: 2 in the Fescue Prairie near Stavely in southwestern Alberta, 1 in the Mixed Prairie at Onefour in southeastern Alberta, and 1 in the Palouse Prairie near Kamloops, British Columbia. Yearling Herefords were tested at 3 sites and Hereford cow-calf pairs at 1 Stavely site. At each site, three paddocks radiated from the pond that were stocked with 10 yearlings or cow-calf pairs randomly assigned to either clean water (water delivered to a trough from a well, river, or pond), pond water pumped to a trough (pond$_{trough}$), or direct access into the pond (pond$_{direct}$) The trials were repeated at each site for 3 to 6 years. P^H from clean water sample from Stavely was (8.50 ± 0.04) whereas in pond water was (7.87 ±0.06). In case of Oneford P^H in clean water (8.76 ± 0.05) and pond water (8.83 ± 0.33 mg/liter).

On the third site Kamloops contained P^H (8.46 ± 0.24) whereas pond water contained (9.00 ± 0.04).

Therefore, the present study was mainly aimed to conduct research and to get knowledge about the physical and chemical nature of drinking water found in Quetta city. The water samples were collected from three different sources viz., Tube well, Karaiz and Spring of Quetta city during 2011. A total of eight physicochemical parameters i.e. air temperature, water temperature, electrical conductivity (EC), pH, Ca, Mg, Na and K were investigated by standard methods. Results showed that the average value of air temperature was recorded as 20, 23 and 26°C at Tube well, Karaiz and spring water locations, respectively. Average water temperature was reported as 22.66, 10.66 and 5.00 °C, electrical conductivity was noted as 278.33, 342.66 and 475.33 µS/cm and pH was recorded as 6.44 and 6.59 lowest to highest in Tube well, Karaiz and Spring water respectively. Average Ca content was 1.45, 2.10 and 5.80 mg L; Ca +Mg was 1.82, 0.85 and 1.27 mg L; Na was 24.16, 49.76 and 62.86 mg L; K was 22.98, 34.32 and 43,86 mg L in Tube well, Karaiz and Spring water respectively. All the investigated parameters (except pH and EC) exhibited that all water samples are neutral and non-polluted, which can be used both for drinking and agricultural purposes. But the pH and conductivity of the tested samples indicated that water from spring and tube well is under deterioration and should be treated before to use mainly for drinking purposes. (Achakzai *et al*., 2014)

Breede (2006) showed that according to National Academy of Science average water p^H is 7.0 whereas expected level of p^H is 6.8-7.5 but possible problem will be occurred when p^H level remains below 5.1 and above 9.0.

The pH of a water body is very important in determining the water quality since it affects other chemical reactions such as solubility and metal toxicity (Fakayode, 2005). pH values ranges from 6.37-6.83. It was found to be acidic in nature during all seasons and no significant

Water pH denotes either alkalinity or acidity. High-saline water is not the same as alkaline water. A pH of 7 would be neutral; a number higher than 7 indicates alkalinity; below 7 designates acidity. Most North Dakota waters are mildly alkaline with a pH value between 7 and 8. Acidic water (pH below 7) is not common in most of North Dakota; however, some reports indicate acidic water in the western part of the state in

proximity to lignite coal veins. Various degrees of alkalinity have been reported in the state. High alkalinity may cause digestive upsets, laxative action, poor feed conversion, and reduced water and/or feed intake (Lardy *et al*., 2008).

Brew *et al*. (2009) reported water analysis result from three different sources where critical level for chloride was 0.10-0.20 ppm. Fountain water contained 19.0 ppm whereas in tank water this value was 22.0 ppm. In case of pond water amount of chloride was 13.0 ppm. From three different sources such as Fountain, tank and pond water also contained different p^H that was 7.1, 7.0 and 7.4 respectively. In case of total TDS, the same sources of water contained 89.6, 145.9, and 238.7 ppm, respectively.

The livestock can support variations of pH between 6.5-8.5 unit.pH (Curran *et al*., 2007). The dairy cow prefers water with pH between 6.0-8.0 unit. pH (Olkowski, 2009). Exceeding this threshold, the water has lye taste, and under 6.5-unit pH the taste of water is acidulous-prickly due to of humic acids, mineral and especially due to the presence of carbon dioxide in high quantity (Man, 1989, 2007; Draghici, 2001; Popescu, 2010). Usually the underground water has alkaline reaction. Unfavorable action of low or high pH were associated with decreasing milk production concomitant with fat content, decreased average daily gain and increase the susceptibility at infection, installation of some metabolic disorder and reducing fertility (Adams *et al*., 2009). At ruminants the consumption of water under 5.5 unit pH produce metabolic acidosis (Grant, 1993) but, after Ishle V. seems to be only a contributory factor, alongside intake and environmental factors.

Alkaline water with pH higher than 8.5 unit pH lead to heightened risk of metabolic alkalosis occurrence (Swistock, 2012), B-vitamin deficiencies, and symptoms similar to mild acidosis (Grant, 1993). Other authors such as Bagley *et al*., (1997), Man (1989, 2002) indicates other negative repercussions of water consumption with high pH: digestive disorder, diarrhea, poor feed conversion and reduced water/feed intake. When cows drink alkaline water, rich diet in alfalfa, buffers and minerals, they are more likely to the occurrence of mild alkalosis (Grant, 1993).

Dependent of water pH, the effect can be corrosive on water supply system influencing at the same time the effectiveness of chlorination (Hersom *et al*., 2008)

2.2.3 Total hardness

Total hardness is given by all calcium and magnesium salts that are found in water and is considered overall indicator of water mineralization (Straus, 1981). Depending on salts concentration from water (carbonate, bicarbonate, sulfates, silica, nitrate, phosphates, by calcium and magnesium, along with potassium, sodium, iron, manganese, etc., the waters can have variable hardness, depending on which can divided in soft water (0-60), semi hard (6-120), hard water (12- 180) and very hard water (over 180) (El Mahdy *et al*., 2016)

Breede (2006) showed that according to National Academy of Science average Total hardness in water is 208ppm whereas, expected level is 0-180ppm.

Consuming the water whose hardness is too high or too low represents a permanently topic for research, controversially in terms of action and the effect on health condition of animals (Draghici, 2001). The high hardness can cause altering of health condition by the presence of renal calculus, gastric disorders, chronic catarrh of the digestive mucosa and even methaemoglobinaemia especially when animals accustomed to a type of water are forced to consume water with highhardness (El Mahdy, 2013). Lardy (2008) points out that the: consumption of water with high hardness is not a factor in their appearance, but affects water palatability.

On other hand, hard water caused by high calcium levels can influence the incidence of milk fever in a dairy herd (El Mahdy, 2016)

Opposed these reactions, was notice that the water with low hardness is favorable, positively influencing the milk production (Popescu *et al.,* 1981;1985).

When hardness equals alkalinity, salts of calcium and magnesium combined with carbonates and bicarbonates are indicated. When alkalinity is less than hardness, salts of calcium and magnesium are more likely to be sulfates instead of carbonates and if the alkalinity exceeds the hardness indicate the presence of sodium and potassium salts in addition to calcium and magnesium (German *et al*., 2008).

2.2.4 Chlorides

Chlorides from water can have telluric origin, in which case the values obtained after performance the analyzes are relatively constant, but, dry periods entail an increase in the values of this parameter in which case is not suspected the existence of a source of contamination (El Mahdy, 2013) or, may get into surface water from several sources like: wastewater from industries and municipalities, effluent wastewater from water softening, road salting, agricultural runoff and produced water from oil and gas wells (Iowa Department of Natural Resources DNR 2009).

Chlorides above 250ppm can imprint a salty taste to water which could result in reduced water intake and milk production. High amount of chlorides present in water should be considered when formulating diets, to prevent the excess which could be detrimental to rumen function (Swistock, 2016).

Sodium chloride poisonings in cattle are a result of administering of a feeding-stuff as rich in salt. Direct consumption of salt and low potassium quantity in feed, changes occurred in the water palatability, high consumption of water with a high concentration of sodium chloride, insufficient watering front, whose manifestations consist of occurrence of toxic encephalosis: hyperexcitability (tremor, muscle cramps, colic), followed by inhibition (amaurosis, paraparesis) (El Mahdy, 2016)

Episodes of poisoning after consumption of water with high amount of sodium chloride in adult cattle is manifested by gastrointestinal irritation, accompanied by emesis, diarrhea, the presence of mucoid faeces, thirst, salivation (Man, 2007), animals appear unwell, lose appetite and are reluctant to drink water, initially increase the urination followed by small amounts but concentrated, nasal discharge, abdominal pain, animals prefers to stay lying down (Curran, 2014) and nervous signs such as: star gazing, tremors, blindness, circling, walking backwards, head pressing, wobbly in the legs; knuckling at the fetlocks and convulsions and even death (Bradford, 2014)

Breede (2006) showed that according to national academy of science average Chloride in water is 20 ppm whereas, expected level is 0-250ppm. Free or residual chlorine concentrations up to 0.5 to 1.0 ppm have not affected ruminants adversely. Municipal water supplies with 0.2 to 0.5 ppm have been used successfully. Chlorine in farm systems with short contact time have caused no apparent problems for cattle.

Sodium by itself, poses little risk to livestock, but its association with sulfate represents a major concern, reason why the acceptable limits should be below 400 ppm, because values greater than 400 ppm can have negative effect dependent on alkalinity and the pH of the water. Over 800 ppm sodium can cause diarrhea and a drop in milk production in dairy cows (Tennis, 2007). High sodium levels in water may require adjustments to the amount of salt (NaCl) added to dairy ration. Lack of drinking water sources and the negative repercussions resulting from the administration of water with high salt content, can be reduced by administration of betaine (Mavromichalis, 2013).

2.2.5 Iron

Weiss (2008; 2010) claims that the although feed: hay, silage contains large amounts of iron, above 500 ppm rarely causes adverse reactions because of insoluble form, ferric ion (Fe^{+3}), but, if the concentration is more than 0.3 ppm in drinking water, this may cause problems for cows (El Mahdy, 2016). Amounts greater than 0.3 ppm induce an unpleasant taste of water, which leads to voluntary water consumption decrease concomitantly with the milk production (Swistock, 2012), but, the study performed by Mann *et al*., (2013) reveals the fact that iron intake up to 1.250 mg per day for 14 days is safe for early lactation dairy cattle, not affect the chemical composition of milk but processed milk from those cows was susceptible to flavor changes.

Signs characteristic of chronic iron intoxication is manifested by reducing feed intake and feed conversion efficiency (Man, 2002). Excessive intake of iron in the water consumed of cattle, after Linn, (2008) has an adverse effect due to increasing the reactivity of oxidative species (oxidative stress) that harms the cell membranes and interrupt several biochemical reactions in the body.

Oxidative stress in cattle was incriminated in increased incidence of metritis and mastitis (Tomlinson, 2014) fetal membrane retention, decreases absorption of essential minerals, decreased immunity, increase the risk of infections and affected milk production.

Breede (2006) showed that according to national academy of science average Iron concentration in water is 0.8ppm whereas, expected level is 0-0.3ppm but possible

problem will be occurred above 0.3ppm. Usually the adverse effects of iron are indirect through association with secondary deficiencies resulting from antagonistic action

High iron, manganese, or molybdenum content may increase needs for copper (Broadwater, 2007), copper deficiency is in most cases a result from an excess of iron in the diet of dairy cows (Draghici, 2001), but in the same time decrease absorption of manganese from the diet (Prairie *et al*., 2014), magnesium and calcium that lead to decrease in productive performance and health of cows. The iron in quantities greater than 0.3 mg/dm^3 has negative impact on absorption of Zn and therefore Higgins, (2008) considers that one of the critical analysis is the analysis of the level of iron in the water, because, ferrous iron (Fe^{2+}) dissolved in water is presumed to be highly absorbable with an estimated absorption rate approaching 100%. After Linn, (2008) pH and the presence of sulfates from water plays decisive role on the form and solubility of iron. The less soluble ferric form (Fe^{+3}) combined with OH is found at pH values below7, and at a pH above 9.5-unit pH the greatest amount of iron can be found as ferric form combined with OH. Water taste may be altered at values greater than 200 ppm/dm3 sulfates present in the water when iron combines with them in a higher percentage than with OH.

CHAPTER III

MATERIAL AND METHODS

3.1 Experimental design:

The entire study of water quality determination was divided into three major steps. The first step included site selection for sample collection. second step was microbiological study containing parameters like counting of Coliform, *E. coli* bacteria, Giardia and Balantidia. Third step was study of physicochemical properties like pH, TDS, Hardness, Iron and Chloride
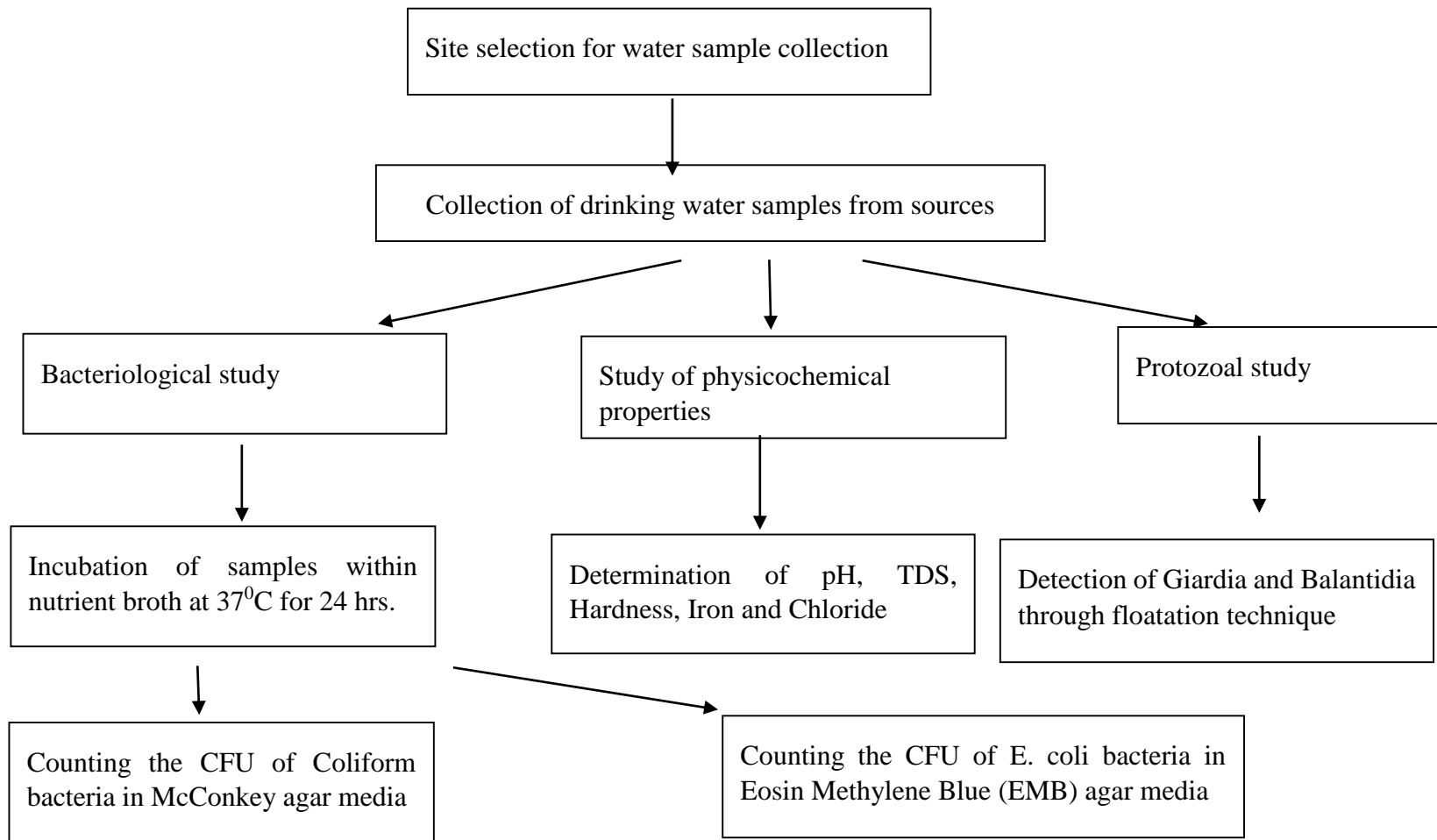
Figure 1: Flowchart of experimental design of this study

3.2 Study sites:

the present study was aimed to assess the quality of water supplied to the dairy farms in several selected regions of Dhaka city. the study areas were located at 60 feet area, mirpur-1, mirpur-2, kalshi, and SAU (Sher-e- Bangla Agricultural University) farm. 5 replicas of each source were collected using sterile PTFE (polytetrafluroethylene) bottle (500ml), thus total number of samples were twenty. description of sampling site is following in detailed

Plate 1: The map of study area from where water samples were collected.

(a) 60 feet area:

Name of farm: Meghdut agro farm

Owner name: Kobir

Number of total cow: 8

Total intake of water: about 82 litter per cow per day

Source of water: underground supply water

Diarrheal disease history: rare

Sample labeling: this area is indicated as S_1(Source 1). 4 replicas of this source are labeled as $S_1 R_1$, $S_1 R_2$, $S_1 R_3$ and $S_1 R_4$.

(b) Mirpur-1:

Name of farm: Ronju dairy

Owner name: Ronju

Number of total cow: 15

Total intake of water: about 75 litter per cow per day

Source of water: underground supply water

Diarrheal disease history: yes, often found

Sample labeling: this area is indicated as S_2(Source 2). 4 replicas of this source are labeled as $S_2 R_1$, $S_2 R_2$, $S_2 R_3$ and $S_2 R_4$.

(c) Mirpur-2:

Name of farm: Alam dairy

Owner name: Shah alam

Number of total cow: Number of total cattle 50 where dairy cattle 32

Total intake of water: about 70-90 litter per cow per day

Source of water: underground supply water

Diarrheal disease history: yes, often found

Sample labeling: this area is indicated as S_3(Source 3). 4 replicas of this source are labeled as S_3R_1, S_3R_2, S_3R_3 and S_3R_4.

(d) Kalshi:

Name of farm: Abu talha dairy farm

Owner name: Monir

Number of total cow: Number of total dairy cow 11 and no. of calf 12

Total intake of water: about 80-90 litter per cow per day

Source of water: well

Diarrheal disease history: yes, rare found

Sample labeling: this area is indicated as S_4(Source 4). 4 replicas of this source are labeled as $S_4 R_1$, S_4R_2, S_4R_3 and S_4R_4.

(e) SAU Campus Farm:

Number of total cow: Number of total cow 12

Total intake of water: about 75-90 litter per cow per day

Source of water: Underground supply water

Diarrheal disease history: yes, rare found

Sample labeling: this area is indicated as S_5(Source 5). 4 replicas of this source are labeled as S_5R_1, S_4R_2, S_5R_3 and S_5R_4.

3.3 Sampling:

Collection of water samples was done according to the standard methods for the examination of water and waste water (APHA, 2001 and IS 10500: 2012). water samples were collected from predefined sites during January 28 to February 1. Four replication of each water source were collected from pre-defined site. Water samples were taken in 500 ml pre-acidic washed PTFE bottles. Bottles were filled to brink with water samples, tightly closed and labeled. Water samples for microbial analysis were collected in 30 ml sterilized glass bottles. Sampling bottles were kept in ice box, carried to the medicine and public health laboratory and 4^0C temperatures was maintained there.
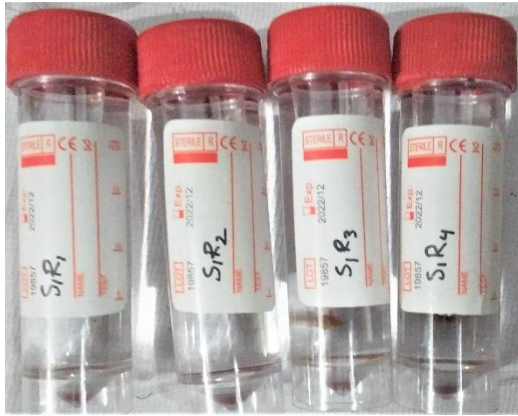
(a) 60 feet area

(b) Mirpur-1

(c) Mirpur-2

(d) Kalshi

(e) SAU Campus Farm

Plate 2: Photographs of collected samples

3.4 Glass wares and other appliances:

The following glass wares and appliances were used during the course of the study:

i. Test tubes (with or without Durham's fermentation tube and stopper)
ii. Petridishes
iii. Conical
iv. Flask,
v. Pipette (1 ml, 2 ml, 5 ml, 10 ml)
vi. Micro-pipettes (1ml, 200μl, 100μl, 10 μl)
vii. Eppendorf tube
viii. Slides
ix. Cover slips
x. Immersion oil, compound microscope,
xi. Bacteriological loop
xii. glass spreader
xiii. Sterilized cotton & cotton plug
xiv. Test tube stands and rack
xv. Water bath
xvi. Bacteriological incubator
xvii. Refrigerator
xviii. Sterilizing instruments
xix. Thermometer
xx. Ice carrier
xxi. Hand gloves
xxii. Spirit lamp
xxiii. Match lighter
xxiv. Laminar air flow
xxv. Hot air oven
xxvi. Centrifuge tubes and machine
xxvii. Electronic weight balance
xxviii. Electric stirrer and magnet
xxix. Auto clave machine.
xxx. Multi parameter -EUTECH PCSTestr
xxxi. UV- Visible spectrophotometer, Iron Cell Test kit

3.5 Bacteriological Media:

Different types of agar media and liquid media are used in bacterial culture. mentioned following.

3.5.1 Agar media:

   i.   MacConkey (MC) agar
  ii.   Eosin Methylene Blue (EMB) agar

3.5.2 Liquid media (broth):

The liquid media used for this study were

   i.   Nutrient broth
  ii.   Methyl-Red

3.5.3 Chemicals and reagents:

The chemicals and reagents used for this study were;

    i.   Phosphate buffered saline (PBS)
   ii.   3% Hydrogen peroxide
  iii.   Methyl red
  iv.   floating solution
   v.   other common laboratory chemicals and reagents.
  vi.   silver nitrate solution
 vii. Fe_{3+} solution
viii. HNO_3 solution
  ix.   Ethylene diamine tetra acetic acid (EDTA)
   x.   Ammonium thioglycolate
  xi.   Thioglycolic acid

3.6 Preparation of various bacteriological culture media:

3.6.1 Nutrient Broth:

Nutrient Broth was prepared by Suspended 25 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Sterilized by autoclaving at 15 lbs pressure (121°C) for 30 minutes. The broth was filled in test tubes & incubated at 37ºC for overnight to check their sterility and stored at 4ºC in the refrigerator until used.

3.6.2 MacConkey agar media:

49.53 grams of Bacto MacConkey agar (HiMedia, India) was suspended in to 1000 ml of cold distilled water and was heated for boiling to dissolve the medium completely. It was then poured in to sterile petridishes and allowed to solidify. After solidification of the medium in the plates, the plates were then incubated at 37ºC for overnight to check their sterility.

3.6.3 Eosine Methylene Blue (EMB) agar:

Thirty six grams powder of EMB agar base (HiMedia, India) was suspended in 1000 ml of distilled water. The suspension was heated to boil for few minutes to dissolve the powder completely with water. The medium was autoclaved for 30 minutes to make it sterile. After autoclaving the medium was put in to water bath at 45ºC to cool down its temperature at 40ºC. From water bath 10-20 ml of medium was poured in to small and medium sized sterile petridishes to make EMB agar plates. After solidification of the medium in the plates, the plates were incubated at 37ºC for overnight to check their sterility.

3.6.4 Methyl Red and Voges–Proskauer (MR-VP) broth:

A quantity of 3.4 gm of MR-VP medium (HiMedia, India) was dissolved in 250 ml of distilled water, distributed in 2 ml quantities in test tube and then autoclaved. After autoclaving, the tubes containing medium were incubated at 37ºC for overnight to check their sterility and then stored at 4ºC for future use.

3.6.5 Floatation fluid:

Floatation fluid was made by mixing 400 grams of Sodium Cloride, 500 grams of Sugar into 1000 ml of water. Thus flotation fluid was prepared with a specific gravity of 1.28.

3.6.6 Phosphate Buffered Saline (PBS):

For preparation of phosphate buffered saline, 8 gm of sodium chloride (NaCl), 2.89 gm of disodium hydrogen phosphate (Na2HPO4.12H2O), 0.2 gm of potassium chloride (KCl) and 0.2 gm of potassium hydrogen phosphate (KH2PO4) were suspended in 1000 ml of distilled 16 water. The solution was heated to dissolve completely and pH was adjusted with the help of pH meter. The solution was then sterilized by autoclaving and stored at 4ºC for future use.

3.7 Isolation and counting of bacteria:

For isolation and counting of Coliform bacteria and E. coli the following procedure are maintained.

3.7.1 Primary culture of microorganism in nutrient broth:

Primary growth of all kinds of bacteria present in the collected samples was performed in nutrient broth. The samples were inoculated in nutrient broth and incubated for overnight at 37^0 C for the growth of the organisms.

3.7.2 Serial dilution for bacterial culture (10-fold dilution method):

Serial dilution of the water sample from nutrient broth was done to lowering the bacterial count for the total coliform (TCC) and E. coli count. It was done by taking 8 (1-8) Eppendorf tube filled with 900µl of PBS. 100µl of stock sample was transferred from the stock sample to the Eppendorf tube next to the stock tube. Then 100µl of diluted sample is transferred from the first Eppendorf tube to the next. Successive dilution should be made in the same way to the last tube and from the last tube 100µl of diluted sample should be discarded. From the 5th tube 50µl of liquid sample should be transferred to the MacConkey agar to elucidate the total coliform count. Enumeration of E. coli was done by transferring same amount of liquid sample in the EMB agar media.

3.7.3 Bacterial culture in MacConkey agar media:

After 10 fold serial dilution, from the 5th Eppendorf tube 50µl of liquid sample should be transferred to the MacConkey agar through micro-pipette then spreaded by glass spreader. Then the petridishes were kept in the incubator for overnight at 37^0 C. After 24 hrs. the petridishes were removed from the incubator and colony were counted.

3.7.4 Bacterial culture in EMB agar media:

After 10 fold serial dilution, from the 5th Eppendorf tube 50µl of liquid sample should be transferred to the EMB agar through micro-pipette then spreaded by glass spreader. Then the petridishes were kept in the incubator for overnight at 37^0 C. After 24 hrs. the petridishes were removed from the incubator and colony were counted.

3.7.5 Identification of isolated E. coli by using specific biochemical tests:

3.7.5.1 Catalase test:

For this study 3 ml of catalase reagent (3% H_2O_2) was taken in a test tube. Single colony from the pure culture of E. coli was taken with a glass rod and merged in the reagent. The tube was observed for bubble formation. All of the isolates were catalase positive; formation of bubble within few seconds was the indication of the positive test, while the absence of bubble formation indicated negative result (Cheesbrough, 2006).

3.7.5.2 Methyl Red test:

The test was conducted by inoculating single colony from the pure culture of the test organism in 5 ml sterile MR-VP broth. After 5 days' incubation at 37ºC, 5 drops of methyl red solution was added and observed for color formation. Development of red color was positive and indicated an acid pH of 4.5-6 resulting from the fermentation of glucose. Development of yellow color indicated negative result (Cheesbrough, 2006).

3.8 Protozoal examination through floatation technique:

5 ml of sample was taken and mixed with 10ml of floatation fluid. This solution was poured in a cup through the tea strainer. This solution was then taken in to 20 ml centrifugal tube and tube was filled with sugar solution about 1 inch from the top of the tube. Counterbalance was done centrifugation was done for 5 minutes at 1200 rpm. The test tube was then removed from the centrifuge and 1 inch was filled with sugar solution. A coverslip was placed on the test tube and it was allowed to stand for 10 minutes. After that the coverslip was removed and observed under the microscope at 10X to 40X.

Plate 3: Centrifugation of water samples with floatation fluids

3.9 Examination of physicochemical parameters:

To determine the water quality physicochemical properties like pH, TDS, hardness, iron and chloride were tested in the laboratory of ACI animal health diagnostic laboratory ltd.

3.9.1 pH testing:

The pH of the water samples was estimated at sampling site in water suspension of 1:2 (Water sample: Distilled water) ratio using portable multi parameter -EUTECH PCSTestr 35 (Jackson, 1998).

3.9.2 Total dissolved Solid:

The TDS was also determined multi parameter -EUTECH PCSTestr 35 in water suspension of 1: 2 ratios and the value was expressed in mg/l.

3.9.3 Estimation of Chloride (Cl):

About 50 ml of the water sample was pipetted out into a porcelain evaporating dish (Frank *et al*., 2000). Then, the same quantity of distilled water was placed into another dish, for color comparison. To each of this 1ml of potassium chromate indicator was added. Then, standard silver nitrate (dissolving 2400 gm of silver nitrate crystals in 1 litre of distilled water) solution was added to the sample from a burette and a few drops at a time, with constant stirring, until the first permanent reddish coloration appeared. This was determined by comparing with the distilled water. The used volume of silver nitrate was recorded.

Calculation:

$$\text{Chloride (ppm)} = \frac{\text{(Volume of AgN03 used) x 500}}{\text{volume of sample}}$$

3.9.4 Estimation of Iron:

Concentration of Iron was determined by the following procedure (Annem, 2014).

i. From a 1000 ppm stock solution of Fe_{3+} in 1% HNO_3 solution of concentrations of 0.5, 1.0, 2.0, 3.0, 4.0 ppm was prepared.

ii. The standards were treated according to Iron Cell Test kit instructions and the absorbance was measured for each sample at 565 nm. Absorbance vs. concentrations was plotted and the y-intercept was obtained slope and correlation were measured for each sample at 565 nm.

iii. From the water samples, 10 mL was dispensed into a small beaker and the pH was measured. The pH (must be within the range 1-10).

iv. If necessary the pH was adjusted with sodium hydroxide (6.0 M) solution or (6.0M) optimally, the pH was adjusted to 7.

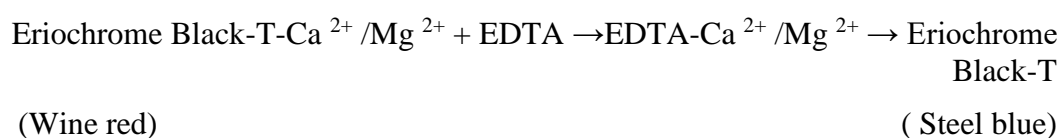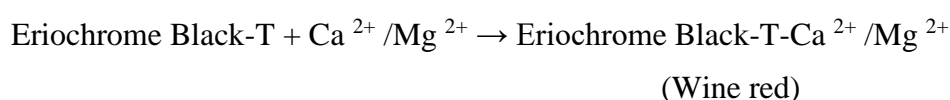v. Further work was performed on a 25 mL aliquot of the sample.

vi.   If there were suspended solids, the 25mL aliquot was filtered using a 0.45 μm polyethylene or Teflon filter.

vii.   The 25 mL aliquot was treated with 0.1 mL of HNO3 (0.1%v/v).

viii. Then, 5.00 mL was pipette into a pre-prepared test tube containing the buffer ammonium thioglycolate and thioglycolic acid. (Note: this buffer stabilizes the pH to 7.0.)

ix.   The test tube was tightly capped and mixed well until the reagent and sample were completely combined.

x.   The samples were left for 3 min. If the iron was present we will observe the formation of a purple solution.

xi.   The sample was measured in the UV- Visible spectrophotometer with absorbance at 565 nm.

xii.   The dissolved iron concentration was calculated from the above calibration curve.

3.9.5 Estimation of hardness of water:

Hardness of water was estimated by EDTA method by following principle and procedure

principle:

Total hardness is due to the presence of bicarbonates, chlorides and sulphates of calcium and magnesium ions. The total hardness of water is estimated by titrating the water sample against EDTA using Eriochrome Black-T (EBT) indicator. Initially EBT forms a weak EBT-Ca^{2+}/Mg^{2+} wine red coloured complex with Ca^{2+}/Mg^{2+} ions present in the hard water. On addition of EDTA solution, Ca^{2+}/Mg^{2+} ions preferably form a stable EDTACa^{2+}/Mg^{2+} complex with EDTA leaving the free EBT indicator in solution which is steel blue in colour in the presence of ammonia buffer (mixture of ammonium chloride and ammonium hydroxide, pH 10).

Eriochrome Black-T + Ca^{2+}/Mg^{2+} → Eriochrome Black-T-Ca^{2+}/Mg^{2+}

(Wine red)

Eriochrome Black-T-Ca^{2+}/Mg^{2+} + EDTA →EDTA-Ca^{2+}/Mg^{2+} → Eriochrome Black-T

(Wine red)   (Steel blue)

Procedure:

20 ml of the given water sample is pipetted out into a clean conical flask. 5 ml ammonia buffer and 2 drops of EBT indicator are added and titrated against EDTA from the burette. The end point is the change of colour from wine red to steel blue. The titration is repeated to get concordant titre value.

Calculation:

1 ml of 0.01 M EDTA ≡ 1 mg of $CaCO_3$

V_1 ml of EDTA ≡ V_1 ml of EDTA

Calculation of total hardness:

$$\text{Total hardness} = \frac{\text{Volume of EDTA solution consumed} \times 1000}{\text{Volume of the hard water taken}} \text{ ppm}$$

CHAPTER IV

RESULT AND DISCUSSION

The result of this study has illustrated the quality of drinking water supplied in the dairy farm in selected regions of Dhaka city where the sites are 60 Feet area, Mirpur-1, Mirpur-2, Kalshi and SAU campus. Quality of water was studied considering microbiological parameters and physicochemical parameters. Coliform bacteria, *E. coli*, *Giardia spp*., and *Balantidia spp* are concerns of microbiological parameters. Likewise, pH, TDS, hardness, iron and chloride are the concerns of physicochemical parameters.

4.1. Bacteriological examination:

All the samples were firstly incubated in nutrient broth then, cultured in McConkey agar media to count the CFU of Coliform bacteria. the stock sample from nutrient broth cultured in EMB agar media for isolation of E. coli and before counting the *E. coli* it was confirmed by biochemical test.

4.1.1. Culture in nutrient broth:

All the sample cultured in nutrient broth showed turbidity after incubation overnight which confirms the growth of bacteria.

4.1.2. Culture in the McConkey agar media:

In McConkey agar media reddish to pinkish, whitish, dark centered brown colored colony was found which are characterized for coliform bacteria. Reddish, pinkis colony indicated the lactose fermenting coliform where whitish and brown color colony indicated the non-lactose fermenting coliform bacteria (Aryal S, 2021).
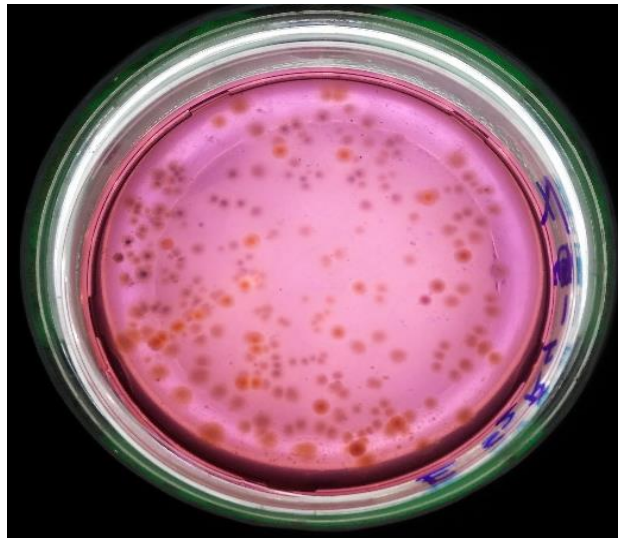
Plate 4: Colonies of Coliform bacteria in McConkey agar

4.1.3. Culture in the EMB agar media:

After overnight incubation in EMB agar media greenish colonies were tentatively confirmed as E. coli which was confirmed by biochemical test.

Plate 5: Colonies of *E. coli* in EMB agar

4.1.4. Catalase test:

In catalase test formation of bubble within few seconds indicated the positive test for *E. coli.*

Plate 6: Bubble formation in catalase test

4.1.5. Methyl red test:

In Methyle red test development of red color was positive for *E. coli* bacteria.

Plate 7: Red color in Methyl red test.

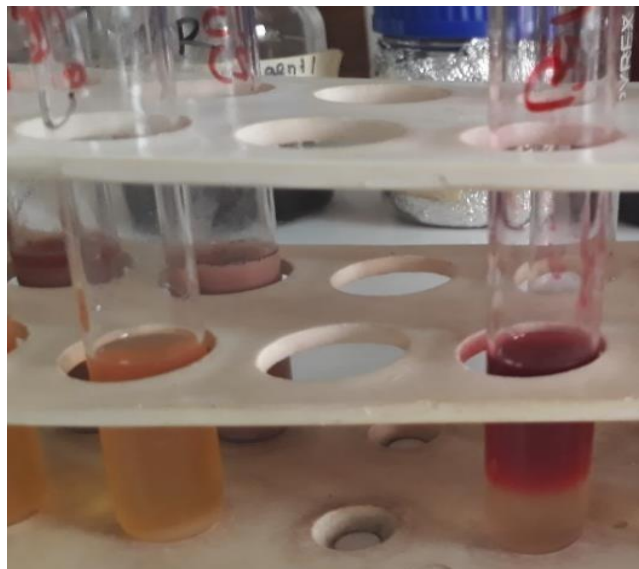### 4.1.6. Counting of Coliform and E. coli:

Table 1. indicate the Coliform and E. coli bacterial population for water samples collected from water sources of five representative locations in Dhaka city. All the water samples collected from 60 Feet area, Mirpur-1, mirpur-2, Kalshi and SAU campus farm contained coliform and *E. coli* bacteria. prevalence of coliform and E. coli was 100%.

Table 1: Coliform and E. coli bacteria counted in water samples of study areas in Dhaka city with their prevalence

Study Area	Water Sample	Coliform CFU/ml	*E. coli* CFU/ml	Prevalence percentage
60 Feet	$S_1 R_1$	2.1×10^8	6.2×10^7	100
	$S_1 R_2$	1.9×10^8	5.6×10^7	
	$S_1 R_3$	2.4×10^8	6×10^7	
	$S_1 R_4$	1.9×10^8	5.2×10^7	
Mirpur-1	$S_2 R_1$	3×10^8	7.6×10^7	100
	$S_2 R_2$	2.94×10^8	7×10^7	
	$S_2 R_3$	2.4×10^8	6×10^7	
	$S_2 R_4$	2.2×10^8	7.6×10^7	
Mirpur-2	$S_3 R_1$	1.6×10^8	7.2×10^7	100
	$S_3 R_2$	1.7×10^8	6×10^7	
	$S_3 R_3$	2.2×10^8	6.4×10^7	
	$S_3 R_4$	1.8×10^8	6.4×10^7	
Kalshi	$S_4 R_1$	1.94×10^8	6.4×10^7	100
	$S_4 R_2$	3×10^8	6×10^7	
	$S_4 R_3$	2×10^8	5.6×10^7	
	$S_4 R_4$	2.1×10^8	6.2×10^7	
SAU Campus	$S_5 R_1$	1.94×10^8	6.6×10^7	100
	$S_5 R_2$	1.5×10^8	5.6×10^7	
	$S_5 R_3$	1.7×10^8	5.4×10^7	
	$S_5 R_4$	1.8×10^8	4.4×10^7	

Table 2: Bacterial load in supplied water in dairy farms of Dhaka city.

Water from different sources	Coliform (CFU/ml)		*E. Coli* (CFU/ml)	
	Mean	SE Mean	Mean	SE Mean
60 Feet Area	1.98×10^8	5.0×10^6	5.75×10^7	2.2×10^6
Mirpur-1	2.63×10^8	1.9×10^7	7.05×10^7	3.7×10^6
Mirpur-2	1.82×10^8	1.3×10^7	6.50×10^7	2.5×10^6
Kalshi	2.26×10^8	2.4×10^7	6.05×10^7	1.7×10^6
SAU Campus	1.73×10^8	9.0×10^6	5.50×10^7	4.5×10^6

Here SE= Standard Error, CFU= Colony Forming Unit.

In this study area of Dhaka city, the load (CFU/ml) of coliform bacteria in water ranges from 1.73×10^8 to 2.63×10^8. The highest load of coliform found in the water of mirpur-1 and the lowest coliform was found in the water of SAU campus farm (Table 2).

In like manner the load (CFU/ml) of E. coli bacteria ranges from 5.50×10^7 to 7.05×10^7 where, the lowest E. coli was found in the water of SAU campus farm and the highest value observed in mirpur-1(Table 2).

(Khan *et al*., 2016) carried out an experiment to observe the presence of Coliform bacterial species from drinking water samples obtained from randomly selected dairy forms at Quetta. The bacterial load in ml^{-1} water sample of buffalo farms the mean number of 197 colonies was counted while bacterial counts were recorded as 3.29X104 and the mean number of 167 colonies ml^{-1} was recorded at dairy farms. While bacterial counts were recorded as 2.93×104. Overall, drinking water samples collected from different dairy farms in Quetta contaminated with Coliform bacteria. The bacterial load/population in water samples of different dairy farms at Quetta was detected higher than standard bacterial concentration level by WHO.

(Hannan *et al*., 2010) determine the bacteriological status of drinking water. They evaluated 100 samples of drinking water from some areas of Lahore by the Membrane Filtration Technique (MFT) using CHROM agar. Escherichia coli was grown from 42% samples (all Eijkman positive). Coliform organisms were grown from 54% specimens. Grant (1993) assess water for animals as follows: safe drinking water for dairy

contained total bacteria under 200/100 ml; total Coliform, fecal coliform and fecal streptococci less than 1/100 ml at. Contaminated drinking water with bacteria over 1,000 000/100 ml can cause health problems, although Broadwater (2007) considers that over 500/100 ml total bacteria counts may indicate water quality problems and the water with over 1,000 000 total bacteria counts should be avoided as a source of water for cows.

4.2. Result of microscopic examination for Giardia spp. and Balantidia spp.

After execution of floatation method, the samples collected from each study area showed no trofozoites and cysts under compound microscope (Table 2). only some debries, fiber etc. are observed under compound microscope (Plate 8). Since the water sources of this study are underground water, here there is less possibility of contamination with faeces contained Giardia and Balantidia. The water sources like river, pond etc. near grazing field or bathan area are usually contaminated with faeces.

Plate 8: Protozoal examination under compound microscope

Archer *et al*. (1995) did not detect Giardia in any of 17 samples from six wells in Wisconsin. Hibler (1988) found Giardia cysts in 19% of springs and 3% of wells sampled. Lee (1993) reported the contamination of two wells in Pennsylvania by surface streams less than 100 feet from the wells;Giardia was recovered from all samples collected from the wells. Hancock *et al*. (1997) collected 463 groundwater

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samples from 199 sites in 23 states in the United States; Giardia cysts were found in 14% of the springs, 1% of the vertical wells, 36% of the horizontal wells, and 25% of the infiltration galleries. The mean levels in positive water samples was 8 cysts/100 L (range = 0.1 to 120/100 L).

Table 3: Protozoal status of water samples concerned of trofozoites and cysts of Giardia spp. and Balantidia spp.

Study Area	Water Sample	Giardia	Balantidia	Prevalence percentage
60 Feet	$S_1 R_1$	Nili	Nil	0
	$S_1 R_2$	Nil	Nil	
	$S_1 R_3$	Nil	Nil	
	$S_1 R_4$	Nil	Nil	
Mirpur-1	$S_2 R_1$	Nil	Nil	0
	$S_2 R_2$	Nil	Nil	
	$S_2 R_3$	Nil	Nil	
	$S_2 R_4$	Nil	Nil	
Mirpur-2	$S_3 R_1$	Nil	Nil	0
	$S_3 R_2$	Nil	Nil	
	$S_3 R_3$	Nil	Nil	
	$S_3 R_4$	Nil	Nil	
Kalshi	$S_4 R_1$	Nil	Nil	0
	$S_4 R_2$	Nil	Nil	
	$S_4 R_3$	Nil	Nil	
	$S_4 R_4$	Nil	Nil	
SAU Campus	$S_5 R_1$	Nil	Nil	0
	$S_5 R_2$	Nil	Nil	
	$S_5 R_3$	Nil	Nil	
	$S_5 R_4$	Nil	Nil	

Physicochemical parameters of water sample

Water pH

The pH is a scale used to quantify the acidity or alkalinity of water. It is a key parameter of the water bodies that affects solubility and may lead to an increase or decrease in the toxicity. The pH of all water samples lies in the range of 6.70 ± 0.14 to 6.13 ± 0.05 (Table 4 and Figure 2). The sample collected from Mirpur-2 contained (6.70 ± 0.14) the highest amount of pH which was statistically different from all other sources. The lowest amount of water pH was found (6.13 ± 0.05) in 60 feet area which was statistically identical with the sample collected from Mirpur-1 and followed by sample collected from Kalshi (6.40 ± 0.08) and SAU campus (6.46 ± 0.10).

Table 4: pH in drinking water supplied in dairy farms at different sites of Dhaka city.

Water from different sources	Water pH			
	Mean ± SD (n=4)	SE Mean	Min	Max
60 Feet Area	$6.13^c \pm 0.05$	0.03	6.10	6.20
Mirpur-1	$6.20^c \pm 0.00$	0.00	6.20	6.20
Mirpur-2	$6.70^a \pm 0.14$	0.07	6.60	6.90
Kalshi	$6.40^b \pm 0.08$	0.04	6.30	6.50
SAU Campus	$6.46^b \pm 0.10$	0.05	6.40	6.60
LSD (0.05)	0.13			
CV (%)	1.37			

In a column, means with a similar letter (s) are not significantly different and means with different letter (s) are significantly different by LSD at 5% level of significance. Where, SD= Standard deviation; CV= Coefficient of Variation; LSD= Least significant different; SE= Standard Error; Min= Minimum and Max= Maximum.

Willms *et al*. (2002) was found water pH from different sources ranges from 9.00 ± 0.04 to 8.46 ± 0.24 that was higher than our findings. Fakayode (2005) also showed pH values in water ranges from 6.37-6.83 that was similar with our investigations. Brew *et al*. (2009) reported water analysis result from three different sources such as Fountain,

tank and pond water also contained different p^H that was 7.1, 7.0 and 7.4 respectively. The livestock can support variations of pH between 6.5-8.5 unit (Curran *et al.*, 2007). The dairy cow prefers water with pH between 6.0-8.0 unit (Olkowski, 2009). Exceeding this threshold, the water has lye taste, and under 6.5 pH the taste of water is acidulous-prickly due to of humic acids, mineral and especially due to the presence of carbon dioxide in high quantity (Man, 1989, 2007; Draghici, 2001; Popescu, 2010).
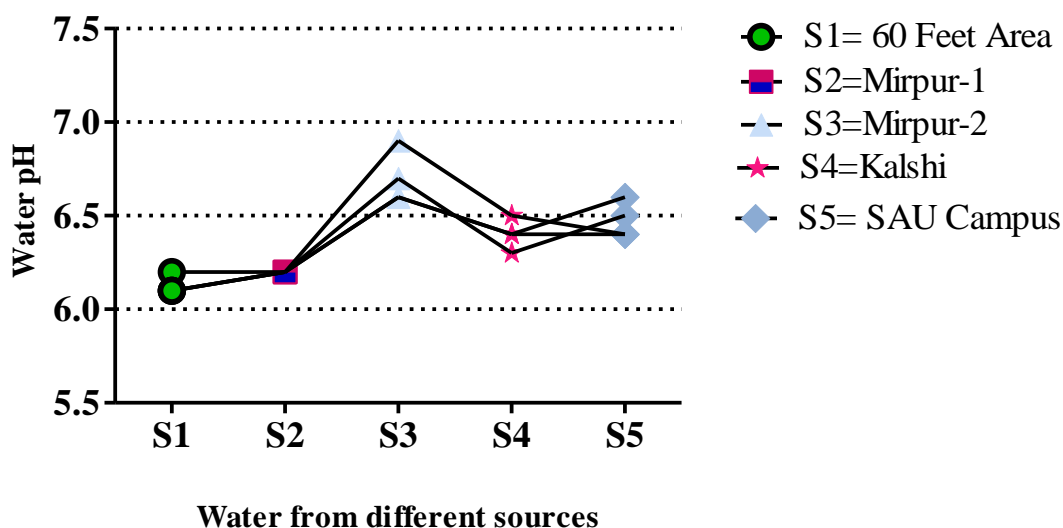

Figure 2: Level of pH in water in different sites of study area.

Total Dissolved Solid

The amount of TDS in water supplied from different sites of Dhaka city ranges 397.00 ± 6.27 to 307.50 ± 6.46 (Table 5 and Figure 3). The sample collected from 60 feet area contained (397.00 ± 6.27) the highest amount of TDS which was statistically identical with the sample collected from Mirpur-2(392.50 ± 6.41ppm). The lowest amount of TDS was observed (307.50 ± 6.46ppm) in the sample collected from SAU Campus which was followed by the water collected from Mirpur-1 (363.00 ± 6.78ppm) and Kalshi (363.00 ± 2.45ppm) Water with a high TDS indicates more ionic concentration, which is of inferior palatability and induce an unfavorable physicochemical reaction in the consumers (Reshu, 2017). Threshold concentration of TDS is 2500 ppm and the limiting concentration is 5000 ppm (Beede *et al.*, 2008). The water with salinity under 1000 ppm is consider safe, higher values having negative effects on health status and animal products(N.R.C., 2001).

Table 5: TDS in drinking water supplied in dairy farms at different sites of Dhaka city.

Water from different sources	Total dissolved solids (TDS) in Water			
	Mean ± SD (n=4)	SE Mean	Min	Max
60 Feet Area	397.00[a] ± 6.27	3.14	390.00	405.00
Mirpur-1	363.00[b] ± 6.78	3.39	355.00	370.00
Mirpur-2	392.50[a] ± 6.41	3.23	385.00	400.00
Kalshi	363.00[b] ± 2.45	1.22	360.00	365.00
SAU Campus	307.50[c] ± 6.46	3.23	300.00	315.00
LSD (0.05)	8.91			
CV (%)	1.62			

In a column, means with a similar letter (s) are not significantly different and means with different letter (s) are significantly different by LSD at 5% level of significance. Where, SD= Standard deviation; CV= Coefficient of Variation; LSD= Least significant different; SE= Standard Error; Min= Minimum and Max= Maximum.

Several studies have shown that TDS between 4000 to 5000 ppm negatively affect daily average gain, decrease milk production in lactating cows which cause a reduction in weights at calves at weaning (Dyer, 2012). When TSD value is between 4400-6000 ppm cattle has lower weight gains than cattle drinking normal water (TDS = 1300 ppm), only in conditions where, content of energy from feeds is low during heat stress. Researchers (Sharma *et al*., 2017; Kewalramani *et al*., 2018; Tausifi *et al*., 2018) also indicated that cattle consumed for high salinity water decreased their weight and milk production compared to those drinking natural water. high saline water consumption of pregnant and lactating cattle also warns because of its negative impact on the health of animals and the fetus(Al-Saffawi *et al*., 2020)
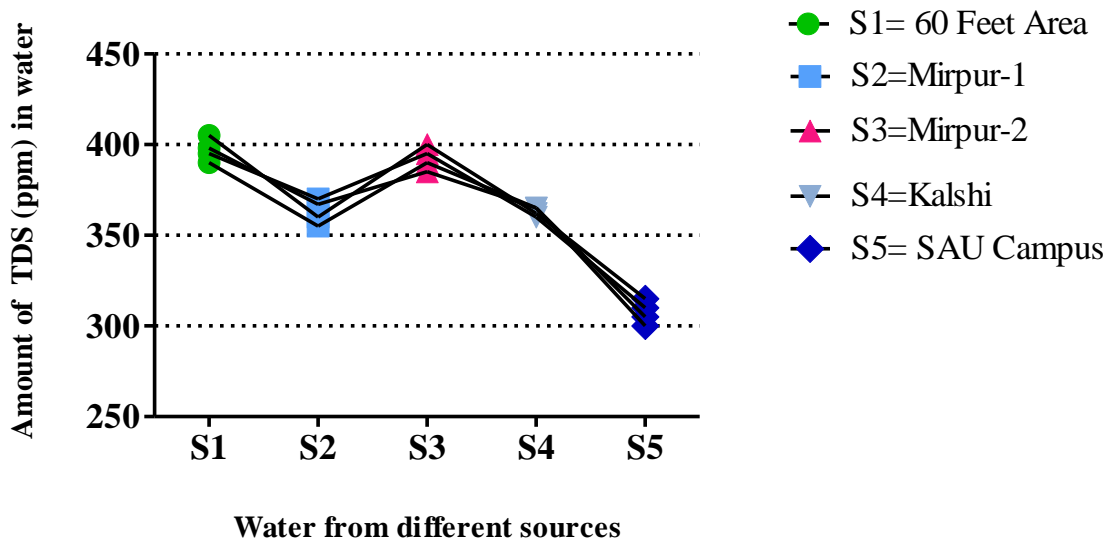
Figure 3: Concentration of TSD in water in different sites of study area.

Total Hardness:

Total hardness means all calcium and magnesium salts that are found in water and is considered overall indicator of water mineralization. Hardness of water supplied from different sites of Dhaka city ranges 165.00 ± 2.45ppm to 75.00 ± 4.08ppm (Table 6 and Figure 4). Statistically the highest amount of hardness was observed in the water sample collected (165.00 ± 2.45ppm) from Mirpur-2 which was statistically similar with the sample collected (158.25 ± 7.09ppm) from Mirpur-1 whereas, the lowest amount of hardness was observed in the water sample collected from Kalshi (75.00 ± 4.08pmm). According to El Mahdy *et al*. (2016), the water can have divided in soft water (0-60), semi hard (6-120), hard water (12- 180) and very hard water (over 180) by considering the total hardness. Breede (2006) showed that according to National Academy of Science average Total hardness in water is 208ppm whereas, expected level is 0-180ppm.

Table 6: Hardness (CaCO₃) in drinking water supplied in dairy farms at different sites of Dhaka city.

Water from different sources	Hardness ($CaCO_3$) in Water (ppm)			
	Mean ± SD (n=4)	SE Mean	Min	Max
60 Feet Area	$151.50^b \pm 13.96$	6.9821	138.00	165.00
Mirpur-1	$158.25^{ab} \pm 7.09$	3.5444	153.00	168.00
Mirpur-2	$165.00^a \pm 2.45$	1.2247	162.00	168.00
Kalshi	$75.00^c \pm 4.08$	2.0412	70.00	80.00
SAU Campus	$149.25^b \pm 3.77$	1.8875	144.00	153.00
LSD (0.05)	11.32			
CV (%)	5.37			

In a column, means with a similar letter (s) are not significantly different and means with different letter (s) are significantly different by LSD at 5% level of significance. Where, SD= Standard deviation; CV= Coefficient of Variation; LSD= Least significant different; SE= Standard Error; Min= Minimum and Max= Maximum.
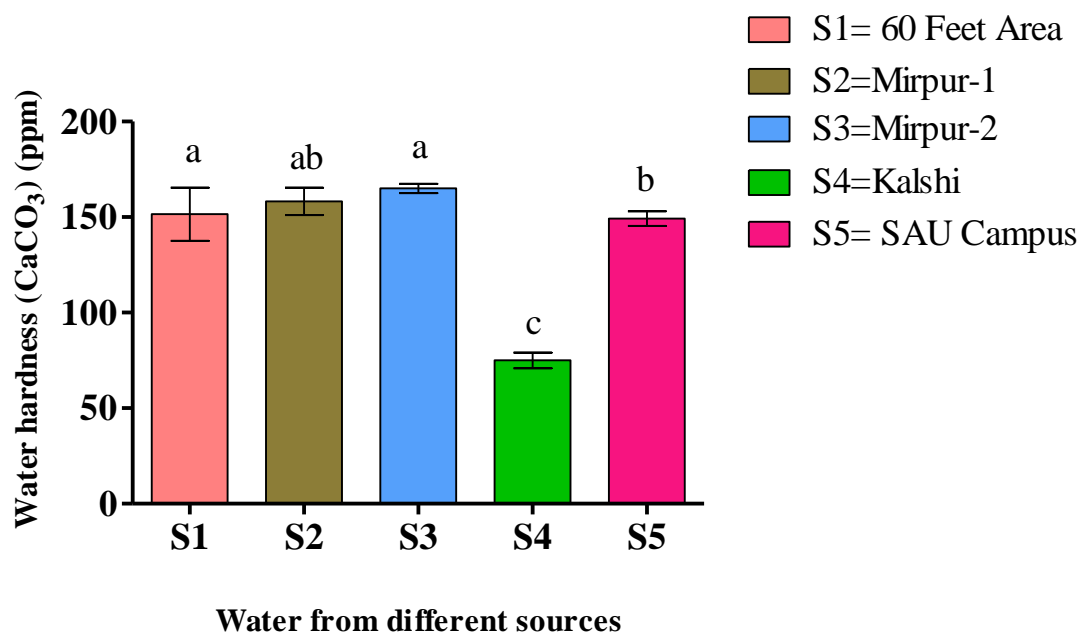

Figure 4: Level of total hardness in water in different sites of study area.

Chloride content:

Concentration of chloride in water supplied from different sites of Dhaka city ranges 0.95 ± 0.06ppm to 0.38 ± 0.03ppm (Table 7 and Figure 5). Statistically the highest concentration of chloride was observed in the water sample collected (0.95 ± 0.06ppm) from 60 feet area which was followed by the sample collected (0.67 ± 0.02ppm) from SAU Campus whereas, the lowest concentration of chloride was observed in the water sample collected from Mirpur-2 (0.38 ± 0.03pmm) which was also followed by Kalshi (0.52 ± 0.02) and Mirpur-1 (0.52 ± 0.03) respectively. According to National Academy of Science average Chloride in water is 20 ppm whereas, expected level is 0-250ppm. Free or residual chlorine concentrations up to 0.5 to 1.0 ppm have not affected ruminants adversely. Municipal water supplies with 0.2 to 0.5 ppm have been used successfully. Chlorine in farm systems with short contact time have caused no apparent problems for cattle (Breede, 2006).

Table 7: Chloride in drinking water supplied in dairy farms at different sites of Dhaka city.

Water from different sources	Concentration of Chloride (ppm)			
	Mean ± SD (n=4)	SE Mean	Min	Max
60 Feet Area	$0.95^a \pm 0.06$	0.03	0.89	1.00
Mirpur-1	$0.52^c \pm 0.03$	0.02	0.48	0.56
Mirpur-2	$0.38^d \pm 0.03$	0.01	0.35	0.41
Kalshi	$0.52^c \pm 0.02$	0.00	0.50	0.55
SAU Campus	$0.67^b \pm 0.02$	0.01	0.65	0.70
LSD (0.05)	0.06			
CV (%)	6.06			

In a column, means with a similar letter (s) are not significantly different and means with different letter (s) are significantly different by LSD at 5% level of significance. Where, SD= Standard deviation; CV= Coefficient of Variation; LSD= Least significant different; SE= Standard Error; Min= Minimum and Max= Maximum.
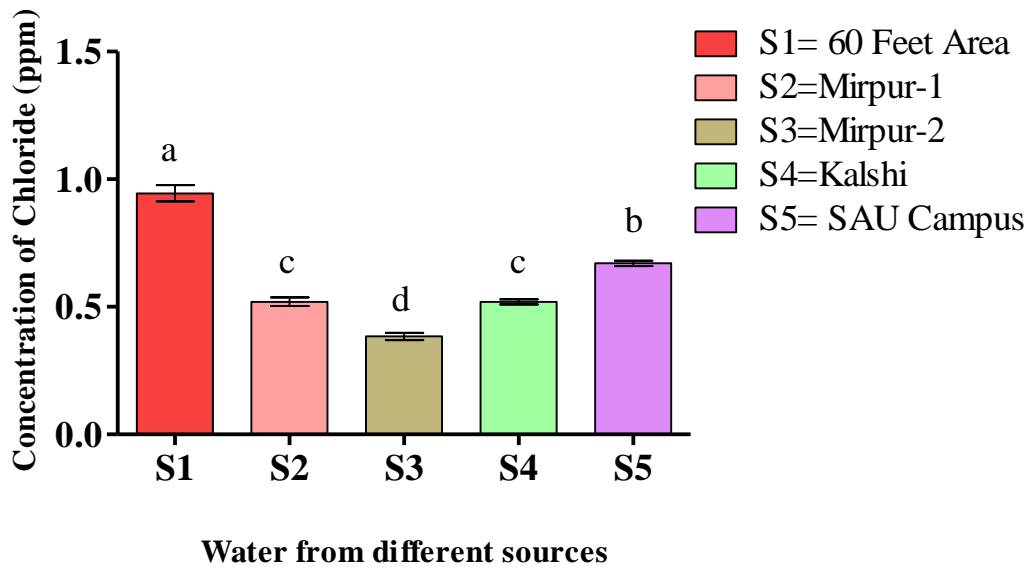
Figure 5: Concentration of Chloride in water in different sites of study area.

Iron content

Concentration of iron in water supplied from different sites of Dhaka city ranges 0.97 ± 0.04ppm to 0.78 ± 0.02ppm (Table 8 and Figure 6). Statistically the highest concentration of iron was observed in the water sample collected (0.97 ± 0.04ppm) from Mirpur-1 which was followed by the sample collected (0.67 ± 0.02ppm) from Kalshi (0.92 ± 0.09) and SAU Campus (0.93 ± 0.08). The lowest concentration of chloride was observed in the water sample collected from Mirpur-2 (0.78 ± 0.02ppm) which was also followed by 60 feet Area (0.85 ± 0.03). This result was higher than the reported value of (El Mahdy, 2016; Swistock, 2012; Higgins, 2008). The concentration is more than 0.3 ppm in drinking water, this may cause problems for cows (El Mahdy, 2016). Amounts greater than 0.3 ppm induce an unpleasant taste of water, which leads to voluntary water consumption decrease concomitantly with the milk production (Swistock, 2012), but, the study performed by Mann *et al*., (2013) reveals the fact that iron intake up to 1.250 mg per day for 14 days is safe for early lactation dairy cattle, not affect the chemical composition of milk but processed milk from those cows was susceptible to flavor changes. The iron in quantities greater than 0.3 has negative impact on absorption of Zn (Higgins, 2008)

Table 8: Iron in drinking water supplied in dairy farms at different sites of Dhaka city.

Water from different sources	Concentration of Iron in Water			
	Mean ± SD (n=4)	SE Mean	Min	Max
60 Feet Area	$0.85^{bc} \pm 0.03$	0.02	0.82	0.89
Mirpur-1	$0.97^{a} \pm 0.04$	0.02	0.92	1.00
Mirpur-2	$0.78^{c} \pm 0.02$	0.01	0.76	0.81
Kalshi	$0.92^{ab} \pm 0.09$	0.05	0.82	1.00
SAU Campus	$0.93^{ab} \pm 0.08$	0.04	0.83	1.00
LSD (0.05)	0.0910			
CV (%)	6.77			

In a column, means with a similar letter (s) are not significantly different and means with different letter (s) are significantly different by LSD at 5% level of significance. Where, SD= Standard deviation; CV= Coefficient of Variation; LSD= Least significant different; SE= Standard Error; Min= Minimum and Max= Maximum.
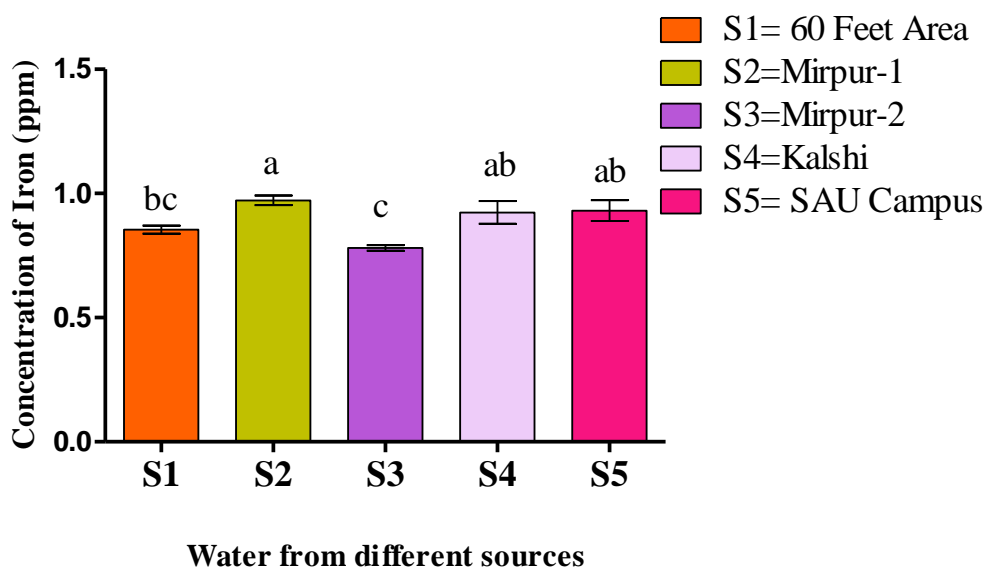

Figure 6: Concentration of Iron in water in different sites of study area.

CHAPTER V

SUMMARY AND CONCLUSION

As a potential part of livestock, dairy farming is a rapidly growing sector having a bright prospect. Drinking water is one of the most vital factors to maintain good health, production and physiology. This study was conducted to know the quality of drinking water supplied to the dairy farm in some selected area of Dhaka city. The research was conducted at laboratory of Medicine and public health department (SAU), and ACI Animal Health Diagnostic Laboratory. Duration of this study was from June, 2020 to November, 2020. Water samples were collected from dairy farm of five sites of Dhaka city like 60 Feet area, Mirpur-1, Mirpur-2, Kalshi and SAU campus farm. Samples were collected in two sets, one set for determination of microbiological quality (Coliform, E. coli, Giardia and Balantidia) and another set to determine physicochemical quality (pH, TDS, Hardness, Iron and Chloride) in ACI animal health diagnostic laboratory.

To know the load of Coliform bacteria firstly the water samples are incubated overnight in nutrient broth and turbidity confirmed the bacterial presence. Then stock samples from nutrient broth were cultured in McConkey agar media. After overnight incubation reddish to pinkish, brown and golden color colonies were found those were coliform bacteria (Plate 4). All the water samples collected from 60 Feet area, Mirpur-1, mirpur-2, Kalshi and SAU campus farm contained coliform and prevalence were 100% (Table 1). The load (CFU/ml) of coliform bacteria in water ranges from 1.73×10^8 to 2.63×10^8 which were higher than the threshold level for safe drinking water. The highest load of coliform found in the water of mirpur-1 and the lowest coliform was found in the water of SAU campus farm (Table 2).

In a similar way to the E. coli status, 10 fold diluted stock samples were cultured in EMB agar media. after overnight incubation greenish metallic sheen colonies were found which were characteristic for E. coli in EMB agar. For confirmation Catalase test and Methyl red test were performed which provided positive results. The prevalence of E. coli was 100%. The load (CFU/ml) of E. coli bacteria ranges from 5.50×10^7 to 7.05×10^7 which exceeded the safe limiting level. The lowest E. coli was found in the water of SAU campus farm and the highest value observed in mirpur-1(Table 2).

To determine the presence or prevalence of Giardia and Balantidia in water of study area floatation techniques were performed and slides were observed under compound microscope but water samples of each site showed no cysts or trofozoites of *Giardia spp.* and *Balantidia spp.* So, the prevalence percentage was zero (Table 3).

The pH of all water samples lies in the range of 6.70 ± 0.14 to 6.13 ± 0.05. The sample collected from Mirpur-2 contained (6.70 ± 0.14) the highest amount of pH which was statistically different from all other sources. The lowest amount of water pH was found (6.13 ± 0.05) in 60 feet area. The sample collected from 60 feet area contained (397.00 ± 6.27) the highest amount of TDS. The lowest amount of TDS was observed (307.50 ± 6.46ppm) in the sample collected from SAU Campus. Hardness of water supplied from different sites of Dhaka city ranges 165.00 ± 2.45ppm to 75.00 ± 4.08ppm. The highest amount of hardness was observed in the water sample collected (165.00 ± 2.45ppm) from Mirpur-2. The lowest amount of hardness was observed in the water sample collected from Kalshi (75.00 ± 4.08pmm). Concentration of chloride in water supplied from different sites of Dhaka city ranges 0.95 ± 0.06ppm to 0.38 ± 0.03ppm. Statistically the highest concentration of chloride was observed in the water sample collected (0.95 ± 0.06ppm) from 60 feet area. The lowest concentration of chloride was observed in the water sample collected from Mirpur-2 (0.38 ± 0.03pmm). Concentration of iron in water supplied from different sites of Dhaka city ranges 0.97 ± 0.04ppm to 0.78 ± 0.02ppm. Statistically the highest concentration of iron was observed in the water sample collected (0.97 ± 0.04ppm) from Mirpur-1. The lowest concentration of chloride was observed in the water sample collected from Mirpur-2 (0.78 ± 0.02ppm).

The overview of the study areas is like that the water of 60 feet area contains highest concentration of TDS and chloride where pH concentration is lowest. The water of Mirpur-1 is highest in concentration of Coliform, E.coli, and iron.

The compendium of this study illuminated that the water quality of study sites in Dhaka city is over all good except the consideration of bacteria and iron. The water of Mirpur-1 contains highest level of Coliform and E. coli bacteria and the water of SAU campus contains lowest level of these bacteria, though both sources are above the safe level for cattle.

In case of physicochemical parameters pH, hardness, TDS and chlorides are ranges within safe level in all the study sites of Dhaka city. But Iron concentration is higher

than the expected level in all study areas, specially Mirpur-1 contains highest level of Iron among the study sites.

CHAPTER VI

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