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MICROBIAL QUALITY OF RAW COW MILK AND ASSOCIATED FACTORS ALONG THE DAIRY VALUE CHAIN IN JIMMA ZONE, SOUTHWEST ETHIOPIA

A MASTER THESIS SUBMITTED TO DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCE AND TECHNOLOGY, FACULTY OF PUBLIC HEALTH, INSTITUTE OF HEALTH, JIMMA UNIVERSITY; IN PARTIAL FULFILLMENT FOR THE REQUIREMENT OF MASTERS IN ENVIRONMENTAL HEALTH SCIENCE

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Jimma, Ethiopia

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Approval sheet

As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared under our guidance by Leykun Berhanu entitled as "Microbial quality of raw cow milk and associated factors along the dairy value chain in Jimma zone Southwest, Ethiopia". We recommended that it could be submitted as fulfilling the thesis requirement.

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As member of the board of examiners of the MSc. thesis open defense examination, we certify that we have read and evaluated the thesis prepared by Leykun Berhanu and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirement for the degree of Master of Science in Environmental health Science.

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External Examiner   Signature   Date

Declaration

I declare that this thesis is my original work which has not been presented in any institution anywhere for the award of any academic purpose.

_____   _____   _____

Name   Signature   Date

ABSTRACT

Background: *Raw milk is a natural product of mammals used as food by human beings that does not undergo heat treatment. It may contain pathogenic microorganism which can seriously affect the health of individuals.*

Objective: *To determine the microbial quality of raw cow milk and associated factors along the dairy value chain in Jimma zone, Southwest, Ethiopia, 2018.*

Method: *A cross-sectional study design was employed from April to May 2018. A total of 150 milk samples and 300 environmental samples were collected randomly from dairy farms, milk distribution centers and milk retailer outlets found in selected district towns and town administrations of Jimma zone. The total mesophilic aerobic bacteria, E. coli O157:H7, total and fecal coliform bacteria were analyzed. One representative milk handler from each milk production stages was interviewed to assess the knowledge, attitude and practice of milk handlers by using pretested structured questionnaire. Descriptive statistics and multiple linear regression model were used to analyze the data.*

Result: *the mean total mesophilic aerobic bacterial count at dairy farm, milk distribution centers and milk retailer outlets were 4.96±0.59, 6.29±0.19 and 7.25±0.14 log CFU/ml respectively. There was statistically significant difference (p<0.05) among the milk mean total mesophilic aerobic bacterial count along the supply chain and their respective mean coliform bacteria were 3.49±1.71, 3.75±2.74 and 6.85± 0.30 log CFU/ml respectively. There was statistically significant difference (p<0.05) among the milk coliform count of raw milk along the supply chain. The mean fecal coliform count of water sample in dairy farms, milk distribution centers and milk retailer outlets were 4.04±0.34, 3.72±0.53 and 2.20±0.51 log CFU/ml respectively. The mean coliform count of milk contact surfaces at dairy farm, milk distribution centers and milk retailer outlets were 4.61±0.38, 4.71±0.52 and 4.75±0.51 log CFU/cm² respectively. The overall mean score of knowledge, attitude and practice of milk handlers were 62.44%±11.53, 57.98%±9.22 and 57.42%± 10.78 respectively.*

Conclusion and recommendation: *Milk quality in terms of microbial counts seems to be significantly decreased after sending off by farmers. About one third of the analyzed samples classified as unacceptable microbial quality. Educational status and attitude of milk handlers and the quality of water used to wash milk contact surfaces and hands of milk handlers were the major factors affecting the microbial quality of raw cow milk in the study area. Hence, measures should be taken to enhance the knowledge and attitude of milk handlers and to improve the quality of water used in the milk processing to prevent consumers from milk borne illness.*

Key words*: Jimma zone, Dairy value chain, milk microbial quality, total bacterial count, water quality, milk handler.*

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ABBREVIATIONS

ANOVA	Analysis of Variance
B. cerus	*Bacillus cerus*
C. jejuni	*Campylobacter jejuni*
CDC	Center for Diseases Control
CFU	Colony Forming Unit
CFS	Center for Food Safety
CFU/ml	Colony Forming Unit per milliliter
cm^2	Centimeter Square
CSA	Central Statistical Agency
E. coli	*Escherichia coli*
EMB	Eosin Methylene Blue
EU	European Union
KAP	Knowledge, Attitude and Practice
L	liter
MCC	Milk Collection Center
mL	milliliter
PCA	Plate Count Agar

S. aureus	*Staphylococcus aureus*
S. Typhimurium	*Salmonella Typhimurium*
SCC	Somatic Cell Count
SD	Standard Deviation
SPSS	Statistical Package for Social Science
STEC	Shiga Toxin producing *Escherichia coli*
TBC	Total Bacterial Count
TMABC	Total Mesophilic Aerobic Bacterial Count
TPC	Total Plate Count
TVC	Total Viable Count
US FDA	United States Food and Drug Administration
US	United States
WHO	World Health Organization

CHAPTER ONE

1. INTRODUCTION

1.1 Back ground information

Raw milk is a natural product of mammals used as food by human beings that does not subjected to any processing intended to alter the quality or composition (Soboleva, 2013). Milk contains essential components including water, carbohydrate, all the B vitamins, vitamin A and D and calcium and phosphorous. Casein accounts more than three-fourth of the milk protein. It contains calcium phosphate, which is a very important nutrient for human and animals. Milk can be obtained from cow, goats, sheep and other mammals. Cow milk, buffalo, goats and sheep milk accounts for 91%, 5.9%, 1.6% and 1.7% of the world's total milk production respectively (Eping, 2006).

As milked from the healthy cow, milk is very low in bacterial numbers, but the bacterial count increased in any stages in the production process. The major source of contamination is fecal contamination from soiled animal bodies, bacterial contamination from poor milking practices such as soiled hands, soiled equipment, and failure to detect mastitis pathogen, physical contamination from dungs, insects and animal hair. When the counts of bacteria become high it produces enzyme that degrade protein, fats and other components of milk which results in deterioration of the quality of milk (Food Standard Agency, 2006).

Pathogenic microorganism can get access to the raw milk into two major ways. The first one is endogenous contamination which relates to the health of the animal. This may be occurred from contact of the milk from the blood of the animal or due to the presence of microorganism causing mastitis in the udder of the cow. The other one is exogenous contamination where milk contamination occurs from the external environment. For instance soiled animal's body, poor milking practice and failure to clean teats prior to milking (C, Verraes *et al*., 2015).

The care taken during transfer and storage of raw milk also determines the load of microbes in raw milk. To do so, milk must be cooled immediately to not more than 8℃ when it collected daily, or not more than 6℃ if collection is not daily to minimize bacteria multiplication. The barn and the milking area should be clean to avoid contamination of raw milk. Sufficient clean water must also be available in the milking area for the cleaning of animal teats and udder, milk contact equipment, floors and hands of the milker. Milk handlers must wear clean clothes. Their hands must be thoroughly washed before milking (Food Standard Agency, 2013).

Ethiopia is enriched with a cattle population in Africa. But the milk yield is low as compared with other countries. The contribution to the national economy is reduced from time to time. Due to this reason, the national milk consumption is very low compared with other developing countries. From the total milk produced in the country three- fourth is consumed locally. Milk production, transportation, storage and marketing are traditional and constrained by multiple problems (Misganaw *et al.*, 2017).

Likewise, in Jimma Zone, the current livestock output is not promising to supply the required quantity of milk for the geometrically increasing human population. The livestock production system is mixed crop-livestock production system where livestock production is totally based on local breed cows with no improved management and low output. The average quantity of milk produced per one lactation period of the cow is 203.29±4.75 liters (Hussen *et al.,* 2015).

The microbiological quality of raw milk is still under investigation by many individuals and public health institutions around the world. The ever increasing of raw milk consumer encourages investigators to deeply assess the microbial quality of raw milk to protect consumers from milk borne illness (Soboleva, 2013).

1.2 Statement of the problem

Raw milk can support the growth of pathogenic bacteria that can seriously affect the health individuals who drinks raw milk or milk products made of raw milk. Salmonella, *Escherichia coli (E. coli)* and Listeria are the major pathogenic bacteria responsible for the occurrence of milk borne illness among raw milk consumers. Center for Diseases Control (CDC) reported that, 1500 people became sick in the United States (US) during the fourteen year period. Also CDC find out that an individual who consume raw milk has 150 times more likely to be infected by milk borne illness than those who do not drink. In 2004, selling raw milk was legal in 22 states of the US. Most out breaks (81%) happened in states where selling raw milk was legal. It indicates raw milk is highly linked with milk borne illness (US FDA, 2012).

The level of illness due to consumption of raw milk can be very serious or even fatal. Pasteurization reduces such harmful effect of raw milk. But inadequate pasteurization and post pasteurization contamination may contribute for the occurrence of illness, even it results less number of illnesses than unpasteurized dairy products. Bacteria may be introduced into the milk during milking, transportation, during storage and/or during preparation, which can cause illness when consumed (Heidinger, 2009). Furthermore, many environmental factors may contribute for the reduced quality of milk and these vary from time to time and form one farm to other (Schutz, 2012).

In Pennsylvania, two persons who consume raw milk were confirmed to be infected with Salmonella enterica serotype Typhimurium. The Pennsylvania Department of Agriculture received additional reports of milk borne illness. After analyzing for pathogenic bacteria the raw-milk bulk tank yielded Salmonella Typhimurium (S. Typhimurium) (CDC, 2007).

The epidemiological investigation from Butler County Health Department showed that, among eighteen ill individual, seven cases were confirmed and eleven were suspected for campylobacteriosis. Diarrhea, abdominal cramp, myalgia, chills and fever were the most dominant symptoms. The laboratory analysis also showed that five of the cases were culture positive for *Campylobacter jejuni (C. jejuni)*, two individuals were positive for campylobacteriosis and eleven individuals were asymptomatic (Dement and Tubach, 2011).

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In Ghana higher exposure and probability of illness was reported when raw milk was consumed and the least when boiled milk consumed showed the advantage of boiling milk to reduce milk borne illness (Appiah, 2012). Also in Abidjan, Cote D'Ivoire of 14 individuals who consumed raw milk the probability of exposure to *Bacillus cerus (B. cerus)* and other milk borne illness was high. After milk consumed about 13 of them reported that they encountered a food borne illness indicating that the milk produced represents a risk to consumers (B.A, Yobouet, *et al* 2014*)*.

Similarly, in Abidjan, Cote D'Ivoire, the proportion of raw milk consumption was 51.6% in people who consume milk. Of which 29.9% of the consumed milk failed to meet microbiological standards which are consumed by 652 individuals. A microbiological study showed that 7.2% of samples taken from milker's hand, 4.4% of water used to rinse milk container or milk utensils, 4.4% of environmental samples, 13.2% of samples from milk utensils and 4.9% of the sample from a cow's udder were contaminated with one or more pathogenic bacteria. About 624.6 liter (L) of raw milk would need to be discarded per day if discarding was chosen as the option of risk reduction. This resulted in a potential loss of €623.9 per day (S.M, Kouamé-Sina *et al*., 2014).

In Ethiopia, raw cow milk is consumed more than processed milk. Some perceived that raw milk contains better nutrients than pasteurized milk other believe that raw milk helps to threat gastrointestinal problems. But the microbiological quality of raw milk, knowledge and hygienic status of the communities with respect to production of raw milk was not assessed as well (Tadesse and Bacha, 2014).

In the previous years, few studies were done in the Jimma zone to assess the microbial quality of raw cow milk. But none of them focused on the quality of raw milk at different stages of the milk production process. The microbial quality of water used to wash milk equipment and the quality of milk contact surface is not studied at all. The knowledge, attitude and practice of those persons who have engaged in milk handling activity were not assessed. Moreover, there is no study done on the different factors affecting microbial quality of raw cow milk in Jimma zone.

Thus to fill these gaps, this study is intended to determine the microbial quality of raw milk and its contributing factors at different stages of the milk production process in selected district towns and town administration of Jimma Zone.

1.3 Significance of the study

The finding of the study will provide baseline information on milk microbiological quality and associated factors in Jimma zone. The finding of this study will be used by Jimma zone livestock and fish resource development offices, dairy enterprises, Zonal health bureau, agricultural offices and other concerned bodies to improve milk quality and protect raw milk consumers from milk borne illness.

Furthermore the finding of the study will provide valuable data for risk assessors who wants to estimate the risk of acquiring an illness due to consumption of raw milk.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Milk production and consumption pattern

International dairy federation reported that, in 2015 the global milk production was about 630 million tons, including milk from cow, buffalo and other type of milk. Asia represents the highest world's cow milk production share (29%), followed by the EU (European Union) 24%, north and central America 18%, South America 10%, and Africa, Oceania and other European country represent the least (5%) (IDF, 2016). Agriculture, forestry and fishery statistics also showed that, in 2016 the EU produced approximately 168.3 million tons of milk. From the total quantity of milk produced, cow milk accounts for about 97% while milk from other animals represented 3.1% (Jortay, 2017).

In 2015, Turkey produced 18.6 million tons of milk from cow, sheep, goat and buffalo with no change in 2014 production. Yogurt and white cheese were the most consumed dairy products. Relatively consumption of drinking milk is too low and most of consumed drinking milk is whole milk. The per capita consumption of raw milk in Turkey was 236 kg and it reduced by 84% when compared to 2014 (Duyum, 2016).

In northeastern India, the quantity of raw milk and milk product produced was negligible compared to other countries. The cattle population accounts only 6.91% of the country's cattle population. The number of cattle population has been declining in the last few years. The region has shared only 0.93 percent of the total milk produced in the country during 2012-2013. Consumption of milk and its product is minimal due to food habit and low availability of milk. The milk consumption rate in northeastern India was less than 8% of the total food requirement (Lalrinsangpuii and Lalrengpuii., 2016).

In peri-urban areas of Mali, Bamako, about 64% of the study subjects drink raw milk and 13% of them drink raw milk with bread. For those individuals who consume raw milk, freshly drawn raw milk was preferred by about 12% of the study subjects, 87% of the study subjects

did not consume raw milk. The milk consumption pattern is different between rich and poor. As shown in this study, only 25% of the richest individuals consume raw milk while those poor persons consume these products regularly (Hetzel *et al.*, 2005).

In Debre Zeit, Ethiopia, the daily milk consumption rate was 500 milliliter (ml) per person per day. 2940L of raw milk was sold for food and drink establishments while the 1960 L of milk was sold to individuals. The dairy farmers also consume equal volume of milk in their home (K. Makita *et al.*, 2012).

In western Oromia, the maximum milk production from local cow was 3.3 L per day in Ambo, and the minimum milk production of 1.2 L was recorded in Gimbi. But from cross breed cows, the highest production was 9.3 L in Jimma town and the lowest was 4 L per day in Bedele. From the overall quantity of milk produced, about 8% was consumed as whole milk. Due to the high number of cross breed cows more milk is consumed in Jimma than in Gimbi. In Jimma about 56% of the study subjects gave first priority in milk consumption to children, but 65% of the dairy farmers from Dembi dolly reported that the priority is given for family head. Wives are also given priority by 48% and 40% of the respondents from Bedele and Jimma respectively (Galmessa *et al.*, 2013).

In the Jimma zone, the amount of milk produced from local breed and cross breed cow was 7.01 L and 19.3 L respectively. About 73% of the farmer believe that the demand for milk is high. 63% of the dairy farmers are not licensed for dairy business. This study also showed that, educational level and milk consumption is associated with that educated individuals consume more raw milk than un-educated or less educated (Tadesse, 2016).

2.2. Microbial quality of raw cow milk along the dairy value chain

Microbiological study in Bangladesh showed that, the highest total viable count (TVC), total coliform count and staphylococcus counts were 5.894±0.221, 2.832±0.129 and 2.898±0.162 log colony forming unit per milliliter (CFU/ml) respectively. The highest prevalence of *E. coli* was observed during the study (R, Khaton *et al.,* 2014). A similar study done in Bangladesh

also revealed that, out of 35 milk samples analyzed, 16(47%) of them were contaminated with
E. coli ((10^4 to 10^6 CFU/ml) (Yasmin *et al.*, 2015).

A study done in Madurai, South India revealed that, the mean total plate count, *Staphylococcus
aureus (S. aureus)*, coliform and *E. coli* O157:H7 were 7.1, 3.80, 4.94 and 3.53 log CFU/ml
respectively. Also *S. aureus*, *E. coli* and salmonella were detected from more than 62%, 65%
and 13.3% of the sample respectively (Lingathural and Vellathurai, 2010).

A study done in Pakistan showed that, among 120 milk sample collected from dairy farms and
milk retailer shops the mean TVC were 5.03 and 5.11 log CFU/ml respectively. The study
also showed that, the mean coliform bacteria at the dairy farm and milk retailer outlets were
4.66 and 4.70 log CFU/ml respectively. The mean TVC and coliform count was significantly
different along the dairy farm and milk selling points (Shah *et al.*, 2016).

A study done in Western Zambia showed that, among 86 milk sample analyzed, total bacteria
count, *S. aureus* and *E. coli* counts were observed in 5%, 22% and 13% of the analyzed
samples respectively. Initially the milk was good quality with a mean total bacterial count of
2.63 log CFU/ml. But at selling point this count exceeds log 5.77 CFU/ml. Also, this study
showed that, storing milk in the refrigerator for a long period of time causes the presence of
pathogenic bacteria in all of the analyzed samples (Knight-jones *et al.*, 2016).

In Tanzania, the overall total bacterial and total coliform count were 7 and 6.04 log CFU/ml
respectively (Msalya, 2017). A similar study done in Arusha, Tanzania showed that,
salmonella and *E. coli* were prevalent in 37% and 91% of the analyzed samples respectively.
The mean *E. coli* count at milk producer, distributor and outlet shops were 3.48, 3.90 and 3.82
log CFU/ml respectively (Lubote.,*et al*. 2014).

A study conducted in Rwanda showed that, the mean total bacterial count (TBC) of
transporters, milk collection centers (MCC) and kiosk samples were 5.83, 6.18 and 6.99 log
CFU/ml respectively. The increase in bacterial load was statistically significant, indicating a
general trend of decreasing quality from farm to milk retailer outlets (Doyle *et al.*, 2015).

Another study done in Khartoum and Omdurman, Sudan showed that, the average values of TVC were 9.29 ± 0.66 and 8.23 ± 0.76 log10 CFU/ml for Omdurman and Khartoum respectively. The number of coliform bacteria and *S. aureus* counts were 7.11±0.07 and 7.08±0.54 log CFU/ml in Omdurman town and 6.61 ± 0.74, 6.91 ± 0.78 log CFU/ml in Khartoum respectively. Considering the distribution channel, milk on pickup trucks was highly contaminated with TVC, coliform bacteria and *S. aureus count* of 9.22 ± 0.64, 7.21 ± 0.25 and 7.37 ± 0.57 log CFU/ml respectively. While on donkey carts the TVC, coliform bacteria and *S. aureus* counts were 8.82±0.84, 6.65±0.89 and 6.86±0.81 log CFU/ml respectively. On a dairy farm, TVC, coliform count and *S. aureus* were 9.06±0.64, 6.72±0.54 and 6.77±0.45 log CFU/ml respectively (Rahamtalla *et al*., 2016).

A similar study conducted in three states of Khartoum showed that, 63% of the analyzed samples were *E. coli* positive. From twenty samples 45%, 50% and 60% contaminated samples were detected from Khartoum, Khartoum North and Omdurman farms respectively, with coliforms ranging between 3.86±0.1, 4.18±0.01 and *E. coli* between 3.53±0.1and 3.93±0.01(Ali and Abdelgadir, 2011).

A study conducted in Burkina Faso showed that, the milk quality was good with a mean TVC of log 1 to log 4 CFU/ml from the cow. But at farm level the count reaches to log 6 CFU/ml. Somatic cell count (SCC) did not show significant variation at different stages of the milk production process. Higher pH and lower milk fat and lactose contents were found in market bucket milk than in farm and processing unit tank milks (V, Millogo *et al.*, 2010).

A study done in Algeria showed that, the mean total bacterial count in a sample collected from the dairy farm, MCC and milk selling points were 6.73±0.25, 6.81±0.19 and 7.2±1.05 log CFU/ml respectively (Titouche *et al.*, 2016). A similar study done in the Eastern region of Morocco revealed that, among 80 samples collected from MCC the mean total plate count, total coliform count, fecal coliform and staphylococcus count were 6.15, 3.41, 2.28 and 3.23 log CFU/ml respectively. Comparing the value with the country microbial limit for food indicated that, three-fourth of the analyzed samples were unsatisfactory quality in terms of

total mesophilic aerobic bacterial count. Also about half and 21% of the analyzed samples were unsatisfactory for fecal coliform and *S. aureus* respectively (Belbachir *et al*., 2015).

A study done in Egypt, Cairo showed that, *E. coil O157:H7* was present in all samples analyzed. This study also confirmed that the microbiological quality of raw milk was poor (Hassan *et al.,* 2015). A similar study done in Tanzania showed that, *E. coli O157:H7* was not detected in samples collected from milk retailer outlets (Swai and Schoonman, 2011).

A study done in Tunisia indicated that, the overall sample *E. coli* contamination prevalence and mean count was 35% and 2.41 log CFU/ml respectively (Bali *et al.*, 2013). Also in Djibouti dairy farm the mean value of aerobic mesophilic bacteria, coliform bacteria and *E. coli* was 6.78, 3.91 and 2.58 log CFU/ml respectively (Mohammed *et al.*, 2017)

A study conducted in Dire Dawa revealed that, the total mesophilic aerobic bacterial count and coliform counts were 6.76 and 1.24 log CFU/ml respectively. This study also confirmed that milk quality was poor. As shown in this study lower coliform bacteria was obtained from a sample collected from dairy farmers who used warm water to wash milk equipment and udder of the cow and store milk in aluminum cans than samples collected from dairy farmers used cold water and store their milk in plastic containers (Mesfine *et al*,. 2015).

A study conducted around Bahir Dar, Ethiopia reported that, 7.58 and 4.49 log CFU/ml of total bacterial count and coliform bacteria were counted respectively. Also the hygienic quality of raw cow milk was poor with total bacterial count and coliform bacteria of 8.12 and 4.94 log CFU/ml respectively (Tassew and Seifu, 2011). Similarly a study done in Dewa Cheffa, District of Amhara region reported that, 6.88, 7.10 and 7.54 log CFU/ml of the total bacteria count was obtained from the dairy farm, milk distribution centers and milk retailer outlets respectively. (Amakelew *et al.*, 2015).

In Hawassa the total mesophilic aerobic bacterial count in milk samples collected directly from the udder, dairy farms and milk selling points was 4.57, 7.28 and 10.28 log CFU/ml respectively. This study clearly confirmed that the microbial quality of raw milk as it goes from the udder to the selling point was deteriorated (Welearegay *et al*.,2012). Also in Kersa

District, the mean aerobic mesophilic bacteria, Coliform and staphylococcus counts were 8.48, 5.82 and 5.23 log CFU/ml respectively. Among pathogenic bacteria of public health significance, *S. aureus* and Salmonella species were detected in 34% and 20% raw milk samples collected from individual farmers respectively (Tadesse and Bacha, 2014).

2.3. Microbial quality of water used along the milk production chain

A study done in Slovakia showed that, 78% of the analyzed samples did not correspond to the microbiological criteria of the country which states no *E. coli* and coliform should exist in 100 ml of water sample. 22% and 67% of the sample were positive for *E. coli* and coliform bacteria, respectively (Torkar and Teger, 2004).

A study done in India revealed that, the quality of water used to wash milk equipment contributes significantly more bacterial population from all possible sources of contamination. The bacterial count of the sample collected from stored water were significantly higher than the sample collected from the tap water. This study also confirmed that using contaminated water for personal hygiene, cleaning utensils and animal's udder can affect the milk microbial quality (Pandey *et al.*, 2014).

A study done in South Africa revealed that, the mean ± Standard Deviation (SD) of coliform count and *E. coli* count of the borehole water was 171.11±704.5 and 62.83± 323.9 per 100 ml respectively (Esterhuizen *et al*., 2012). A similar study done in South Africa showed that, 60% for total coliform and 29% for *E. coli* exceeded the South African drinking water quality guideline (Esterhuizen, 2014). In Zambia utilization of untreated surface water and absence of soap to wash milk equipment were the major factors affecting milk quality. These using water to rinse milking equipment at the start and end of milking indicating the occurrence of milk borne illness in the country (Knight-jones *et al.*, 2016).

In Hawassa, southern Ethiopia, the overall prevalence *E. coli* exceeding zero CFU/ml was 39.2%. From water sample collected from wide opening containers about 66.7% of the samples were positive for *E. coli* (Amenu *et al*., 2016). A similar study done in rural households of Ethiopia showed that, among 233 water samples analyzed the overall

prevalence of *E. coli* (>0 CFU/ml) was 233 (55%). This study showed that the quality of water has its own negative effect on the quality of the milk (Amenu *et al.*, 2014).

2.4 Microbiological quality of milk contact surfaces

Inadequately washed milk equipment such as milk cans and bulk tanks are the major source of bacteria in milk after it leave the udder of the cow. Rinsed milking equipment and other milk contact surfaces cause for the occurrence of 10% of bacteria in milk during milking because of the formation of biofilm in the rough inner surface of the milk equipment. In Tanzania, the mean total viable count and total coliform count of milk container surfaces were 9.7 ± 10.5 and 7.8 ± 8.5 log CFU/cm^2 respectively (Gwandu *et al.*, 2018). In a Slovakian dairy farm, about 60% of the farmers wash milk equipment inadequately in which 12% of the swab sample was positive for *E. coli* (Torkar and Teger, 2004).

A study done in Assela and Debre Zeit confirmed that, the risk of milk contamination was reduced by 66% when hot water with detergent was used to clean the milk container compared to usage of detergent and cold water. Also, farmers who used only cold water to clean milk containers had three times increased risk of contamination compared to those who used cold water in combination with detergent. Similarly, farmers who checked for mastitis had three times increased risk of contamination to those who did not check for mastitis. When travel time to collection centers was above 30 minutes, the risk of contamination was about five times greater when compared to travel time less than 30 minutes. And for every 1 L increased in milk delivery to the collection centers, the probability of contamination increased by 5.7% (E, Tigabu *et al.*, 2015)

2.5. Knowledge, attitude and practice of milk handlers

The relationship between educational level of milk handlers and milk microbial quality was studied in Kerman Iran. The finding showed that, the quality of bulk tank milk in dairy farms with the owners who had below high school diploma was lower by 1.40% compared with the owner who held a high school diploma and higher degrees. This study confirmed the effect of

educational status of the milk owner on milk microbiological quality of raw milk (L, Mansouri -Najand and Z, Rezaii, 2015).

A study done in Jordanian military hospitals showed that, the mean score for knowledge, attitude and practice (KAP) among food handlers were 84.82, 88.88 and 89.43 respectively. And the overall KAP mean percentage score was 87.88 (Sharif *et al*., 2013). Similarly a study done in Malaysia hospital showed that, the mean score of KAP of food handlers were 83, 87.2 and 90.7 respectively (Norhaslinda *et al*., 2016).

A study done in Brazil indicated that, the microbial quality of raw milk was affected by knowledge of animal handling, schooling of milker's, milker attitude and behavior. About 6.97% of the variation in reduced microbial quality of raw cow milk were due to the aforementioned factors (Munera-Bedoya *et al.*, 2017).

In Kenya the mean knowledge score of milk handler was 60.0± 9.4%. Herdsmen had the lowest knowledge score of 49.4 ± 9%. Women at milk collection centers had the highest score along the chain with a mean of 68.8± 9.8%. Those milk handlers working with milk retailer outlets had a score of 61.9± 9.3% (Odongo *et al.*, 2017).

In Arusha, Tanzania about 45% of the consumers were aware of the potential milk borne pathogen and concerned with milk safety, but 65% of consumers were not aware that salmonella and *E. coli* can be transmitted from animal to human through the drinking of raw milk (Lubote *et al.*, 2014). A similar study done in Ghana revealed that, 31% of the study subjects did not know the importance of boiling for preventing milk borne illness. Regarding their practice towards the safe handling of milk 19% of the respondents did not wash their hands before milking and 92% of the respondents did not wash teats before milking (Addo *et al.*, 2011).

Unpublished survey in Kenya revealed that, more than 80% of the farmers were practicing good hygiene practices such as washing the milk cans with hot water and soap (Kabui, 2012). A similar study done in rural and peri-urban farms in Nakuru, Kenya revealed that, hand washing was practiced by all farmers in peri-urban (Orwa *et al.*, 2017).

In Kersa district Jimma Zone milking process was undertaken in a barn which were not in a good sanitation standard. More than 80% of the respondents were complaining about a shortage of animal health services. Regarding the frequency of cleaning milking utensils 67%, 17% and 16% of the respondents clean their milking utensils once, twice and three times per day respectively. About 13% of the respondents wash udder before and after milking and more than 90% of them use bare hands to dry the cows' udder. 3 % of the study subjects use individual towel and the rest 5% use shared towel to dry the cow's udder (Tadesse and Bacha, 2014).

2.6 Food borne illness associated with consumption of raw milk

Raw milk favors the development of pathogenic bacteria because of its optimum pH and the high availability of nutrients that support the growth of microorganism. Currently investigators tried to identify microorganism relevant in the dairy industry. In most cases *salmonella*, *S. aureus*, *E. coli O157:H7*, *L. monocytogenes*, Bacillus species and *C. jejuni* are the commonest pathogen found in raw milk causing a number of cases each year. Currently, investigators are aware of the newly emerged pathogenic microbes which is available in unpasteurized milk and milk products. Among these *E. coli*, Listeria, Salmonella, campylobacter and Yersinia are the major pathogens causing numerous food borne illness. *E. coli* are specially dangers to immune compromised individuals, elderly and children. These bacteria can cause illness, lifelong adverse health problems and ultimately deaths. A report from center for enteric diseases South Africa showed, 557 confirmed listeriosis cases were registered from all the provinces related with drinking of raw milk (V, Singh *et al*., 2011).

Most strain of *E. coli* are part of the normal flora of the human and other warm blooded animal which are considered harmless, but certain strain can cause severe illness. Shiga Toxin Producing *E. coli* (STEC) is the category of bacteria that produce a powerful toxin which can cause the health problem. STEC is an emerged human pathogen causing fatal hemolytic syndrome in human. According to CDC estimation, approximately 70,000 cases of STEC associated illness were occurred in the US each year (Yoon and Hovde, 2008).

In Iraq, 28.5% of the analyzed samples were positive for *S. aureus*, 24.56% of them were confirmed to have *S. aureus* enterotoxin genes (Khudor *et al.*, 2012). Similar study done in Palestine also showed that, out of 100 sample analyzed 37% were toxin genes positive for *S. aureus* which may contribute for the occurrence of food poisoning among raw milk consumers (G, Adwan *et al.*, 2005).

2.7 Conceptual framework of the study

Figure 1: Conceptual framework of the study showing the relationship between dependent and independent variables.

CHAPTER THREE

3. OBJECTIVES

3.1 General objective

To determine the microbial quality of raw cow milk and associated factors along the dairy value chain in Jimma Zone, Southwest Ethiopia.

3.2 Specific objective

-To determine the microbial quality of raw cow milk from production to selling points

-To investigate the effect of water quality on the microbiological quality of raw cow milk

-To examine the effect of cleanliness of milk contact surfaces on the milk microbial quality

-To identify the role of knowledge of milk handlers on the microbial quality of raw cow milk

-To determine the effect of attitude of milk handlers on the microbial quality of raw cow milk

-To determine the effect of practice of milk handlers on the microbial quality of raw cow milk

CHAPTER FOUR

4. METHODS AND MATERIALS

4.1 Study area

The study was conducted in selected district towns and town administrations of Jimma zone in Oromia region, southwest of Ethiopia. According to the 2007 national population and housing census, about 2.5 million population was counted. Of which female accounts 47% and more than 90% of the population are rural residents. A total of 521, 506 households were reported resulting in an average of 4.77 persons to a household. Topographically it varies from 1000 to 3360 m above sea level. Annual rainfall is one of the highest in the country, reaching up to 2800 mm per year. Coffee is the major crop which significantly contributes to the national economy. According to central agricultural census commission, 2002, a total of 1, 718,284 head of cattle, 466,154 sheep, 194,677 goats, 74,774 horses, 40, 555 donkeys and 30, 541 mule population were counted in this zone (CSA, 2007).

From 22 district towns and town administrations of the zone, Jimma town administration, Agaro town administration, Sekoru town, Serbo town, Yebu town, Seka town, Dedo town and Shebe town were purposively selected based on their location within milk shed and high potential for dairy farming (Tadesse *et al*., 2016; Duguma *et al*., 2017).
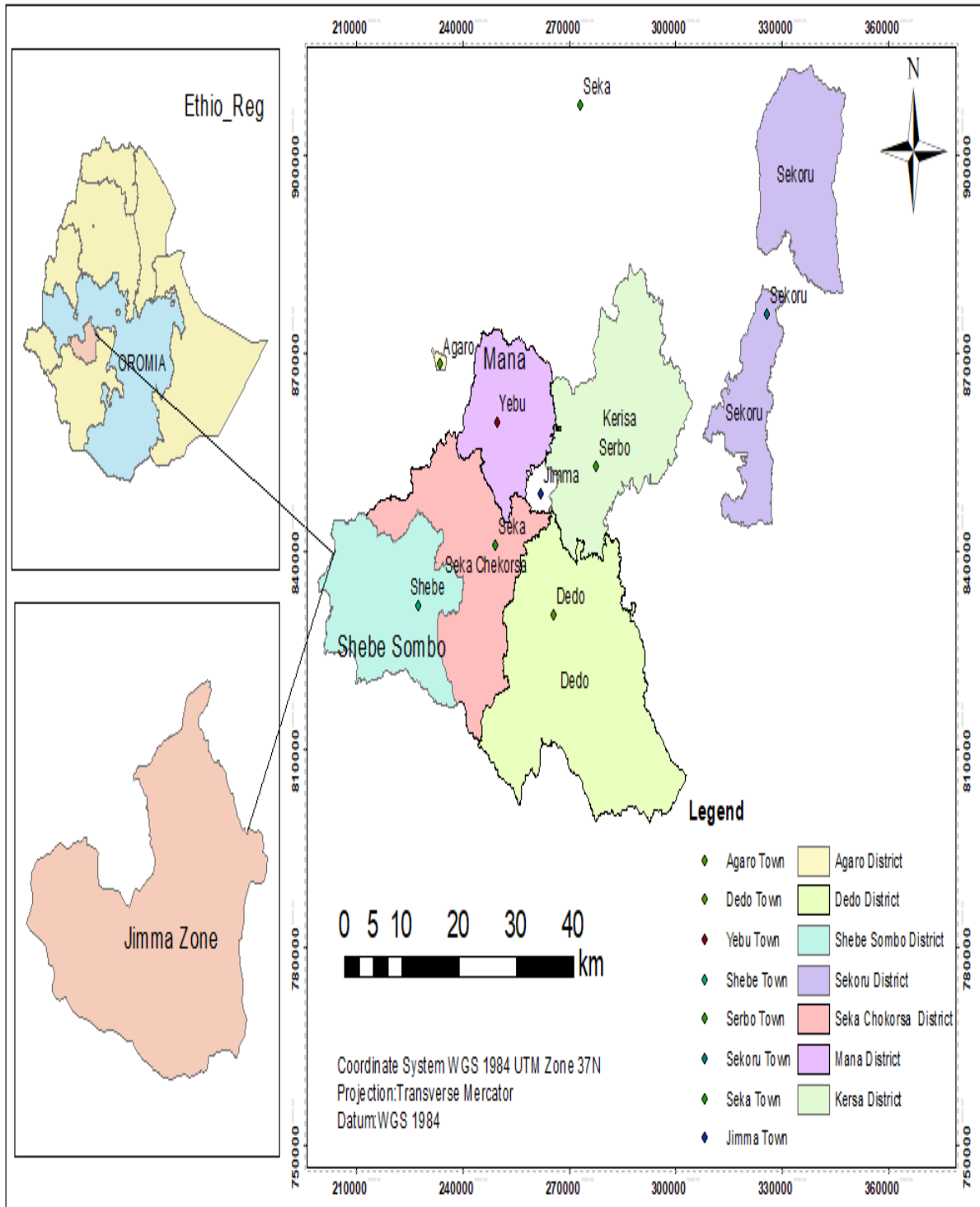
Figure 2: Map of the study area

4.2 Study design and period

A cross-sectional study design was used to assess the microbiological quality of raw milk and associated factors along the dairy value chain in Jimma zone south west, Ethiopia. The study was conducted from April to May 2018, during the 'Belg' season of the year.

4.3 Population

4.3.1 Source population

All dairy farms, milk distribution centers, retailers and milk handlers found in Jimma zone were the source population.

4.3.2 Sampling population

The sampling populations were all dairy farms, milk distribution centers, retailers and milk handlers found in eight district towns and town administration of Jimma zone.

4.3.3 Study population

The study populations were all dairy farms, milk distribution centers, retailers and milk handlers who are actually included in the study.

4.3.4 Study unit

Dairy farm, milk collection center, milk retailer shop and person

4.3.5 Inclusion and exclusion criteria

4.3.5.1 Inclusion criteria

Dairy farms, milk collection centers and milk retailers who are linked to each other in the dairy chain and those person who work in dairy farm, milk collection centers and milk retailer outlets for at least six month and who are above the age of 18 were included in the study.

4.3.5.2 Exclusion criteria

Dairy farms, milk collection centers and milk retailer shops without milk during sample collection and those who are not volunteer to be included in the study were excluded from the study.

4.4 Sample size and sampling technique

In Ethiopia there is no record regarding the number of dairy farms, milk collection centers and retailers (T, Tolosa *et al*., 2016). In Jimma zone also, the exact number is unknown. As a result a total of 150 milk samples (50 from each dairy farm, milk distribution centers and milk retailer out lets) were collected aseptically from selected district towns and town administrations of the zone. 150 water samples (50 samples each from dairy farm, milk distribution centers and milk retailer out lets were collected. And also a total of 150 swab samples (50 samples each from dairy farms, milk distribution centers and milk retailer out lets) were collected randomly. The knowledge, attitude and practice of milk handlers working at each milk processing stages was also assessed by interviewing a randomly selected milk handlers in the milk processing units.

To collect the required number of samples from selected district towns and town administrations of the zone, first representative Kebeles were selected from each towns. The Kebeles were selected purposely based on cattle population and high potential for milk processing which was identified in consultation with responsible body from district agents. Then a random sampling procedure was followed to collect a sample from each site (Azeze and Tera, 2015).

4.5 Sample and data collection

4.5.1. Milk sample collection

Milk sample was collected from milk storage tanks at dairy farms, milk distribution centers and milk vendors. About 25 milliliter (ml) of raw milk samples was collected aseptically with

sterile universal plastic screw capped bottle placed in a cold box with ice packs as per the recommendation of ET ISO 707, (2012). Thereafter, the samples were transported to Medical microbiology laboratory of Medical Laboratory Science department in Jimma University for analysis within 4 hours of collection (Ethiopian Standards Agency, 2012).

4.5.2. Environmental sample collection

4.5.2.1 Swab sample collection

Surface swab technique was used as described in the Compendium of Methods for the Microbiological Examination of Foods (APHA, 1992). The sampling procedure was performed by swabbing a delimited area of $100cm^2$ from milk storage tanks which was washed and made ready for storing milk. A sterile polypropylene template was used to sample each $100cm^2$ surface. The wetted swab head was rubbed slowly into two directions at right angles to each other, e.g. horizontally and vertically. The area was swabbed for approximately 20 seconds. The total surface swab for each food contact surfaces was $100 \ cm^2$. All swab samples were placed in an ice cold box and transported immediately to Medical microbiology laboratory in Jimma University for analysis within 4 hours of collection (New South Wales food authority, 2003; Lani *et al.*, 2014).

4.5.2.2 Water sample collection

About 150 water samples each with 250 ml was collected from water storage tanks by using sterile autoclave proof glass sample bottles. The sample was placed in cold box with ice pack, labeled and transported to the laboratory of Environmental health science and technology department of Jimma University for analysis within 4 hours of collection (Abdul *et al*., 2010).

4.5.3 Data collection

The knowledge, attitude and practice of milk handlers were assessed by using pre tested structured questionnaire.

4.6 Sample analysis

4.6.1. Microbial determination in milk samples

To estimate the microbial load per ml of milk sample, 25 ml of each sample was transferred to 225 ml of sterilized buffered peptone water to make one in ten stock solution of the sample (Nanu *et al.*, 2007). Serial dilution was prepared by transferring one ml stock solution into the first test tubes which initially contain 9 ml of sterilized buffered peptone water (Welearegay *et al.*, 2012). The test tubes were homogenized by using vortex mixer (Fisher Scientific, USA) (Tadesse and Bacha, 2014). Then the homogenates were serially diluted up to 10^{-4} and 0.1 ml aliquot of the appropriate dilution was spread plated in duplicate on sterilized petri dish by using sterile spreader. Then the plates were incubated at 37℃ for 24 to 48 hours (R, Khaton *et al.*, 2014). Finally the colony was counted by using colony counter (Gallen hamp colony counter, England) and reported as CFU/ml. Dilutions with the total number of colonies on a plate between 30 to 300 per plates were selected for colony calculation (Welearegay *et al.*, 2012).

To isolate *E. coli* O157:H7 in milk samples, 0.1 ml of the aliquot was spread plated on two separate petri-dishes. Then the plates were incubated at 37℃ for 24-48 hrs. After incubation colorless colonies were identified as presumptive *E. coli* O157:H7 whereas the pink/red colonies were identified as coliform bacteria. To identify hygiene indicator *E. coli,* few pink/red colonies were taken and sub-cultured on Eosin Methylene Blue (EMB) agar and incubated for additional 37℃ for 24 hours. Then colonies with green metallic sheen were considered as *E. coli.* The colorless colony was also taken and sub cultured on EMB agar and incubated for additional 37℃ for 24 hours. After incubation the colony with green metallic sheen were identified as *E. coli* O157:H7. Then few colonies were taken and tested for gram staining and all of the gram negative *E. coli* were taken and tested for oxidase and catalase test (Swai and Schoonman, 2011; R, Khaton *et al.*, 2014).

Then, the number of colony count per ml of milk sample was calculated by using the following formula.

$$\frac{CFU}{ML} = \frac{\text{no of Colony counted on plates}}{\text{volume plated(ml)} * \text{dilution factor}}$$ (Wubet *et al*., 2014).

4.6.2. Microbial determination of Environmental samples

4.6.2.1 Water sample analysis

Exactly 100 ml of the water sample was measured and filtered into sterile filter paper with a 0.45 micrometer pore size which retain bacteria and allow the passage of water molecule. Then the filter paper was placed on petri-dish which initially contain wetted absorbent pad on it (A, Rompre *et al.,* 2002). Then the petri dish was incubated at 37 and 44℃ for total coliform and fecal coliform respectively. Yellow colony from both 37 and 44℃ incubated plates were identified as total and fecal coliform bacteria respectively. Plates having a bacterial count of 20 to 60 for fecal coliform and 20 to 80 for total coliform was considered as ideal countable range to calculate the number of colony per 100 ml of water sample filtered. The maximum countable range were 200 colony (Myers *et al.*, 2007).

To calculate the total and fecal coliform bacteria per 100 ml of water sample the following formula was used (Environmental Agency, 2010).

$$\frac{\text{count}}{100 \text{ ML}} = \frac{\text{No of colony forming unit} * \text{dilution factor} * 100}{\text{volume of sample filtered}}$$

4.6.2.2 Swab sample analysis

Up on arrival to the laboratory the swab head was rinsed into sterile 10 ml buffered peptone water to make the first dilution (10^0). From this dilution, one ml was taken and transferred to the second and continued in this manner to each of the remaining test tubes containing nine ml of sterilized buffered peptone water. The process was continued until the desired dilution was obtained. To enumerate coliform bacteria, 0.1 ml from the two consecutive dilution was spread plated on separate MacConkey sorbitol agar dispensed petri-dish to get the accurate colony count. After incubation at 37℃ for 24 hours, the pink colony (sorbitol fermenter) was identified and counted as coliform bacteria. To identify *E. coli* the suspected colony was sub-

cultured on EMB agar and then gram staining, oxidase and catalase test was undertaken (Lani *et al.*, 2014).

The result was reported as log CFU/cm^2 (Sneed *et al.*, 2004). The number of coliform for each swab sample was calculated using the following general formula (Public Health of England, 2017).

$$\text{Count/swab} = \frac{C * n3}{V(n1 + 0.1n2)d}$$

Where: -

C is the sum of colonies counted on both plates

V is the volume applied to each plate

n1 is the number of plates counted at the first dilution

n2 is the number of plates counted at the second dilution

n3 is the original volume of neat suspension (*i.e.* 10 ml)

d is the dilution from which the first count was obtained

Finally the count was divided by 100 which is the total area in which surface sample is taken to get the count per centimeter square.

4.7. Study variables

4.7.1 Dependent variables

-Microbial quality of raw cow milk

4.7.2 Independent variables

-Socio-economic status of milk handlers (Sex, age, educational status, milk handling experience)

-Water coliform count

-Microbial load of milk equipment

-Knowledge about milk handling

-Attitude towards milk handling

-Milk handling practice

4.8. Operational definition

Raw milk: a natural secretion of mammary glands of cow that does not undergo any heat treatment.

Milk handler: Any individuals who are above the age of 18 and who have direct or indirect contact with raw milk along the chain. For instance milker in dairy farm, one who load and unload milk container in milk distribution system or who weight milk and sold to the consumer in milk retailer out lets.

Milk retailer outlet: Any establishment which receive milk from milk distribution centers and sold for individuals either while it is raw or processed. It includes cafeterias, restaurants and other places.

Dairy/milk value chain: The flow of milk starting from milking at dairy farm to the milk retailer out lets. It shows the safety and quality issues happening in dairy farm, milk collection centers and milk retail out lets.

Poor microbial quality of raw cow milk: The presence of total mesophilic aerobic bacterial and coliform bacteria in raw cow milk at the level exceeding the limit set by (CFC, 2014) and (Food Standards Australia New Zealand, 2018).

Microbial quality of raw cow milk due to TMABC could be:

Satisfactory: if the count of total mesophilic aerobic bacteria in raw milk is $<10^5$ CFU/ml.

Boarder line: if the count ranges from 10^5 to $\leq 10^7$ CFU/ml.

Unsatisfactory: if the count of total bacteria in raw milk exceeds 10^7 CFU/ml.

Microbial quality of raw cow milk due to coliform bacteria could be evaluated as:

Satisfactory: if the count of coliform bacteria in raw cow milk is $<10^2$ CFU/ml

Border line: if the count ranged from 10^2 to 10^4 CFU/ml

Unsatisfactory: if coliform count exceeds 10^4 CFU/ml

Microbial quality of raw cow milk due to STEC could be:

Satisfactory: if STEC is not detected in 25 ml of milk sample

Unsatisfactory: if STEC is detected in 25 ml of milk sample

Knowledge of milk handlers: The grading of score to evaluate knowledge of the respondents was taken from literature. The questions had two possible answers, yes and no. Each correct solution carried 2 marks while wrong solution carried 1 mark. In the case of negatively quoted questions, reverse scoring was used. Respondents who scored less than or equal to 50% were categorized as having poor knowledge, categorized as average if the they scored 51 to 69% and categorized as having good knowledge if they scored 70% and above (Norhaslinda *et al.*, 2016).

Attitude of milk handlers: The evaluation of attitude of milk handlers was also depends on literature. The questions had five possible answers strongly agree, agree, neutral, disagree and strongly disagree which carries 4, 3, 2, 1 and 0 marks respectively. For negatively quoted questions reverse scoring was used. Then the subjects were classified as having good attitude if they scored 70% and above, named as having fair attitude if they scored 51 to 69% and poor if they scored less than or equal to 50% (Norhaslinda *et al.*, 2016).

Practice of milk handlers: The criteria used to evaluate the practice of milk handlers was also obtained from literature. The questions had always, often, sometimes, rarely and never responses which carries 4, 3, 2, 1 and 0 marks respectively. For negatively quoted questions reverse scoring was used. Accordingly, respondents classified as having good practice if they scored greater than or equal to 70% and fair if scored 51 to 69% and classified as having poor practice if they score less than or equal to 50% (Norhaslinda *et al.*, 2016).

Water quality: Water used to wash milk equipment and hands of milk handler is considered to be good quality if no fecal coliform present in 100 ml of water sample and poor quality if fecal coliform is detected in 100 ml of water sample analyzed (Ethiopian Standards Agency, 2013).

Milk equipment cleanliness: milking equipment can be considered as clean if the coliform bacteria present in milk sample is not more than l0 CFU/cm^2 (1 log CFU/cm^2) otherwise considered as unclean (Trindade *et al*., 2014).

4.9. Data management and statistical analysis

In each day of data collection, the data was entered into epi data version 3.1. Finally it was transported to statistical package for social science (SPSS) version 23 for analysis. Similarly the laboratory results were recorded in each day of counting. Each bacterial count was transformed to log values. Descriptive measures including frequency, percentage, mean and range were used to analyze both the laboratory investigation and survey data. The data was presented by using table and bar graph. To determine the significance of differences ($P<0.05$) of the mean microbial count between different milk value chain one way analysis of variance

(ANOVA) was used. The presence of linear relationship was also determined by using Pearson correlation. The correlation coefficient (r) and p-value was considered to interpret the presence and magnitude of linear relationship. To determine the effect of the various explanatory variables on outcome variables, multiple linear regression model was employed. P-value less than 0.05 was considered as cut-off point.

Assumption checking: The presence and/or absence of outlier, normality, homogeneity of variance, multicollinearity and linearity of the data set was tested before the regression analysis. There is no extreme numbers in the data set. The normality of the data set was checked by using the normal P-P plots in the SPSS. Here, almost all of the measurements were close to the straight line produced by the mean of all observation. The scatter plots also showed some trends of relationship between variables. To test the homogeneity of variance in the data set, levene test was used. As indicated in the test, the p value was higher than the significance level (0.05), indicating that the variance are equal. To check weather one explanatory variable affect the other explanatory variable, the variance inflation factors and the tolerance value was considered, all the variance inflation factor values were less than 10 and all the tolerance value for each variables were >0.1. The variance inflation factors were ranged from 1.046 to 1.409 and tolerance was varied from 0.710 to 0.956. (See annex VIII)

4.10 Quality assurance

Laboratory instruments and measurements were calibrated and standardized. Pretest was carried out with 10% of the study subjects to test the appropriateness of the questionnaires to be used prior to the actual data collection. Close supervision was conducted during the actual data and sample collection. In each day of data collection period, the questionnaire was checked for its completeness and internal consistency. All sample analysis was carried out in duplicate with its control. All Medias and reagents used were up to date and all sample analysis was carried out inside level II safety cabinet (BDK, Genkingen) to protect myself, the surrounding environment and the sample. All culture media and equipment was sterilized by using autoclave (Astell, England). The adequacy of autoclaving process was assured by using sterilization indicator. The white strip on sterilization indicator was changed to black if the

sterilization is adequate. If the sterilization is adequately completed, the white color of the sterilization indicator was changed to black. *E. coli* O157:H7 (ATCC 35218) and sterilized water was used as a positive and negative control respectively (Koochakzadeh *et al.*, 2014).

4.11 Dissemination and utilization of finding

The findings of this study will be presented to department of Environmental health science and technology, Institute of Health, Jimma University. The finding will also be presented to Jimma zone and town livestock and fish resource office. It will also be disseminated to those dairy farms and enterprises involved in milk production and distribution activities. And finally the finding will also be published on reputable journal.

4.12 Ethical consideration

Ethical clearance was obtained from ethical review board of Institute of Health Science, Jimma University. Written consent and support letter was obtained from zonal and district administration. Additionally, consent was obtained from the respondents and all the information obtained from each study participant was hold confidential.

CHAPTER FIVE

5. RESULT

5.1 laboratory result

5.1.1. Microbiological quality of raw cow milk along the dairy value chain

Total mesophilic aerobic bacterial count: the mean total mesophilic aerobic bacterial count at the dairy farm, milk distribution centers and milk retailer outlets were 4.96 ± 0.34, 6.29 ± 0.19 and 7.25 ± 0.14 log CFU/ml respectively. The TMABC ranges from 4.49 to 5.48 at dairy farm and 6.85 to 7.48 at milk selling points. The result from analysis of variance indicated that there was statistically significant difference ($p<0.05$) between the milk mean total mesophilic aerobic bacterial count of raw milk along the milk supply chain.

Coliform count: the mean coliform bacteria at the dairy farm, milk distribution centers and milk retailer outlets were 4.43 ± 0.40, 5.67 ± 0.39 and 7.00 ± 0.18 log CFU/ml respectively. Coliform bacteria ranged from 3.70 to 5.40 at dairy farm and 6.63 to 7.26 log CFU/ml while it reaches at milk selling points. There was statistically significant difference ($p<0.05$) among the mean coliform bacteria count of raw milk.

E. coli O157:H7: the mean *E. coli* O157:H7 at dairy farm**,** milk distributor and milk retailer outlets were 3.49 ± 1.71, 3.75 ± 2.74 and 6.85 ± 0.30 log CFU/ml respectively. The result from analysis of variance showed that the mean count was significantly different ($p<0.05$) among the stages of milk supply chain.

Table 1: Microbial counts in raw cow milk sample along the dairy supply chain in Jimma zone, southwest Ethiopia, 2018.

Sample site	Mean ±SD and range						p-value
	TMABC		Coliform count		E. coli O157:H7		
	Mean	Range	Mean	Range	Mean	range	
Dairy farm (n=50)	4.96±0.34	4.49 -5.48	4.43±0.40	3.70-5.40	3.49±1.71	0- 5.09	0.000 for all cases
Milk distribution centers(n=50)	6.29±0.19	5.65- 6.48	5.67±0.39	4.70-6.27	3.75±2.74	0- 6.33	
Milk retailer out lets(n=50)	7.25±0.14	6.85-7.46	7±0.18	6.63-7.26	6.85±0.30	6.15-7.26	
Over all	6.17±0.97	4.49-7.46	5.7±1.1	3.70-7.26	4.70±2.40	0-7.26	

5.1.2 Evaluation of microbial quality of raw cow milk sample along the milk supply chain

Among 50 milk samples collected from the dairy farm, half of them were classified as satisfactory level of quality in terms of TMABC. All milk samples collected from milk distributor were categorized as borderline level of quality. Almost all of the milk sample collected from milk retailer outlets were unsatisfactory as shown below.
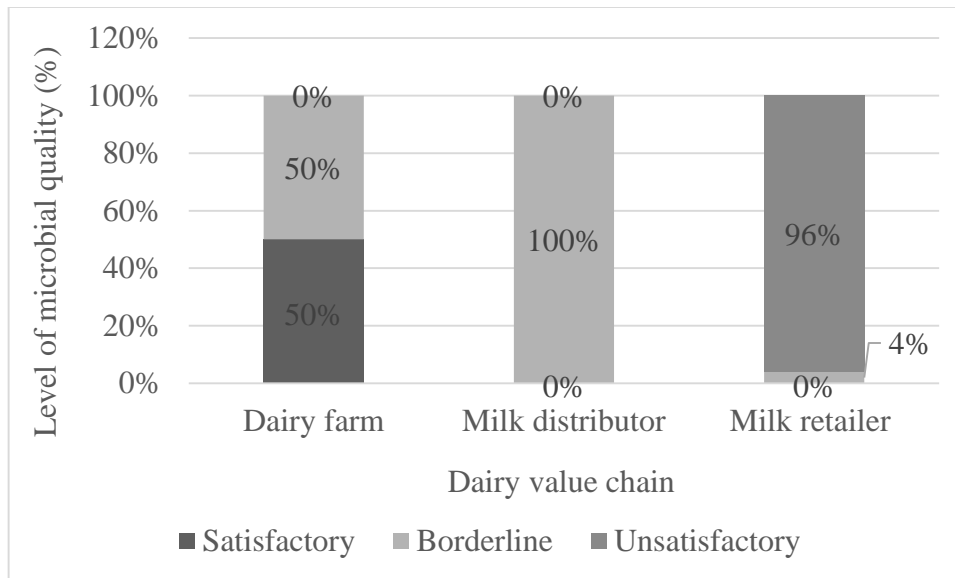
Figure 3: Evaluation of microbial quality of raw cow milk at different stages of milk production process in Jimma zone south west Ethiopia, 2018.

As shown below among 50 milk sample analyzed in dairy farm, 84% of them contain one or more cells of suspected cells of *E. coli O157:H7* and hence categorized as unsatisfactory quality. The rest 16% of them were satisfactory in which no cells of *E. coli O157:H7* were observed. 66% in milk distribution centers and all of the sample in milk retailer out lest were unsatisfactory quality.
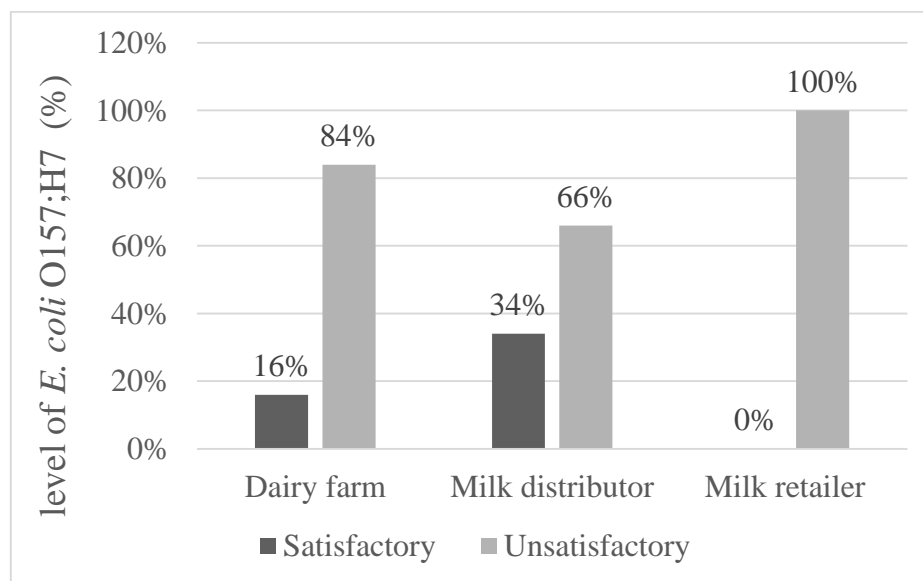
Figure 4: Level of *E. coli* O157:H7 in raw milk sample collected along the milk supply chain in Jimma zone, southwest, Ethiopia.

As shown in figure 5, overall 25 (16.7%), 77 (51.3% and 48 (32%) of the total sample analyzed were categorized as satisfactory, borderline and unsatisfactory level of quality for TMABC respectively. 10 (6.7%) and 140 (93.7%) of the analyzed samples were borderline and unsatisfactory quality respectively, for coliform bacteria according to Australia and New Zealand food standards.
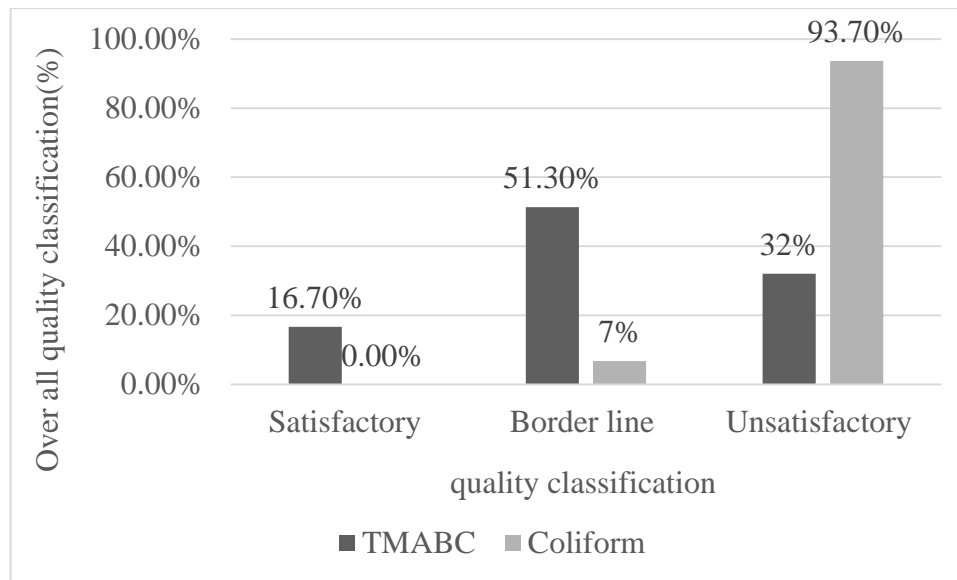
Figure 5: Overall microbial quality evaluation of raw cow milk along the supply chain in Jimma zone southwest, Ethiopia.

As shown below, all of the analyzed samples were positive for coliform bacteria. From these about 56.7% of them were positive for *E. coli* as confirmed by the presence of green metallic sheen on EMB agar.

Table 2: Growth of hygiene indicator *E. coli* on various media in milk samples collected along the milk supply chain in Jimma zone, Southwest, Ethiopia, 2018.

Milk value chain	Growth of coliform on macConkey agar	Growth of *E. coli* on EMB (%)
Dairy farm	All samples positive	25(50%)
Milk distributor	All samples positive	27(54%)
Milk retailer	All samples positive	33(70%)
Over all	All samples positive	85 (56.7%)

Over all among 150 milk samples analyzed, 82.7% of them were presumptively positive for *E. coli* O157:H7. Of which about 60% of them were positive for *E. coli* O157:H7 on EMB agar

Table 3: Growth of *E. coli* O157:H7 on MacConkey sorbitol agar and EMB agar in milk samples collected from dairy farm, milk distribution centers and milk retailer out lets in Jimma zone, Southwest, Ethiopia, 2018.

Milk value chain	MacConkey sorbitol positive (%)	EMB positive (%)
Dairy farm	42(84%)	23(55%)
Milk distributor	33(66%)	26(78.8%)
Milk retailer	50(100%)	29(58%)
Over all	124(82.7%)	78(62.4%)

5.1.3 Microbiological quality of water along the dairy value chain

The mean fecal coliform count of water samples collected at the dairy farm, milk distribution centers and milk retailer out lets were 4.04±0.34, 3.72±0.53 and 2.20±0.51 log CFU/100ml respectively. And the mean total coliform bacteria at the dairy farm, milk distributors and milk retailer outlets were 4.44±0.55, 4.32±0.57 and 4.57±0.41 log CFU/100ml respectively. Fecal coliform ranged from 3 to 4.57 at dairy farm and 1.70 to 2.70 log CFU/100ml at milk retailer outlets. The result from analysis of variance indicated that the mean fecal coliform count was affected by the various stages along the chain ($p < 0.05$). But the mean total coliform count did not show significant difference along the milk value chain ($p > 0.05$).

Table 4: Microbial counts in water samples collected along the milk value chain in Jimma zone, south west Ethiopia, 2018.

| Sample site | Mean ±SD and Range | | | | p-value |
| | Total coliform | | Fecal coliform | | |
	Mean	Range	Mean	Range	
Dairy farm(n=50)	4.44±0.55	2.81-4.93	4.04±0.34	3.0-4.57	p=0.000*(fecal coliform)
Milk distribution centers(n=50)	4.32±0.57	3.02-4.92	3.72±0.53	3.0-4.59	
Milk retailer out lets(n=50)	4.57±0.41	3.73-5.14	2.20±0.51	1.70-2.70	p=0.068 (total coliform)
Over all (n=150)	4.44±0.55	1.70-4.59	3.32±0.93	2.81-4.95	

*-Significant difference

As shown below, the mean fecal coliform and total coliform count of water sample increases as milk transported from dairy farm to milk retailer outlets.
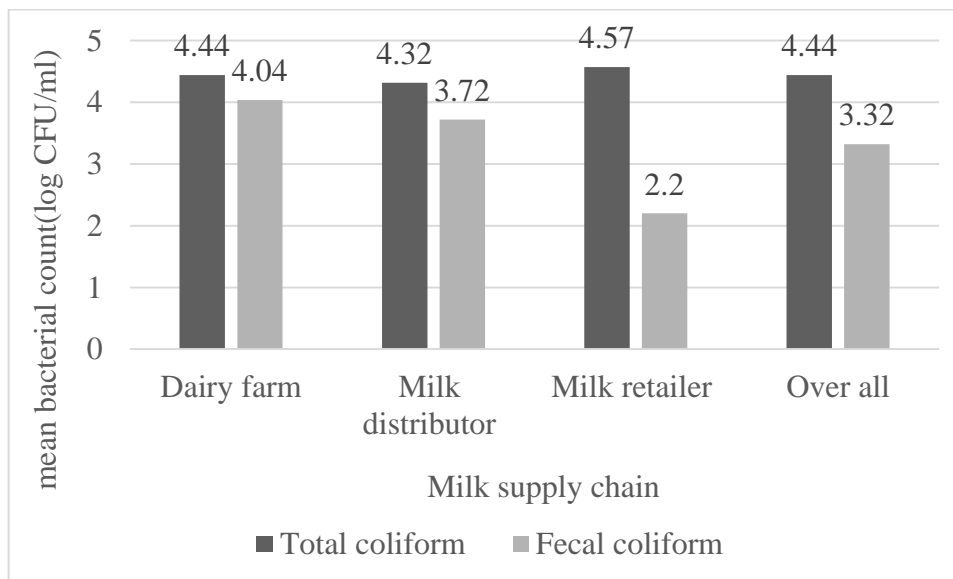
Figure 6: Microbiological quality of water used to process milk along the milk supply chain in Jimma zone south west, Ethiopia 2018.

5.1.4 Microbiological quality of milk contact surfaces along the milk supply chain

The mean coliform count of milk contact surfaces in the milk sample collected from the dairy farm, milk distribution centers and milk retailer out lets were 4.61±0.38, 4.71±0.52 and 4.75±0.51 log CFU/cm^2. The result from ANOVA indicated that, the mean coliform bacteria was not affected by the various stages of the milk production process (p>0.05).

Table 5: Coliform counts in milk storage tanks along the milk value chain in Jimma zone, south west, Ethiopia, 2018.

| Sample site | Mean ±SD and Range | | | p-value |
| | Coliform (log CFU/cm^2) | | EMB *(E. coli)* | |
	Mean	Range	% positive sample	
Dairy farm(n=50)	4.61±0.38	3.66 to 5.37	14(28%)	
Milk distribution centers(n=50)	4.71±0.52	3.56 to 5.36	18(36%)	
Milk retailer out lets(n=50)	4.75±0.51	3.26 to 5.29	22(44%)	0.318
Over all (n=150)	4.69±0.48	3.26 to 5.37	54(36%)	

5.2 Survey result

5.2.1. Socio-demographic characteristics of the respondents

Out of 150 respondents included in the study, 104 (69%) of them were male while the rest were female. 64 (43%) of the respondents aged between 35 and 50 years. 64 (42.7%). 61 (40.7%) of the study subjects had no education and completed their elementary school respectively. About half of the study subjects were single. And about 40% of them had milk handling experience of 1 to 2 years as shown below.

Table 6: Socio demographic characteristics of milk handlers working in milk processing area in Jimma zone, south West Ethiopia, 2018.

Variables	Category	Frequency	Percentage
Sex	Male	104	69
	Female	46	31
	Total	150	100
Age	18-35	53	35
	35-50	64	43
	>50	33	22
	Total	150	100
Educational status	No education	64	42.7
	Elementary	61	40.7
	Secondary	25	16.7
	Total	150	100
Marital status	Single	73	49
	Married	69	46
	Divorced	6	4
	Widowed	2	1
	Total	150	100
Religion	Christian	98	65
	Muslim	52	35
	Total	150	100
Ethnicity	Oromo	80	53.3
	Amhara	11	7.3
	Dawuro	25	16.7
	Gurage	15	10
	Gumuz	1	0.7
	Yem	18	12
	Total	150	100

Milk handling experience in years	<1	24	16
	1-2	62	41.3
	3-5	47	31.3
	6-10	17	11.3
	Total	150	100

5.2.2 KAP of milk handlers along the milk supply chain

As shown below, the mean knowledge score of the milk handlers working in dairy farm, milk distribution centers and milk retailer outlets were 60%±11.74, 66.31%±10.74 and 61.10%±11.28 respectively. The overall mean score of KAP of the respondents was 62.44%±11.53, 57.98%±9.22 and 57.42%± 10.78 respectively. The analysis of variance showed that the mean knowledge score of the respondents differs significantly ($p<0.05$). Also the mean attitude score of the respondents showed significant difference ($p<0.05$) along the milk production chain.

Table 7: Mean score of KAP of milk handlers in Jimma zone, south west Ethiopia, 2018.

Variables	**Milk value chain**	**Mean score** ±SD	**P-value**
Knowledge	Dairy farm(n=50)	60.00±11.74	0.012
	Milk distributor(n=50)	66.31±10.74	
	Milk retailer (n=50)	61.10±11.28	
	Overall score(n=150)	62.44±11.53	
Attitude	Dairy farm	52.95±8.12	0.000
	Milk distributor	57.68±7.08	
	Milk retailer	63.30±9.37	
	Over all mean score	57.98±9.22	
Practice	Dairy farm	51.96±8.56	0.000
	Milk distributor	59.68±10.88	
	Milk retailer	60.54±10.75	
	Overall mean score	57.42±10.78	

5.2.3 Evaluation of KAP of milk handlers

As shown in the table below, among 150 respondents included in the study about one third of them had good knowledge and 23 (15.3%) of them had a poor of knowledge. Regarding their attitude 11.3% of the respondents had good of attitude towards milk handing and 34 (22.7%) of them had a poor level of attitude. Considering their practice 17(11.3%) and 44 (29.3%) of them had good and poor of milk handling practice respectively.

Figure 7: Evaluation of KAP of milk handlers in Jimma zone south west Ethiopia, 2018.

5.3. Correlation of variables included in the study

The degree and magnitude of linear relationship between variables were tested using correlation analysis. As shown below all the variables had a positive relationship with the total bacterial count of raw cow milk with the exception of water coliform count. The degree of linear relationship varied from fair to excellent as shown below.

Table 8: Correlation of variables

Variables	Correlation coefficient (r)	p-value	Remark
Microbial load of milk equipment	0.134	0.103*	--------
Water coliform count	-0.752	0.000	Very good to excellent correlation
Knowledge about milk handling	0.052	0.530*	-------
Attitude towards milk handling	0.442	0.000	Fair to moderate
Milk handling practice	0.319	0.000	Fair to moderate

 * no correlation

5.4 Factor affecting microbiological quality of raw cow milk

After checking the assumption for linear regression all the variables (sex, age, educational status, marital status, ethnicity, religion, milk handling experience, knowledge about milk handling, attitude towards milk handling, practice of milk handling, water coliform count and microbial load of milk equipment) were entered into the multiple linear regression model.

The twelve explanatory variables in the standard model were significantly predictive of the dependent variables according to the ANOVA table (p <0.05). The overall correlation coefficient of the variables was R=0.81. Indicated that, there is positive and moderate to excellent relationship between variables included in the model. Also the model's degree of explaining the variance in the dependent variables was $R^2 = 0.656$. This means that the proportion of variation in microbial quality of raw cow milk that is explained by the regression of all the explanatory variables is 65.6%. These two values indicated that the model predicts the dependent variables well.

Table 9: Model summary

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics Sig. F Change
1	.810ᵃ	.656	.626	.59407	0.000

a. Predictors: (Constant), Sex, Age, Religion, Marital status, Educational status, Ethnicity, Milk handling practice, Milk handling experience, swab coliform, Knowledge about milk handling, attitude of milk handlers, water coliform count

The order of importance of explanatory variables in predicting the dependent variables is indicated by the absolute value (β) as shown below. Among the twelve explanatory variables water coliform count had the highest beta value β=-0.593 indicating that water quality is the most important variables in predicting the microbial quality of raw milk. The least important

variable was the knowledge of milk handlers towards milk handling as indicated by a lowest β value. (β=0.03)

The multiple linear regression model indicated that educational status of the milk handlers, water coliform count and attitude of milk handlers were the three major explanatory variables that have a great influence on the microbial quality of raw cow milk.

As the number of water coliform count increased by one unit, the microbial quality of raw cow milk decreases by 0.619. It clearly showed that, improving the quality of water has its own contribution to improve the quality of raw cow milk. Also as educational status of the respondents increased by one unit, the microbiological quality of raw cow milk increased by 0.187. It indicated that increasing the educational status of the respondents play a great role in improving the quality of raw cow milk along the dairy value chain. Similarly as the attitude of the respondents increased by one unit, the microbiological quality of raw cow milk increased by 0.018.

Table 10: Multiple linear regression results

Model	Unstandardized Coefficients		Standardized Coefficients	p- value
	B	Std. Error	Beta	
(Constant)	5.943	.783		.000
Sex	.206	.117	.098	.081
Age	-.108	.075	-.083	.151
Educational status	.187	.076	.140	.014*
Marital status	-.056	.088	-.035	.528
Religion	.139	.104	.068	.185
Ethnicity	-.038	.020	-.099	.063
Milk handling experience	.038	.059	.035	.524
Swab coliform count	.119	.109	.058	.275
Water coliform count	-.619	.062	-.593	.000*
Knowledge about milk handling	.000	.005	-.003	.954
Attitude towards milk handling	.018	.006	.171	.005*
Milk handling Practice	.004	.005	.044	.426

Dependent variable: Microbial quality of raw cow milk

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-significant difference, MH- milk handling /milk handler

Based on the regression analysis, the fitted regression model was:

MICROBIAL QUALITY OF RAW COW MILK=5.943-0.619 WATER COLIFORM +0.187 EDUCATI

ONAL STATUS OF MILK HANDLERS+ 0.018 ATTITU

DE OF MILK HANDLERS

CHAPTER SIX

6. DISCUSSION

Cow's milk may be contaminated from different sources. Proliferation of the already existed microbes in raw milk and the introduction of other microbes from the external environment leads to the deterioration of raw milk after it leaves the udder of the cow. Microbiological guideline for ready to eat food prepared by CFS recommended that, a total mesophilic aerobic bacterial count of less than log 5 CFU/ml is classified as satisfactory level, while those milk samples having a mean total count of log 5 to less than log 7 CFU/ml as borderline and those having a mean count of greater than log 7 CFU/ml as unsatisfactory level of microbial quality. In this study the mean total mesophilic aerobic bacterial count of dairy farm sample was 4.96 log CFU/ml which is satisfactory level of quality, indicating good microbiological quality. Hence consuming raw milk at this stage may be harmless. But, in most cases milk reaches to the consumers at milk retailer points in which the milk is transported under unhygienic condition where no cooling device and adequate protective transportation means are unavailable. At this time the microbial count increases due to the cell division of the already existed microbes or due the introduction of other microbes from the external environment. Being a good quality at this stage may not guaranty to reduce the health effect of consuming raw milk. Rather, it requires care taken during the transfer and storage of milk until it reaches to the consumer (CFS, 2014).

In this study, the mean TMABC at dairy farm was 4.96 log CFU/ml. Slightly higher result (5.03 log CFU/ml) was reported by (Shah *et al.*, 2016). This may be due to the difference in climatic condition of the study area. Higher temperature favors the growth of bacteria and once the milk leaves the cow's udder they multiply quickly leading the spoilage of milk. But the lower result (2.62 log CFU/ml) was obtained by (Knight-jones *et al.*, 2016). This value is almost a half lower than the present study indicating good microbiological quality of raw cow milk which can be consumed without adverse health effect. Although (V, Millogo *et al.*, 2010) reported 6 log CFU/ml of TMABC in milk samples collected from a dairy farm. This may be due to seasonal variation in contamination level of raw milk.

The present study indicated that, the mean coliform bacteria count at dairy farm was 4.43 log CFU/ml. According to (Food Standards Australia New Zealand, 2018), it means that the quality is unacceptable. This may be due to inadequate processing or post processing contamination of raw milk from the external environment.

Slightly higher (4.66 log CFU/ml) value was reported by (Shah *et al.*, 2016). This may be due to the difference in climatic condition of the area. The high temperature of the milk leads to the elevation of the number of bacteria in milk. But the higher result (6.04 log CFU/ml) was obtained by (Lubote *et al*., 2014). The presence of coliform bacteria in dairy farm indicated that, the milk is contaminated with fecal matter associated with poor environmental hygiene condition including; using poor quality water, unhygienic milk handling practice and use of inadequately washed milk equipment.

In the present study, the mean TMABC at milk distribution centers was 6.29 log CFU/ml. The microbiological quality of raw milk collected from milk distribution centers is of borderline level of quality according to (CFS, 2014) indicating that the quality are not unsatisfactory but also not satisfactory, are at the upper limit of acceptability and which indicates the potential for development of public health problems and unacceptable risk. But lower, result (5.78 log CFU/ml and 5.63 log CFU/ml) was reported by (Knight-jones *et al.*, 2016) and (Amakelew *et al.*, 2015) respectively. The reason for this deviation may be due to the difference in efficiency of cleaning milk contact surface particularly milk transportation tanks and absence of cooling device to safely transport milk from the production area to the distribution centers. Also this may be due to the difference in sample size. Higher total bacteria count in milk distribution centers may indicate unhygienic collection, transportation and improper handling/washing of milking equipment. On the contrary, higher result (6.81 log CFU/ml) was reported by (Titouche *et al.*, 2016).

The current study showed that the mean TMABC of milk sample collected from milk retailer outlets was 7.25 log CFU/ml. Based on (CFS, 2014), the microbial quality of the samples collected from milk retailer outlets were unsatisfactory. This indicates that the milk is potentially injurious to health and/or unfit for human consumption and requires immediate

remedial action. This stage is important from a public health point of view since the majority of the individuals gets raw milk from selling points than any other milk production stages.

3.03 times higher result was reported by (Welearegay *et al*., 2012). It indicated that the milk is highly contaminated by microbial population. This might be due to seasonal variation. But, (Titouche *et al.*, 2016) reported 7.2 log CFU/ml of TMABC. Poor milking practice and using contaminated water may enhance the bacterial count in raw milk.

In the present study, 5.67 log CFU/ml of coliform bacteria was reported in milk distribution centers. This finding is almost similar to the study done by (Amakelew *et al.*, 2015), Who report 5.63 log CFU/ml. But lower, result (3.42 log CFU/ml). The higher coliform count in the present study may be due to the absence of cooling device to transport milk from the farm to the distribution centers. The presence of cooling device prevents the multiplication of bacteria in milk (Belbachir *et al*., 2015).

In the present study, the mean coliform count in milk retailer out let is 7 log CFU/ml. But, lower results (4.70 and 5.37 log CFU/ml) of coliform bacteria were reported by (Amakelew *et al.*, 2015) and (Shah *et al.*, 2016) respectively. Higher coliform count in milk selling point indicated that the overall hygienic condition undertaken from the milk produced in the farm until it reaches to the selling points.

Overall, the microbiological quality of raw cow milk decreases along the milk production chain. The mean total mesophilic aerobic bacterial count at dairy farm, milk distribution system and milk retailer outlets were 4.96, 6.29 and 7.25 log CFU/ml respectively. As the milk transport from dairy farm to milk distribution centers and from milk distribution centers to milk retailer points the mean total bacteria count increased by 1.33 and 0.96 log CFU/ml respectively. This is supported by (Knight-jones *et al.*, 2016) where the mean total mesophilic aerobic bacterial count increased by 3.15 when the milk goes from dairy farm to milk selling points. But (Shah *et al.*, 2016) reported that the bacterial count increased by 0.08 log CFU/ml as milk transported from dairy farm to milk selling points. Similarly, as the milk transport from dairy farm to milk distribution centers and from milk distribution centers to milk retailer points the mean coliform bacteria count increased by 1.24 and 1.33 log CFU/ml respectively.

The overall mean coliform bacteria in the present study were 5.70 log CFU/ml. According to (Food Standards Australia New Zealand, 2018) it is classified as unsatisfactory quality indicating that post processing contamination has occurred or there has been inadequate processing. Poor storage condition of the milk, absence of cooling device to transport milk and poor handling practice may be the contributing factors influencing milk coliform count. High coliform count in the present study indicated fecal contamination either animal or human occurred in the milk while it pass different stages of the production line. Environmental contamination may also cause the high count of coliform bacteria in raw milk according to (Shah *et al.*, 2016).

Overall, 48(32%) of the analyzed samples were unsatisfactory level of microbiological quality indicating test remedial action is required. But (Belbachir *et al.*, 2015) reported that about 75% of the analyzed samples were unsatisfactory level of contamination. Hence, consuming this raw milk may produce unwanted health consequence if precautionary measures are not taken.

Water used in the process of milk production should be bacteriologically potable. The purity of adequately treated water supply taken direct from the tap may be assured. But bacterial contamination can be introduced from storage tanks not properly protected against rodents, birds, insects and dusts. Therefore assuring the quality of water used to process milk play a vital role in producing safe milk in all stages of the milk production process. As suggested by (Esterhuizen, 2014), the water used to process milk should be safe as drinking water. In the present study, the presence of coliform bacteria in water has been identified as a risk factor associated with poor quality of raw milk. This is supported by (Torkar and Teger, 2004) who agreed that, the presence of coliform in water used to process milk was the major factors affecting microbial quality of raw milk.

The current Ethiopian drinking water quality guideline recommended that the water supply should be free from any fecal coliform bacteria, the presence of even one colony of fecal coliform bacteria makes the water unsafe for human consumption. Water used to wash milk equipment and other milk contact surfaces should follow the same quality issue that should

be potable as drinking water. In the present study all of the analyzed samples were positive for fecal coliform bacteria showing potential fecal contamination of the water from the surrounding environment (Ethiopian standards agency, 2013). But (Torkar and Teger, 2004) reported that, 67% of the analyzed sample were positive for total coliform bacteria. Similarly (Welearegay *et al.,* 2012) reported that, about 67% and 55% of the analyzed samples exceeds zero CFU/100 ml.

In the present study the overall total and fecal coliform bacteria in water samples were 4.4 and 3.32 log CFU/100ml. But higher result was obtained by (Trindade *et al.*, 2014) who reported that, the mean count of total coliform in water used to process food in school was 48 log CFU/100ml and only 3.1% of the analyzed samples were positive for coliform, indicating poor quality of water than in the present study. In the present study the correlation analysis between water, fecal coliform count and milk microbial quality showed excellent and positive correlation (r=-0.752), indicating that increasing fecal coliform count of water results in reduced quality of raw milk.

This study showed that the quality of stored water used to wash milk contact surfaces and hands of milk handlers contribute significantly (p<0.05) higher than other factors considered in the study. This idea is in agreement with (Pandey *et al.*, 2014) who confirmed that, water used to process milk contributes significantly (p<0.05) more microbial load from all possible sources of contamination. This is because of the fact that, storing water in the container can favors the growth of bacteria. Using such water in milking activity resulted in the transfer of bacteria from the water to the milk. In the present study, the majority of the milk processor used plastic container for storing water. Perhaps this may be the major factors which rise the number of bacteria count in water samples.

Inadequately cleaned milking equipment, milk storage tanks and other milk contact surfaces may also contribute to the elevation of bacterial count in raw milk due to cross contamination. This study showed that all the analyzed samples were positive for coliform bacteria. But (Torkar and Teger, 2004) reported that, only 12% of the analyzed swab samples were positive for coliform bacteria. The difference for this deviation may be in the study area only one third

of the respondents use detergent to wash milk equipment which may cause for the deterioration of microbial quality of raw milk (Torkar and Teger, 2004).

Similarly (Trindade *et al.,* 2014) identified that, 40.7% and 3.3% of the milk surface samples contaminated by coliform and *E. coli* bacteria respectively. In the present study the overall mean coliform count of milk contact surfaces was 3.16 log CFU/cm^2. But, (Gwandu *et al.*, 2018) reported that, 0.089 log CFU/cm^2 coliform bacteria in swab samples. The difference in quality of milk contact surfaces may be due to the difference in the quality of water used to wash milk equipment, due to the difference in washing technique, whether the detergent used or not and whether they used warm water to wash milk equipment.

The poor milk handling practice may contribute to high post-harvest losses. The poor handling practice has been reported to be associated with poor knowledge and practices of food hygiene and safety among milk handlers along the milk supply chain. This study showed that 28%, 11.3% and 11.3% of the respondents had a good level of knowledge, attitude and practice respectively. The overall mean score of knowledge, attitude and practice of the respondents were 62, 58 and 57.4 respectively. Better result was reported by (Norhaslinda *et al.*, 2016). As shown in this study, the mean score of knowledge, attitude and practice of food handlers were 83, 87.2 and 90.7 respectively. This difference may be due to the fact that in the present study majority of the food handlers did not attend an education. Also (Sharif *et al.*, 2013) reported that the mean percentage score of knowledge, attitude and practice of 84.82, 88.88 and 89.43 respectively. And the overall mean percentage score of knowledge, attitude and practice was 87.88.

Almost similar result was obtained by (Odongo *et al.*, 2017) who reported that the average score of knowledge of milk handlers was 62%. Educational status of milk handler may influence the quality of the milk at each of the milk production chain. In the present study educational status of milk handlers significantly contribute to the reduced quality of raw milk in the study area. As the educational status of the milk handlers increased by one unit, the microbial quality of raw cow milk increased by 0.187. The present finding is supported by (L, Mansouri -Najand and Z, Rezaii., 2015).who showed that, the microbial quality of raw milk

with the owner who had below high school diploma lower microbiological quality of raw milk by 1.40% compared to those who had finished.

The present study confirmed that attitude of the respondents greatly influences the microbial quality of raw cow milk. This issue is supported by (Munera-Bedoya *et al.*, 2017) in Brazil who showed, attitude of milk handlers contribute to the raised count of bacterial population in raw milk.

6.1 Limitation of the study

This study mainly focused on the isolation and enumeration of total mesophilic aerobic bacteria and coliform bacteria count in raw cow milk sample and determination of microbiological quality of water and swab sample during the short rainy season of the year. However, microbiological quality may vary between wet and dry seasons. Hence, future researcher may be advised to investigate the quality of milk in both seasons of the year.

The isolation and enumeration of *E. coli* O157:H7 in raw cow milk in the present study was based on the presumptive test. Hence, further biochemical and serological test should be done to confirm the pathogenic strain of *E. coli* and the type of toxin produced from them.

Additionally, quantification of pathogenic bacteria relevant in dairy industry should be investigated.

CHAPTER SEVEN

7. CONCLUSION AND RECOMMENDATION

7.1 Conclusion

Microbiological quality of raw cow milk reduces significantly after sending off by farmers. Initially the microbiological quality of raw cow milk was good, but after it reaches to the retailer outlets the quality becomes poor. Overall about one third of the milk samples grouped under unacceptable level of microbial quality for total mesophilic aerobic bacterial count that require remedial action.

Also more than 90% of the analyzed milk samples classified as unacceptable level of quality in terms of coliform bacteria, indicating the possible contamination of raw milk by fecal matter. Generally the milk produced, distributed and sold in the study area can be considered as poor in terms of total mesophilic aerobic bacteria count.

The microbiological quality of water used to wash milk contact surfaces and hands of milk handlers can also be considered as poor quality in which all of the analyzed samples were positive for fecal coliform bacteria. Similarly the microbiological quality of milk contact surfaces was poor.

The result from multiple linear regression analysis confirmed that the educational status of milk handlers, attitude of milk handlers and the quality of water used to wash milk contact surfaces and hands of milk handlers were the major factors affecting microbiological quality of raw cow milk along the dairy value chain in the study area.

7.2. Recommendation

Based on the finding obtained the following recommendation were forwarded.

Dairy farmers and business owners should:

➢ Clean the water storage tanks adequately and make covering it
➢ Provide personal protective equipment for workers.
➢ Provide adequate detergent to wash milking equipment, milk storage tanks, hands of milk handlers and teats and udder of the cow.
➢ Promote the use of warm water with detergent to wash milk equipment and teats and udder of the cow.
➢ Arrange training for milk handlers in collaboration with other stakeholders.

Zone livestock and fish resource development should:

➢ Provide veterinarian service like counselling and training for farmers and milk handlers
➢ Arrange regular milk quality assessment and take measures when necessary.

Jimma zone water and sewerage office should:

➢ Provide training and other awareness creation program about the safe storage and utilization of water to prevent cross contamination.
➢ Conduct water quality assessment from water storage tanks in milk processing areas and when necessary to support farmers and milk handlers to be aware of the negative consequence of using stored water both from a health perspective and economic loss.

Jimma zone health office should:

➢ Assess the quality of milk to prevent the health of the community from milk borne illness.

➢ Provide training for dairy farm owners, milk handlers and consumers about the care taken during milk handling.

➢ Enable the community to be aware of the negative consequence of consuming raw milk.

➢ Educate the community about the importance of boiling milk before consumption.

➢ Inspect the cleaning efficiency of milk utensils and provide guidance to enable them to use three dish washing system.

➢ Arrange training and awareness creation program to enhance the attitude towards milk handling in collaboration with business owners and other responsible body.

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ANNEXES I

Questionnaire

Consent form

My name is Leykun Berhanu MSc student in Environmental health science and technology department, Jimma University. I am working my thesis on issue entitled "***Microbiological quality of raw cow milk and associated factors along the dairy value chain in Jimma zone southwest Ethiopia***". The main objective of the study is to determine microbial quality of raw cow milk and associated factors along the dairy value chain in Jimma zone south west Ethiopia.

KAP of milk handlers may affect the quality of raw milk along the supply chain. The questionnaire will consist of variables including socio-demographic information, knowledge, attitude and practices of milk handlers on the hygienic food handling practices.

Your answers will be recorded on a survey questionnaire. No personal identifiers will be recorded to the interview. All the data obtained will be kept strictly confidential by using only code numbers. Your participation in the study is upon purely voluntary basis. Your information is vital without which the realization of the research would be impractical. And also what we learn from this study will be used to generate information necessary for the prevention and control of food borne illnesses.

The interview will take 15-20 minute. During the interview period, if you feel inconvenient, you can interrupt and clarify inconvenience, appoint to other time or even withdraw any time after you get involved in the study.

If yes go on.   If no stop

Thank you for your cooperation!!!

Questionnaire developed to assess KAP of milk handlers

General information

Respondent's number_____

District:_____Town_____Kebele_____.

Data collector's name:_____

Knowledge, attitude and practices of milk handlers on hygienic handling practices

A. Socio-demographic characteristics of respondents

1. Sex: A. Male   B. Female

2. Age: A. 18-35   B. 35-50   C. >50

3. Educational status: A. No education   B. Elementary   C. Secondary   D. Tertiary

4. Marital status: A. Single   B. Married   C. Divorced   D. Widowed

5. Religion: A. Christian   B, Muslim   C. Other (specify)_____

6. Income: A. In cash (specify)_____B. In kind (specify)_____

7. Ethnicity: A. Oromo   B. Amara   C. Dawuro   D. Gurage   E. Gumuz   F. Yem

8. How long have you been involved in milk handling (in years)?

A.   <1   B. 1-2   C. 3-5   D. 6-10   E. >10 (specify)_____

B. Questions developed to assess knowledge of milk handlers

1. Wearing gloves is one part of personal hygiene   A. Yes   B. No

2. Wearing apron is one part of personal hygiene.   A. Yes   B. No

3. Wearing cap is one part of personal hygiene.   A. Yes   B. No

4. Wearing mask is one part of personal hygiene.   A. Yes   B. No

5. Washing hands regularly before work is one part of personal hygiene.

 A. Yes   B. No

6. Washing hands regularly after work is one part of personal hygiene.

A. Yes   B. No

7. Washing hands regularly after hand contamination is one part of personal hygiene.

A. Yes B. No

8. Washing hands properly reduce risk of contamination.   A. Yes   B. No

9. Washing hands with only water is not clean enough.   A. Yes   B. No

10. Employees should avoid touching their hair after washing hands.

A. Yes   B. No

11. Employees cannot wear adornments. A. Yes   D. No

12. Employees cannot have long nails and make coloring it.   A. Yes   B. No

13. When employees have wound on hands, use plaster and not touch milk directly

A. Yes   B. No

14. Contamination is the transfer of harmful microorganisms to food from other foods or non-food-contact surfaces.   A. Yes   B. No

15. Use of gloves reduces the risk of transmitting infection to consumers.

 A. Yes   B. No

16. If gloves are broken, you need to change new one.   A. Yes   B. No

17. Using hot water to clean equipment decrease risk of contamination.

   A. Yes   B. No

18. Equipment such as serving jugs can transfer diseases.   A. Yes   B. No

19. Equipment such as drinking cups can transfer diseases.   A. Yes   B. No

20. Cleaning equipment after work can reduce cross contamination.

 A. Yes   B. No

21. Separating dirty and clean zone helps to reduce cross contamination

A. Yes   B. No

22. Diarrhea is a disease which occurred when people eat unclean food.

A. Yes B. No

23. Diarrhea can be transmitted from people to others.   A. Yes   B. No

24. Food borne illness can be caused by bacteria only.   A. Yes   B. No

25. Time is one of the important factors to control growth of bacteria.

A. Yes   B. No

26. Temperature is one of the important factors to control growth of bacteria.

A. Yes   B. No

27. Bacteria in milk cannot overgrowth at 4°C (refrigeration).   A. Yes   B. No

28. Chilling process cannot kill any bacteria.   A. Yes   B. No

29. Cooking can destroy bacteria.   A. Yes   B. No

C. Questions developed to assess attitude of milk handlers

Please show your position of agreement for the following statements encircling A for "agree strongly", B for "agree", C for "neutral", D for "disagree", and E for "strongly disagree"

1. Safe milk handling is an important part of my job responsibility.

A. Strongly agree   B. Agree   C. Neutral   D. Disagree   E. Strongly disagree

2. I will change my milk handling behavior when if I know it is incorrect.

A. Strongly agree   B. Agree   C. Neutral   D. Disagree   E. Strongly disagree

3. I believe food safety knowledge will benefit to my personal life.

A. Strongly agree   B. Agree   C. Neutral   D. Disagree   E. Strongly disagree

4. I believe food safety knowledge will benefit to consumers.

A. Strongly agree   B. Agree C. Neutral   D. Disagree   E. Strongly disagree

5. Producing safe food is more important than tasty food.

A. Strongly agree   B. Agree C. Neutral   D. Disagree   E. Strongly disagree

6. I believe good personal hygiene can prevent food-borne illness.

A. Strongly agree   B. Agree   C. Neutral   D. Disagree   E. Strongly disagree

7. Washing hand before handling milk reduces risk of food poisoning.

A. Strongly agree   B. Agree   C. Neutral D. Disagree   E. Strongly disagree

8. Worker should make sure that their nails are short and clean.

A. Strongly agree   B. Agree   C. Neutral   D. Disagree   E. Strongly disagree

9. Workers with abrasion or cuts on fingers and hands can handle milk without gloves.

A. Strongly agree   B. Agree C. Neutral   D. Disagree   E. Strongly disagree

10. I come to work even I get sick, fever or catch cold.

A. Strongly agree   B. Agree   C. Neutral   D. Disagree   E. Strongly disagree

11. Diarrhea does not affect my job.

A. Strongly agree   B. Agree C Neutral   D. Disagree   E. Strongly disagree

12. Using mask is important in reducing risk of food contamination.

 A. Strongly agree   B. Agree   C. Neutral   D. Disagree   E. Strongly disagree

13. Using apron is important in reducing risk of food contamination.

A. Strongly agree   B. Agree   C. Neutral   D. Disagree   E. Strongly disagree

14. Using cap is important in reducing risk of food contamination.

A. Strongly agree   B. Agree C. Neutral   D. Disagree   E. Strongly disagree

15. Using gloves is important in reducing risk of food contamination.

A. Strongly agree   B. Agree   C. Neutral   D. Disagree   E. Strongly disagree

16. The use of adornments, such as earrings, rings and watches, cannot cause food contamination.

A. Strongly agree   B. Agree   C. Neutral   D. Disagree   E. Strongly disagree

17. It is necessary to check temperature settings of chillers

A. Strongly agree   B. Agree   C. Neutral   D. Disagree   E. Strongly disagree

18. I care more about cheap price than about good quality milk.

A. Strongly agree   B. Agree   C. Neutral   D. Disagree   E. Strongly disagree

19. You can tell if milk is safe to eat by looking at it

A. Strongly agree   B. Agree   C. Neutral   D. Disagree   E. Strongly disagree

20. I will complain to the farm/distributor if there is a problem with milk

 A. Strongly agree   B. Agree   C. Neutral   D. Disagree   E. Strongly disagree

D. Questions developed to assess practice of milk handlers

Please show your position of practice encircling anyone of the following alternatives against the questions below

1. Do you wash your hands before processing milk?

A. Always   B. Often   C. Sometimes   D. Rarely   E. Never

2. Do you use detergent to wash your hands?

A. Always   B. Often   C. Sometimes   D. Rarely   E. Never

3. Do you wash milk equipment?

A. Always   B. Often   C. Sometimes   D. Rarely   E. Never

4. Do you use detergent to wash milk equipment?

A. Always   B. Often   C. Sometimes   D. Rarely   E. Never

5. Do you keep your nails short?

 A. Always   B. Often   C. Sometimes   D. Rarely   E. Never

6. Do you remove all adornments before starting activities?

 A. Always   B. Often   C. Sometimes   D. Rarely   E. Never

7. Do you handle food at work when you have diarrhea?

 A. Always   B. Often   C. Sometimes   D. Rarely   E. Never

8. Do you handle food at work when you have abrasions or cuts on your hands?

 A. Always   B. Often   C. Sometimes   D. Rarely   E. Never

9. Do you wash your hands after go to toilet?

A. Always   B. Often   C. Sometimes   D, Rarely   E. Never

10. Do you use mask at work daily?

A. Always   B. Often   C. Sometimes D. Rarely   E. Never

11. Do you use apron at work daily?

 A. Always   B. Often   C. Sometimes   D. Rarely   E. Never

12. Do you use cap at work daily?

A. Always   B. Often   C. Sometimes   D. Rarely   E. Never

13. Do you use gloves at work daily?

A. Always   B. Often   C. Sometimes   D. Rarely   E. Never

14. Do you take a physical examination every year?

A. Always   B. Often   C. Sometimes   D. Rarely   E. Never

Annex II

Culture media composition and preparation

1. Plate Count Agar

Composition	g/l
Casein peptone	5
Yeast extract	2.5
Dextrose	1.0
Agar	9

Final pH 7.0 ±0.2 at 25ºC

Source: www.grosseron.com/oo/Assets/client/GROSSERON/FT/FT9010032. Accessed on *9/18/2018.*

Preparation

Plate count agar was used for the enumeration of total mesophilic aerobic bacterial count. The media was prepared according to the instructions given by manufacturers in which 17.5 gram of PCA powder (ATICO, INDIA) was dissolved in 1000 ml of distilled sterilized water. Then it was heated to dissolve the powder completely. And it was sterilized by autoclaving at 121℃ for 15 minutes. Finally the media was dispensed on sterile petri-dish.

Source:*www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0325&c=UK.* Accessed on 9/18/2018.

2. MacConkey sorbitol agar

Composition	gram/liter
Peptone (pancreatic digest of gelatin)	17
Protease peptone (meat and casein)	3
Sorbitol	10
Bile salt	1.5
Neutral red	0.03
Sodium chloride	5.0
Crystal violate	0.001
Agar	13.5

Final pH 7.1±@25°C.

Preparation

For the selective isolation and enumeration of *E. coli* O157:H7 in milk sample MacConkey sorbitol was used. The preparation follows the instruction given by the manufacture (SRL, India) in which 50 gram of the powder was dissolved into 1000 ml distilled water. Then the mixture was heated to dissolve completely. Then it was sterilized by autoclaving at 121℃ for 15 minutes. Finally the media was dispensed in sterilized Petri dish.

Source: www.himedialabs.com/TD/M298. Accessed on 9/18/2018

3. M-lauryl sulfate broth

Composition	gram/liter
Peptic digest of animal tissue	39
Yeast extract	6.0
Lactose	30.0
Sodium lauryl sulfate	1.0
Phenol red	0.20

Final pH 7.4±0.2 at 25°C

Preparation

For the isolation and enumeration of *E. coli* and coliform in water sample M-lauryl sulfate broth (Fluka, Analytica, India) was used. The media was prepared according to the instruction given on the package. Accordingly 76.2 gram of the powder was dissolved in 1000 ml sterilized water and sterilized by autoclaving at 121℃ for 15 minute. After sterilization about 0.2 ml of the broth was dispensed on the absorption pad.

*Source:*www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=MM0615&c=UK. *Accessed on 9/18/2018.*

4. Eosin Methylene Blue Agar

Composition	gram/ liter
Peptic digest of animal tissue	10.0
Lactose	10.0
Dipotassium hydrogen phosphate	2.0
Eosin – Y	0.40
Methylene blue	0.065
Agar	15.0

Final pH (at 25°C) 6.8±0.2

Preparation

It is slightly selective media for the isolation of *E. coli* from milk samples. The media was prepared according to the manufacturer's instruction given on it. Here, 37.5 gram of EMB powder (Accumix, Belgium) was dissolved in 1000 ml of distilled water. Mixed well and boiled to dissolve the media. Then the media was sterilized by autoclaving. Finally the media was dispensed on sterilized petri-dish.

Source:www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM0069&org=66. Accessed on 9/18/2018.

5. Buffered Peptone water

Is a pre-enrichment medium designed to help recovery of sub-lethally damaged bacteria before transfer to a selective medium. This pre-enrichment medium is free from inhibitors and is well buffered and provides conditions for resuscitation of the cells that have been injured

by processes of food preservation. Buffered Peptone Water during the pre-enrichment period helps in recovery of injured cells that may be sensitive to low pH.

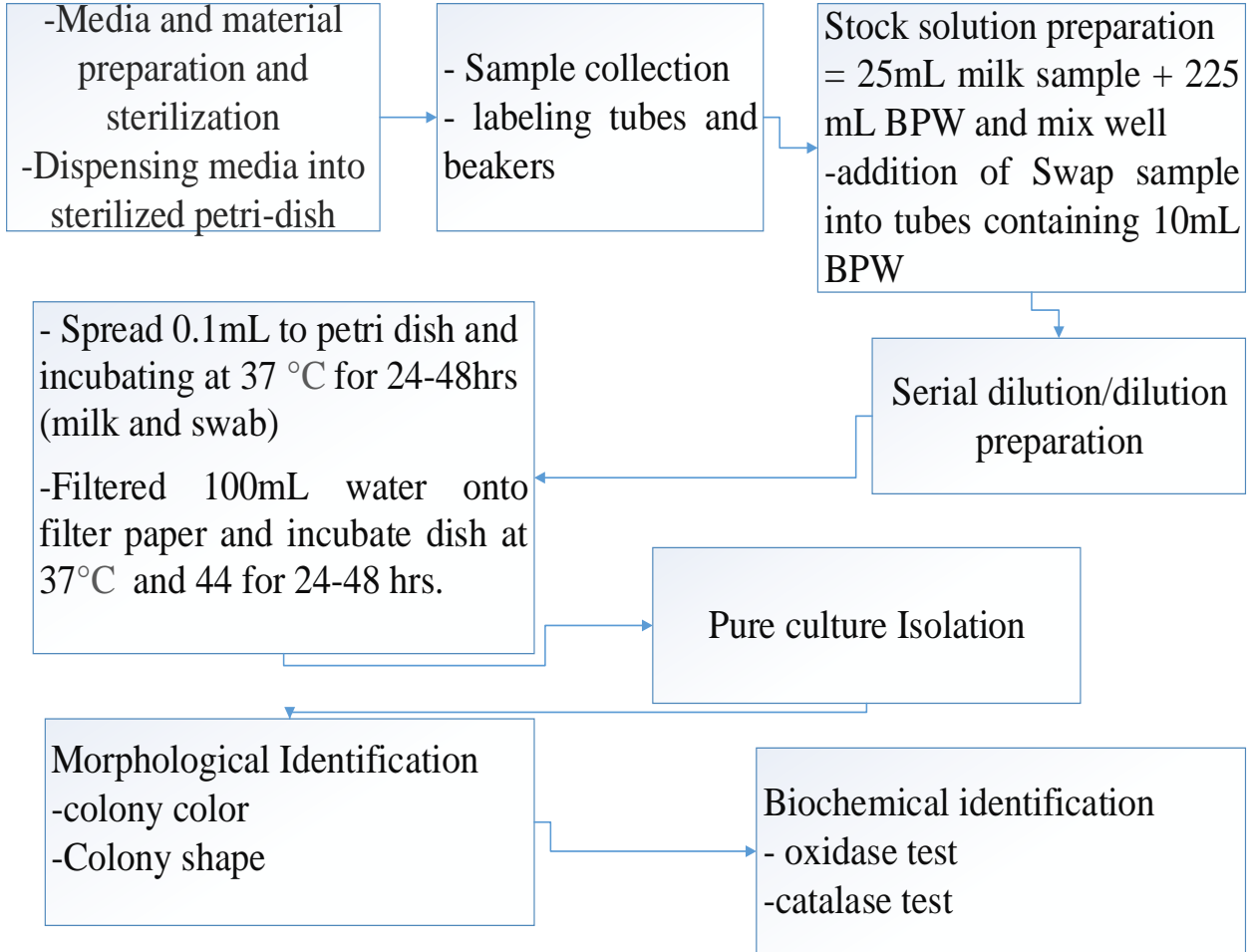| Composition	gram/liter
Peptone (Accumix, Belgium)	1.0
Sodium chloride (alpha chemica, India)	4.3
Disodium hydrogen phosphate	7.20
Potassium di-hydrogen phosphate (UNI-CHEM)	3.60

Final pH 7.0±0.2 @25^{0}C

Preparation

Buffered peptone water was prepared by adding 1 gram of peptone, 4.3 gram of NaCl, 7.20 gram of disodium hydrogen phosphate (Na$_2$ HPO$_4$ anhydrous) and 3.60 gram of potassium di-hydrogen phosphate (KH$_2$ PO$_4$, anhydrous) into beaker containing 1000 ml distilled water. The buffered peptone water was then sterilized by autoclaving at 121^{0}C for 15 minutes.

Source: www.himedialabs.com/TD/MH1275. Accessed on 9/18/2018

ANNEX III

Laboratory procedure followed during sample analysis

| -Media and material preparation and sterilization -Dispensing media into sterilized petri-dish | → | - Sample collection - labeling tubes and beakers | → | Stock solution preparation = 25mL milk sample + 225 mL BPW and mix well -addition of Swap sample into tubes containing 10mL BPW |

- Spread 0.1mL to petri dish and incubating at 37 °C for 24-48hrs (milk and swab)

-Filtered 100mL water onto filter paper and incubate dish at 37°C and 44 for 24-48 hrs.

Serial dilution/dilution preparation

Pure culture Isolation

Morphological Identification
-colony color
-Colony shape

Biochemical identification
- oxidase test
-catalase test

ANNEX IV

Pictures showing sample analysis

Spreading 0.1 ml sample dilution into the macConkey sorbitol dispensed Media
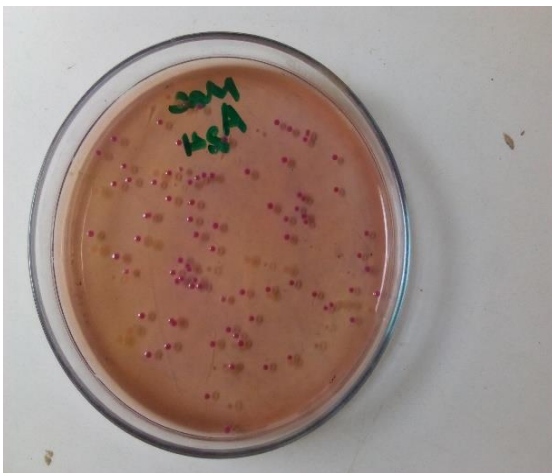
Growth of *E. coli* O157:H7 in
MacConkey sorbitol agar
Colorless colony (presumptive *E. coli*
O157:H7)
-Pink/red colony (Coliform bacteria)
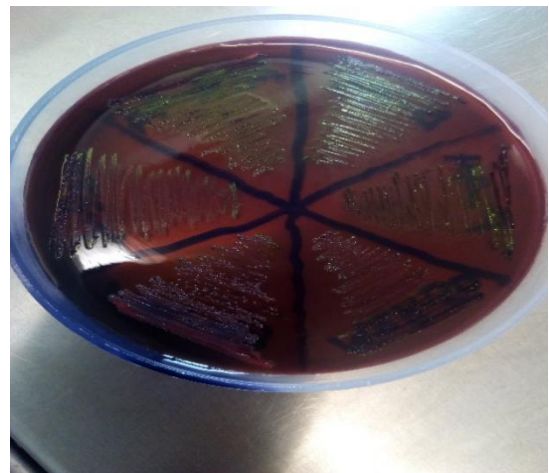
Growth of *E. coli* O157:H7 in EMB agar
Green metallic Sheen colonies are *E. coli* -
O157:H7

Annex V

Microbiological guide line for the interpretation of results for TMABC (CFS, 2014)

Food Category [a]	Examples	Result (colony-forming unit (cfu)/g)		
		Satisfactory	Borderline	Unsatisfactory
1. Ambient stable canned, bottled, cartoned and pouched foods immediately after removal from container [b]	Canned products such as tuna, salmon, corned beef, soups, stews, desserts and fruit; ultra-high-temperature (UHT) products	<10	N/A	Note [c]
2. Foods cooked immediately prior to sale or consumption	Takeaway food, burgers, kebabs, sausages, pizza, ready meals (cook/chill and cook/freeze) after regeneration, dim sum, rice, noodles	$<10^3$	$10^3 - <10^5$	$\geq 10^5$
3. Cooked foods chilled but with minimum handling prior to sale or consumption; canned pasteurised foods requiring refrigeration	Whole pies, sausage rolls, samosas, flans, quiches, chicken portions; canned ham requiring refrigeration, pasteurised foods including fruit juice and soups; desserts	$<10^4$	$10^4 - <10^7$	$\geq 10^7$
4. Bakery and confectionery products without dairy cream, powdered foods	Cakes without dairy cream, soup powders, milk powder, powdered dairy products, other reconstituted powdered foods ready to eat after reconstitution or warming	$<10^4$	$10^4 - <10^6$	$\geq 10^6$
5. Cooked foods chilled but with some handling prior to sale or consumption	Sliced meats, cut pies, pâté, sandwiches without salad, hot smoked fish (mackerel, etc.), molluscs, crustaceans and other shellfish out of shell, non-prepackaged cold beverages with solid ingredients but without dairy components (iced green tea with red bean, etc.)	$<10^5$	$10^5 - <10^7$	$\geq 10^7$
6. Non-fermented dairy products and dairy desserts, mayonnaise and mayonnaise based dressings, cooked sauces	Most butter, fresh cheese (mascarpone, paneer), trifle with dairy cream, satay, cakes with dairy cream, non-prepackaged cold beverages with solid ingredients and dairy components (iced milk tea with pearl tapioca, etc.)	$<10^5$	$10^5 - <10^7$	$\geq 10^7$
7. Food mixed with dressings, dips, pastes	Coleslaw, dips, taramasalata, houmous	$<10^6$	$10^6 - <10^7$	$\geq 10^7$
8. Extended shelf life food products requiring refrigeration	Modified atmosphere packaging (MAP) or vacuum packed products, e.g. meat, fish, fruit and vegetables	$<10^6$	$10^6 - <10^8$	$\geq 10^{8\,d}$
9. Raw ready-to-eat meat and fish, cold smoked fish	Sushi, sashimi, smoked salmon, gravalax	$<10^6$	$10^6 - <10^7$	$\geq 10^7$
10. Preserved food products – pickled, marinated or salted	Pickled or salted fish, cooked shellfish in vinegar, vegetables in vinegar or oil, herbs, spices	N/A	N/A	N/A
11. Dried foods	Fruits, berries, vine fruits, nuts, sunflower seeds, herbs, spices, dried fish	N/A	N/A	N/A
12. Fresh fruit and vegetables, products containing raw vegetables	Whole fruit, pre-prepared fruit salads, vegetable crudités, salads, sandwiches with salad, mixed commodity salads containing raw vegetables, non-prepackaged cold beverages with solid and fresh fruit ingredients (chilled fresh mango juice with pomelo and sago, etc.)	N/A	N/A	N/A

Annex VI

Microbiological guide line for interpretation of results for indicator organisms in ready to eat food in general (Australia New Zealand, 2018)

Indicator	Result (cfu/g)	Interpretation
Enterobacteriaceae[a] (includes coliforms)	>10^4	Unsatisfactory
	$10^2 - 10^4$	Marginal
	<10^2	Satisfactory
Escherichia coli[b]	>10^2	Unsatisfactory

Annex VII

Ethiopian drinking water quality guide line for hygiene indicator bacteria

| Organism	Maximum permissible level	Test method
Total viable organisms, colonies per ml	must not be detectable	ES ISO 4833
Faecal streptococci per 100ml	must not be detectable	ES ISO 7899-1 ES ISO 7899-2
Coliform organisms, number per 100 ml	must not be detectable	ES ISO 9308-1
E. Coli, number per 100 ml	must not be detectable	ES ISO 9308-1 ES ISO 9308-2

Annex VIII

Examples showing the fulfilment of assumption of data

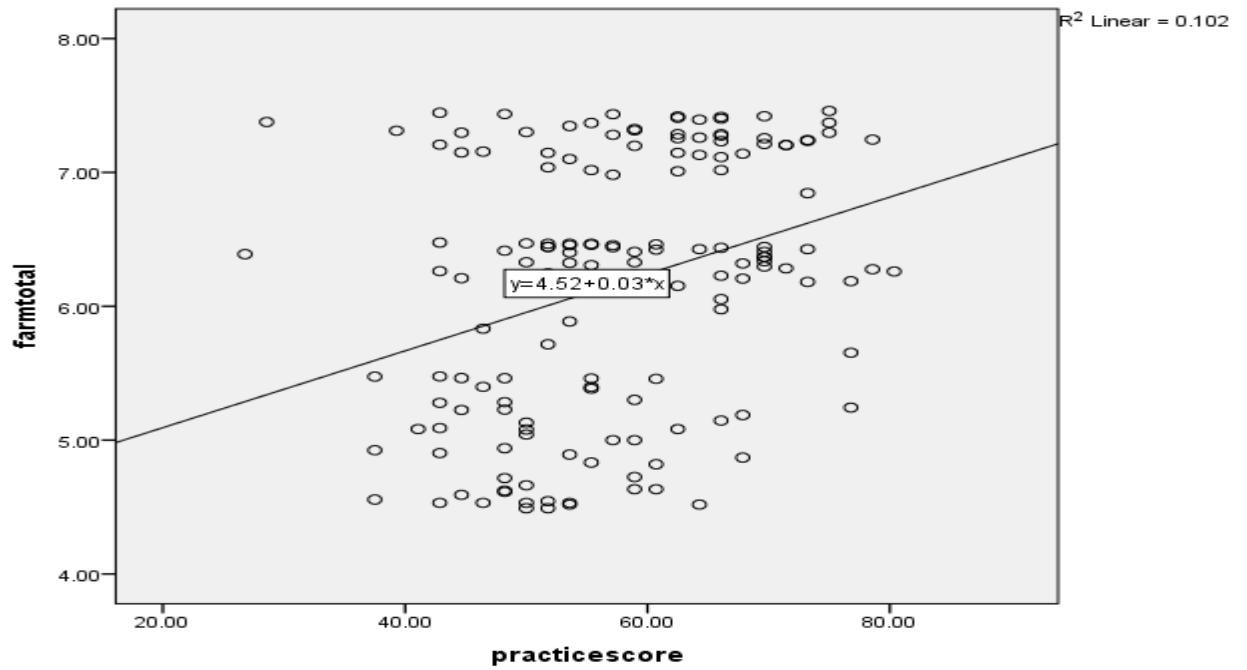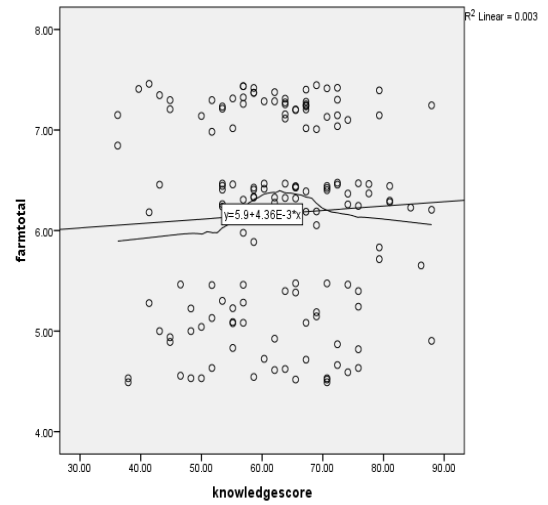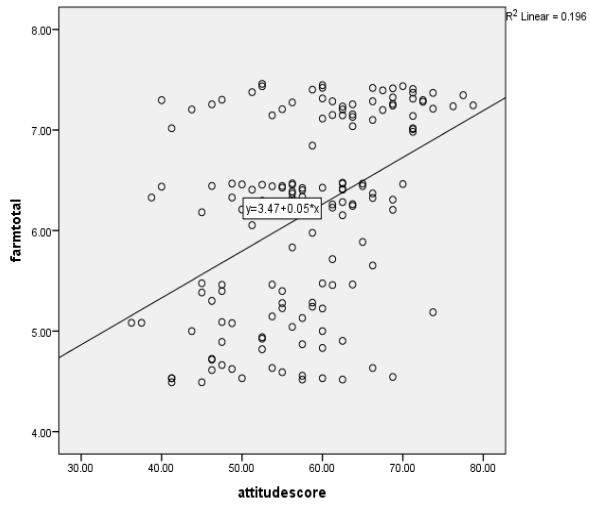## A. Normality
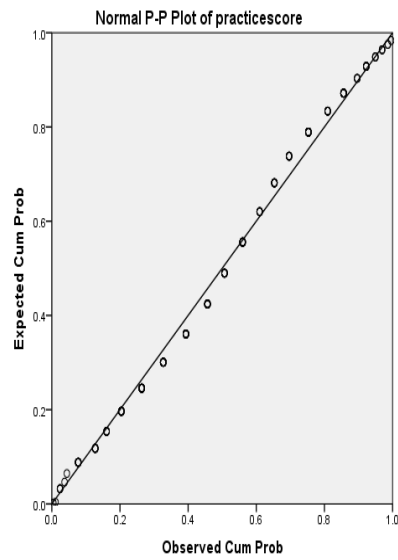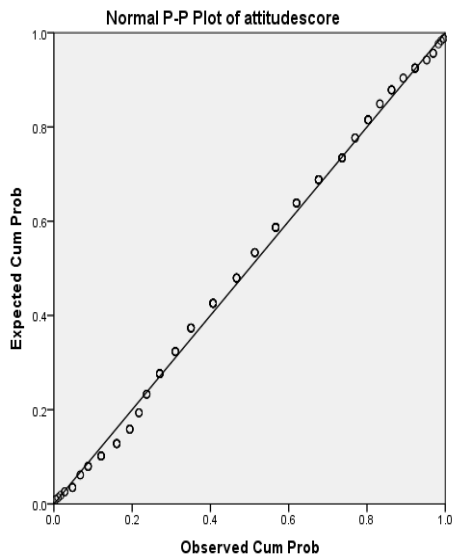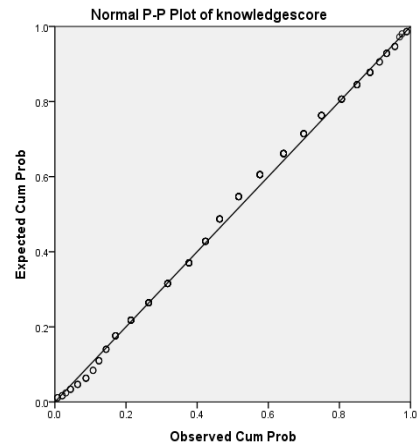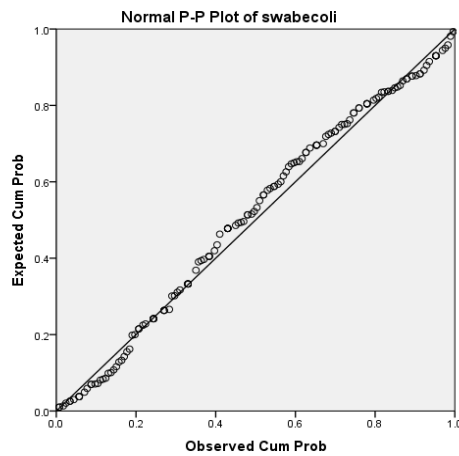
B. Outlier
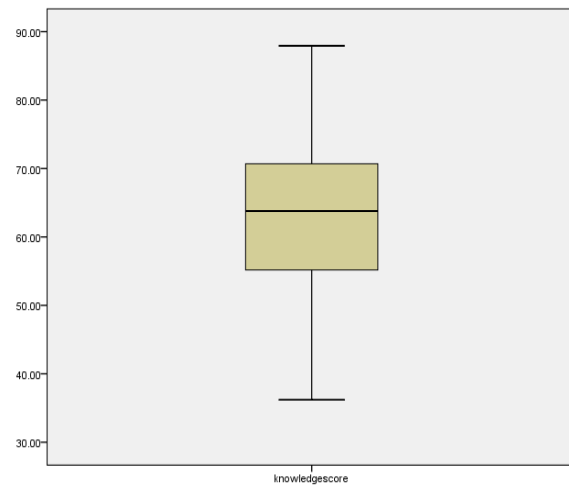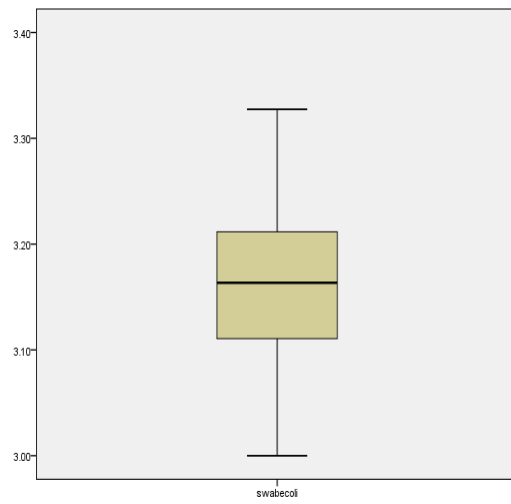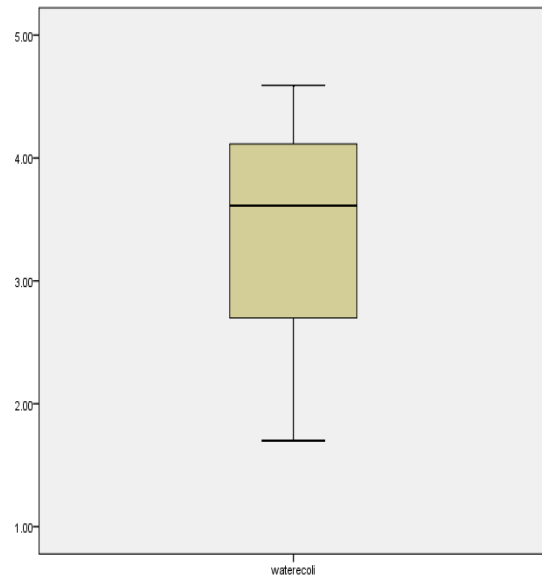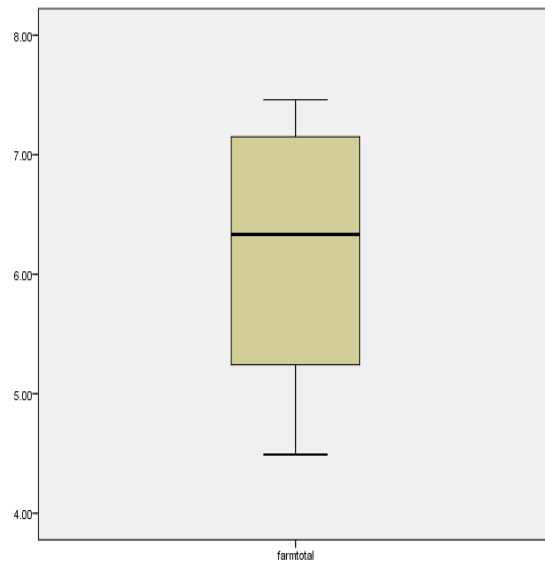
C. **Linearity between the data set**

D. Multicollinearity

The presence of the effect of one explanatory variables on other explanatory variables was assessed. The following table showed the collinearity test. As shown below the tolerance and the variance inflation factors indicated that there is no multicollinearity problem in the data set.

Tolerance should be >0.1 (VIF should be less than 10)

T=1/VIF

Source:

www.statisticssolutions.com/the-multiple-linear-regression-analysis-in-spss/. Accessed on October, 2018.

Model	Collinearity Statistics	
	Tolerance	Variance inflation factor
(Constant)		
Sex	0.803	1.246
Age	.753	1.328
Educational status	.785	1.275
Marital status	.817	1.223
Religion	.956	1.046
Ethnicity	.908	1.102
Milk handling experience	.859	1.164
Swab coliform count	.884	1.131
Water coliform count	.710	1.409
Knowledge about milk handling	.773	1.294
Attitude towards milk handling	.716	1.396
Milk handling practice	.838	1.194

Annex VIIII

Material used

70% alcohol

Absorbent pad

Autoclave (Astell, England)

Beaker with a capacity of 250 ml

Colony counter (Gallen hamp colony counter, England)

Container with icepacks

Continue paper

Cylinder gas

Electrical pump

Examination glove

Filtration apparatus

Forceps

Graduated measuring cylinder with a capacity of 100 ml

Incubator

Inoculating loop

Marker

Membrane filter paper (0.45 micro meter pore size)

Micro pipette (Eppendorf)

Microbiological safety cabinet (BDK, Genkingen)

Microscope

Petri dish

Petri dishes both small and normal size

Pipette tip both one ml and 100 micro liter

Refrigerator

Spreader

Sterilization indicator

Sterilized sample bottle

Test tube rack

Test tubes

Vortex mixer (Fisher Scientific, USA)

Water bath (London)

Weighting balance (Mettler Toledo, Switzerland)