

# **Types and Load of Bacteria Contaminating Drinking Water in Dairy Farms in Khartoum North**

*By*

**Shirin Babeker Alamen Babeker**

(BVM, University of Khartoum, 2003)

*Supervisor:*

**Dr. Elhassan Mohammed Ali Saeed**

(BVSc, MVSc, PhD)

A Dissertation Submitted to the University of Khartoum in Partial  
Fulfillment for the Requirements of the Degree of Master of  
Tropical Animal Health (MTAH)

Department of Preventive Medicine and Public Health

Faculty of Veterinary Medicine

University of Khartoum

**June 2010**

# DEDICATION

*To my mother & father who*

*constantly strive to better*

*my live*

*To my sisters who encouraged and helped me*

*To all my friends*

*To Alamin Hassan Eltayeb family*

*I dedicate this work*

## **Acknowledgements**

I would like to express my thanks to Almighty Allah, who gave me health and patience to achieve this work.

Also, I am greatly indebted to my supervisor Dr. Elhassan Mohammed Ali Saeed, for his supervision and advice through this work.

My thanks are extended to the staff members of the Department of Preventive Medicine and Public Health, Faculty of Veterinary Medicine, University of Khartoum for allowance to use the facilities of the department and for their technical assistance, especially Mr. Hussain Abdel Rahim.

My great thanks are due to Dr. Selma Kamal (Central Veterinary Research Laboratories, Soba) for her advice and academic support.

Last but not the least, my deep and sincere thanks to my best friends, Maiy Mahmoud, Abuzar Alamin and Sameer Mosa for their encouragement, support and help.

## List of Contents

<b>Subject</b>	<b>Page</b>
Dedication	I
Acknowledgements	II
List of contents	III
List of tables	VIII
List of figures	IX
Abstract	X
Abstract (Arabic)	XII
Introduction	1
<b>Chapter one: Literature review</b>	<b>3</b>
1.1 Water for dairy cattle	3
1.1.1 Water definition	3
1.1.2 Water intake	3
1.1.3 Water type	4
1.1.4 The need for water	5
1.1.5 Quality of drinking water and its effect on performance of dairy cattle	5
1.2 Bacterial contamination of drinking water	7
1.2.1 Sources of bacterial contamination	8

1.2.2 Sources of bacterial contamination in dairy cattle water troughs	9
1.2.3 Factors influence the survival rate of bacteria in water troughs	9
1.3 Bacterial water-borne diseases and agents	10
1.3.1 <i>Escherichia coli</i>	10
1.3.2 <i>Salmonella</i> spp.	11
1.3.3 <i>Corynebacterium</i> spp.	11
1.3.4 <i>Shigella</i> spp.	12
1.3.5 <i>Bacillus</i> spp.	12
1.3.6. <i>Aeromonas</i> spp.	12
1.3.7 <i>Staphylococcus</i> spp.	12
1.3.8 <i>Streptococcus</i> spp.	13
1.3.9 <i>Proteus</i> spp.	13
1.3.10 <i>Klebsiella</i> spp.	13
1.4 How water transfers bacterial diseases?	13
1.5 Prevention of bacterial water-borne diseases in dairy cattle farms	14
1.5.1 General control measures	14
<b>Chapter two: Materials and Methods</b>	<b>15</b>
2.1 The study area	15

2.2 Samples	15
2.2.1 Size	15
2.2.2 Collection method	15
2.2.2.1 Collection of samples from the main sources of water	16
2.2.2.1.1 Collection of samples from tap water	16
2.2.2.1.2 Collection of samples from wells	16
2.2.2.2 Collection of samples from storage places	16
2.2.2.3 Collection of samples from surface of water in troughs	17
2.2.2.4 Collection of samples from walls of water troughs	17
2.2.3 Transportation of samples	17
2.3 Sterilization	17
2.3.1 Flaming	17
2.3.2 Hot air oven	18
2.3.3 Red heat	18
Autoclaving (Moist heat)	18
Disinfection	18
2.5. Culture media	18
2.5.1 Solid culture media	18

2.5.1.1 Nutrient agar (Oxoid)	18
2.5.1.2 Blood agar (Oxoid)	19
2.5.1.3 MacConkey's agar (Oxoid)	19
2.5.1.4 Urea agar base	19
2.5.2 Semi-solid media	20
2.5.2.1 Motility medium	20
2.5.2.2 Hugh and Liefson's (O/F) medium	20
2.5.3 Liquid media	21
2.5.3.1 Peptone water	21
2.5.3.2 Nutrient broth	21
2.5.3.3 MR-VP medium (Glucose-phosphate medium)	21
2.5.3.4 Nitrate broth	22
2.5.3.5 Carbohydrate liquid medium	22
2.6 Chemical reagents, indicators and solutions	22
2.6.1 Oxidase test reagent	22
2.6.2 Hydrogen peroxide	22
2.6.3 Kovac's reagent	22
2.6.4 Potassium hydroxide and alphanaphthol	23

2.6.5 Methyl red solution	23
2.6.6 Nitrate test reagent	23
2.6.7 Bromocrysol purple indicator	23
2.6.8 Gram's stain reagents	23
2.6.8.1 Crystal violet solution	23
2.6.8.2 Lugol's iodine	24
2.6.8.3 Acid alcohol	24
2.6.8.4 Weak carbol fuchsin	24
2.6.9 Normal saline	24
2.7 Culturing of samples	24
2.7.1 Primary culture	24
2.7.2 Purification	25
2.8 Identification of isolates	25
2.8.1 Microscopic examination	25
2.8.1.1 Staining method	25
2.8.2 Cultural characteristics	26
2.8.3 Biochemical testing	26
2.8.3.1 Primary tests	26



2.8.3.1.1 Oxidase test	26
2.8.3.1.2 Catalase test	26
2.8.3.1.3 Motility test	27
2.8.3.1.4 Hugh and Leifson's test	27
2.8.3.2 Secondary tests	27
2.8.3.2.2 Nitrate reduction test	27
2.8.3.2.3 MR & VP tests	28
2.8.3.2.4 Urease test	28
2.8.3.2.5 Indole test	28
2.8.3.2.6 Carbohydrate fermentation tests	28
2.9 Bacterial viable count	29
2.9.1 Preparation of the dilutions	29
2.9.2 Preparation of the plates	29
2.9.3 Colony count	30
2.9.4 EPA Drinking Water Standards	30
2.10 Water sanitary measurements in dairy cattle farms	30
Chapter Three: Results	31
3.1 Overall bacterial isolates and bacterial types	31

3.2 Bacterial isolates according to the source of water	31
3.3 The total viable count of bacteria in water from different sources	32
3.4 Water sanitary measurements in dairy farms	32
Chapter Four: Discussion	41
Conclusions	45
Recommendations	46
References	47
Appendix	55

## List of tables

<b>Table No.</b>	<b>Title</b>	<b>Page</b>
1	Isolation frequency and percentages of isolated bacterial from drinking water of dairy cattle farms in Khartoum North	33
2	Frequency and percentage of isolated bacteria according to the source of water samples	34
3	Reading of total and mean viable counts of bacteria in water from different sources	35

## **List of figures**

<b>Figs. No.</b>	<b>Title</b>	<b>Page</b>
1	Bacteria isolated from 104 samples collected from drinking water of dairy cattle farms in Khartoum North	36
2	Bacterial species isolated from main sources of water	37
3	Bacterial species isolated from storage places	37
4	Bacterial species isolated from water in troughs	38
5	Bacterial species isolated from wall of troughs	39
6	Mean viable counts of bacteria in water samples according to the source of water	40

## Abstract

This study was conducted to assess the bacterial contamination of drinking water in dairy farms in Khartoum North, based on bacterial isolation and identification and viable count. A total of 33 farms were randomly selected. Three samples were taken from each farm, one from the main source of water (network or well), the second from the surface of water in drinking trough and the third from the wall of water trough. In addition, five samples were taken from storage places of water (found in only five farms), making the total number of samples 104. All samples were cultured on Blood Agar and MacConkey's Agar for bacterial isolation and on Nutrient Agar for viable counts. A total of 188 isolates were obtained, 57% of them were Gram-positive bacteria. The isolates were identified according to their microscopic, cultural and biochemical properties to 19 bacterial species. The species and their isolation percentages related to number of samples were *Corynebacterium renale* (29.8%), *Aeromonas salmonicida* (17.3%), *Micrococcus luteus* (16.3%), *Klebsiella pneumoniae* spp. *aerogenes* (14.4%), *Aeromonas* spp. (13.5%), *Bacillus mycoides* (12.5%) *Escherichia coli* (11.5%), *Corynebacterium pseudodiphtheriticum* (10.6%), *Staphylococcus epidermidis* (8.7%), *Moraxella urethralis* (8.7%), *Nocardia asteroides* (6.7%), *Corynebacterium diphtheriae* (5.8%), *Proteus* spp. (5.8%), *Bacillus sphaericus* (5.8%), *Staphylococcus warneri* (4.8%), *Aeromonas sobria* (2.9%), *Kingella kingae* (2.9%), *Micrococcus lylae* (1.9%) and *Klebsiella oxytoca* (1.0%). Water samples from troughs were the most contaminated by all bacterial species except *Klebsiella oxytoca*, followed by the samples from walls of water troughs and the least contaminated was the main source of water. *Aeromonas salmonicida*,

*Bacillus mycoides*, *Bacillus sphaericus* and *Micrococcus luteus* were found in all water sources, whereas *Micrococcus lylae* and *Kingella kingae* were found only in water of troughs and *Klebsiella oxytoca* (one isolate) was found in a wall of a trough. The results of the viable count showed considerable variation among sources of water. The mean viable count was  $4 \times 10^3$ ,  $4.4 \times 10^5$ ,  $1 \times 10^6$  and  $5.2 \times 10^6$  cfu /ml for the main source, storage places, surfaces of water in troughs and wall of troughs, respectively.

In conclusion, many bacterial species and high viable counts were found in drinking water of dairy cattle in Khartoum North, some of them are water-borne pathogens this finding points to poor hygienic measures which constitute hazards to dairy production and public health.

## المستخلص

أجريت هذه الدراسة بغرض تقييم التلوث البكتيري لمياه الشرب في مزارع الأبقار الحلوب بمنطقة الخرطوم بحرى وذلك من خلال العزل و التعريف و العد الحى للبكتيريا. أختيرت 33 مزرعة أبقار عشوائياً لأخذ العينات. أخذت ثلاث عينات من كل مزرعة، إحداها من مصدر المياه الرئيسي فى المزرعة و الثانية من سطح مياه حوض الشرب و الثالثة من جدار الحوض. بالاضافة الى خمس عينات من مكان تخزين المياه و الذى وجد بخمس مزارع فقط، ليصبح مجموع العينات 104. زرعت جميع العينات فى وسطى أجار الدم و الماكونكى لتحديد انواع البكتيريا و على وسط الأجار المغذى لإجراء العد البكتيرى الحى. تم الحصول على 188 عزلة بكتيرية، 57% منها موجبة لصبغة الجرام و التى عرفت حسب خصائصها المجهرية و المزرعية و الكيموحيوية الى 19 نوع. كانت الأنواع و نسبة عزلها من العدد الكلى للعينات كالتالى: الوتدية الكلوية (29.8%)، إيروموناس سالمونسيديا (17.3%)، المكورة الدقيقة الصفراء (16.3%)، الكلبسيلا الرئوية نويج الهوائية (14.4%)، أنواع إيروموناس (13.5%)، العصية شبه الفطرية (12.5%)، الإشريشية القولونية (11.5%)، الوتدية الخناقية الكاذبة (10.6%)، المكورات العنقودية البشرية (8.7%)، الموراكزيبلا الإحليلية (8.7%)، نوكارديا أستيريويديس (6.8%)، الوتدية الخناقية (5.8%)، أنواع المتقلبة (5.8%)، العصية الحشرية (5.8%)، المكورات العنقودية الوارنرية (4.8%)، إيروموناس سوبريا (2.9%)، كينجيلا كينجى (2.9%)، المكورة الدقيقة لايلى (1.9%) و كلبسيلا أوكسييتوكا (1.0%). أظهرت عينات الماء من أحواض الشرب أعلى درجة من التلوث البكتيري و عزلت منها كل أنواع البكتيريا المذكورة آنفاً ما عدا كلبسيلا أوكسييتوكا، تلتها عينات جدار الحوض و أقل المصادر تلوثاً كان المصدر الرئيسى لمياه الشرب فى المزرعة. عزلت المكورة الدقيقة الصفراء و العصية شبه الفطرية و العصية الحشرية و إيروموناس سالمونسيديا من كل مصادر العينات و عزلت كينجيلا كينجى و المكورة الدقيقة لايلى من سطح مياه الحوض فقط، بينما عزلت كلبسيلا أوكسييتوكا مرة واحدة من جدار أحد الأحواض. أظهرت نتيجة العد الحى تفاوتاً كبيراً بين مصادر الماء. كان متوسط العد الحى كالتالى :  $10 \times 4^3$  و  $10 \times 4.4^5$  و  $10 \times 1^6$  و  $10 \times 5.2^6$  لكل من المصدر الرئيسى للمياه فى المزرعة و مكان التخزين و سطح مياه الحوض و جدار الحوض على التوالى.

خلصت الدراسة الى ان مياه الشرب بمزارع الالبان بمنطقة الخرطوم بحرى ملوثة بكثير من أنواع البكتيريا و بإعداد كبيرة و بعض هذه الأنواع معروفة بأنها ممرضة و يمكن انتقالها عن طريق الماء. كما تشير هذه النتيجة إلى رداءة الوضع الصحى وخطورة ذلك على صناعة الألبان و صحة الإنسان.

## Introduction

Water is considered as one of the most vital resources. It is the most important nutrient for dairy cattle. Dairy cattle need free access to a clean, quality source of water for optimal production (Wright, 2007). Bovine milk and dairy products have long traditions in human nutrition. Bovine milk contains the nutrients needed for growth and development of the calf, and is a resource of lipids, proteins, amino acids, vitamins and minerals. It contains immunoglobulins, hormones, growth factors, cytokines, nucleotides, peptides, polyamines, enzymes and other bioactive peptides (Keenan and Patton, 1995).

Good quality water is odorless, colorless, tasteless, and free from faecal pollution and chemicals in harmful amounts. The acceptable quality water is defined as that which is suitable for all usually domestic purposes, including personal hygiene (WHO, 2006). It has estimated that up to 80% of all human sicknesses and diseases in the world are caused by inadequate sanitation, polluted water, or unavailability of water. Water is said to be bacteriologically contaminated or polluted either due to presence of certain pathogens or due to high increase of viable count or due to presence of what is called indicator bacteria at certain levels (Theroux and Leroy, 1943).

Source of livestock and human drinking water must frequently be examined for evidence of pollution. Unhygienic water supplies contribute to many animal and human hazards (Lewis, 1985).

According to Mahgoub (1984), *Salmonella*, *Shigella*, *Pasteurella*, *Yersinia*, *Lep-tospira*, *Compylobacter*, *Mycobacterium*, *Pseudomonas*, *Vibrio*, and enteropathogenic *E. coli*, are the important bacteria that can be transmitted



by water to both animals and humans. Animal diseases, which affect alimentary tract and transmitted through oral route such as cattle plaque, mucosal disease, salmonellosis and paratuberculosis, can be transmitted via faecaly-polluted water (Brendan, 1975). Nile and shallow wells have been found to be polluted with faeces (Abd Mageid *et al.*, 1984).

Many of works that has been done in Sudan for drinking water were oriented towards water consumed by humans only, unless humans and animals shared this water. As a matter of fact most water in the rural areas in Sudan is consumed by both humans and animals. Research on water contaminants and their effects on dairy cattle performance is scarce.

### **Objectives**

1. To isolate and identify the aerobic bacteria found in the drinking water of dairy cattle.
2. To determine the bacterial viable counts of water samples from different sources in the dairy farms.
3. To compare between bacterial types and loads of water samples according to source.

# **Chapter One**

## **Literature Review**

### **1.1 Water for dairy cattle**

#### **1.1.1 Water definition**

The free online Dictionary defines water as: a clear, colorless, odorless, and tasteless liquid. Water is the most important nutrient in animal feeding and animal health. It is the most abundant ingredient of the animal body in all phases of growth and development. A calf's body contains 75 to 80% water at birth and about 55 to 65% water at maturity. Of all farm animals, lactating dairy cows require the greatest amount of water in proportion to their size and because water constitutes 86 to 88% of the milk they yield (Bray *et al.*, 1990).

#### **1.1.2 Water intake**

Dairy cattle get the water they need by drinking and consuming feed that contains water, as well as from metabolic water produced by the oxidation of organic nutrients. The water consumption of dairy animals is influenced by many factors including breed, body size, ambient environment, water temperature, humidity, feed supply, salt, and level of production. An average dairy cow drinks about 25 gallons of water a day, but it will drink less if water quality is poor and that will limit its milk production and jeopardize its health (FAO, 2000). There is a link between bad quality drinking water and ill health. Many diseases and disorders in man and animals are attributed to poor quality water (White and Godfree, 1985). Generally, cattle consume 2

to 4 lbs of water for each pound of dry matter consumed and an additional 3 to 5 lbs of water per pound of milk produced. Rations high in salt or protein increase water intake. Milk production and feed intake decline when water intake is not adequate. At environmental temperatures above 70 degrees Fahrenheit, the animal's respiration rate begins to increase, and increasing amounts of water are lost from the lungs and from sweating. Increased losses of water signal the animal to consume more water to replace the losses (Murphy *et al.*, 1983). Water loss from the body occurs via urine, feces, and milk; through sweating; and by evaporation from body surfaces and the respiratory tract. The amount of water lost from a cow's body is influenced by the animal's activity, air temperature, humidity, respiratory rate, water intake, feed consumption, milk production and other factors. Water intake usually refers to free-drinking water plus that available in the feed (Davis *et al.*, 1983).

### **1.1.3 Water type**

For purposes of simplicity, scientists classified water into two major types, ground water and surface water. Ground water originates from deep wells and because of filtering action of soil, deep sand and rocks, it's virtually free of microorganisms. As water flows up along channels, contaminants may enter it and alter its quality (Alcano, 1997). Surface water is found in lakes, streams, and shallow wells. Generally surface water contains more microbes than ground water and rain water since the majority of soil microorganisms are found in upper crust (6 inches) of the earth. Water under natural conditions contains different microbes. The numbers and kinds of microbes present depend on the source of water, the contamination by excreta from

humans, animals and addition of other contaminated materials (Smith, 1981).

#### **1.1.4 The need for water**

The use of water by man, animals and plants is universal. Water is usually demanded for drinking, irrigation for agriculture, industry and trade. Different quality criteria are set for water according to purpose (Hosny, 1981). Dairy cows and milking facilities require a reliable high quality water supply. These dairy facilities require a water supply that can deliver high quality water to the cows at all times in sufficient amounts to meet both physiological and sanitation needs of the facility. Water is necessary for maintaining body fluids and proper ion balance; digesting, absorbing, and metabolizing nutrients; eliminating waste material and excess heat from the body; providing a fluid environment for the fetus; and transporting nutrients to and from body tissues (Linn,1991).

#### **1.1.5 Quality of drinking water and its effect on performance of dairy cattle**

Water quality is an important issue in dairy cattle production and health and it is important for maximum performance of dairy cattle. Water represents a vital part of the nutrient intake of livestock. In quantity, it is greater than feed intake. The temperature of the water affects water consumption and performance (Milam *et al.*, 1986). Water troughs should be located in areas where cows have easy access. Keeping the troughs clean, so that the cows will be more aggressing drinking the water, is a recommended practice. Contamination of the water supply from drainage and the presence of

nitrites, pesticides, algae and certain parasites such as tapeworms and liver flukes add additional stress to cows. Also, water palatability and odor as well as high levels of minerals such as iron and sulfur reduce consumption (Linn *et al.*, 1987). The five properties most often considered in assessing water quality for both humans and livestock are organoleptic properties (odor and taste), physiochemical properties (pH, total dissolved solids, total dissolved oxygen and hardness), along with the presence of toxic compounds (heavy metals, toxic minerals, organophosphates and hydrocarbons), excess minerals or compounds (nitrites, sodium sulfates and iron) and bacteria and algae.

In general terms, the greatest microbial risks are those associated with ingestion of water that is contaminated with human or animal feces. Feces can be a source of pathogenic bacteria, viruses, protozoa and helminthes. Microbial water quality often varies rapidly and over wide range. Short-term peaks in pathogen concentration may increase disease risks considerably and may trigger outbreaks of water-borne disease (WHO, 2006).

Bacterial contamination of water is measured in a laboratory using microbiological techniques to permit any bacteria present in a water sample to grow. Results are then counted and reported as bacterial counts per 100 ml of water. A coliform count over 1/100 ml can cause scours in calves. In adult cows, a count of 15-20/100 ml can cause diarrhea and cows may go off-feed. Positive results for fecal coliform (more than 0 counts/100 ml) indicate a pollution problem that should be investigated and corrected (Wright, 2007). The most acceptable definition of water pollution is the presence of any substance (organic, inorganic, biological, thermal or

radiological) in water at intensity levels which tend to impair, degrade or adversely affect its quality or usefulness for specific purposes (FAO, 1997).

## **1.2 Bacterial contamination of drinking water**

The term contamination is defined as the presence of bacteria in water from intestinal tract of warm-blooded animals including man. The presence of such bacteria means the water may carry disease germs. The fact that water looks clear and sparkling is not assurance of its purity. Disease germs are invisible to the unaided eye (Forrest, 1956).

A previous study in the Sudan has clearly demonstrated close association of biological contamination of drinking water with higher prevalence of diarrheal diseases and certain enteric pathogens (Elshazali and Erwa, 1971). Bacterial contamination is usually measured by the fecal coliform levels in the water. Fecal coliform is an indicator organism; it is easily measured and can signal the presence of other harmful bacteria in water. This sort of bacterial contamination can occur as a result of improper water treatment or poor water storage.

Major type of bacteria in contaminated water is coliform bacteria, a group of Gram-negative bacteria, non-sporing bacilli which inhabit human and animal intestines. They usually ferment lactose to acid and gas. The most important species of this group are *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. Non-coliform bacteria are also common in contaminated water and include *Streptococcus* spp., *Proteus* spp. and *Pseudomonas* spp. (Alcano, 1997). The presence of any member of organisms from coliform group in treated potable water is not acceptable regardless of their source, and that their presence in potable water indicates in proper practices (Kabler *et al.*, 1960).

Contamination of drinking water may introduce a variety of intestinal pathogens: bacterial, viral, and parasitic. Their presence being related to microbial diseases and carriers present at the moment in the community. Intestinal bacterial pathogens are widely distributed throughout the world. Those known to have occurred in contaminated drinking water include strains of *Salmonella*, *Shigella*, enterotoxigenic *Escherichia coli*, *Vibrio cholerae*, *Yersinia enterocolitica*, and *Campylobacter fetus*. These organisms may cause diseases that vary in severity from mild gastro-enteritis to severe and sometimes fatal dysentery, cholera, or typhoid (Van der, 1992).

Many different studies evaluated the microbiological quality of drinking water in relation to water sources. Data from Sierra Leon on the water from surface sources showed that there was often a high level of fecal bacterial contamination (Wright, 1984). While, Esrey *et al.* (1985) stated that the original sources of water may not be unsafe but it becomes contaminated after distribution and storage by fecal matter in unhygienic and inadequate sanitary conditions.

### **1.2.1 Sources of bacterial contamination**

Water received fecal pollution from a variety of sources, including human and animals. The sources of bacterial contamination include:

1. Human and animal wastes which are primary sources of bacteria.
2. Discharge from septic tanks and sewage treatment centers. It was found that the major feature of safe water supply is the separation of sewage (human excreta) from drinking water (Duerden *et al.*, 1988).

3. Natural soil/plant bacteria. Bacteria from these sources can enter wells that open at land surface.
4. Infiltration by flood waters or by surface runoff. Flood waters commonly contain high levels of bacteria.

### **1.2.2 Sources of bacterial contamination in dairy cattle water troughs**

The presence of bacteria in natural aquatic ecosystems is dependent upon the rate of contamination and equilibrium that establishes between bacterial proliferation in that environment and the rate of elimination. Bacterial contamination in dairy cattle water troughs may arise from multiple sources. Water may be contaminated with cud or fecal materials. Extraneous matter including dust, feed, or bedding may also enter the trough. The bacterial contamination is higher in troughs that are proximity to the feed bunk that may have permitted a greater amount of feed to enter the troughs, thus increasing the level of contamination as well as providing a nutrient-rich substrate for bacterial growth and survival at the button of the trough (Ashbolt *et al.*, 1993).

### **1.2.3 Factors influence the survival rate of bacteria in water troughs**

Competition with and predation by other microorganisms is considered to be one of the most important factors influencing the elimination of bacteria from natural aquatic system (Gonzalez *et al.*, 1992; Mallory *et al.*, 1983; Marino and Gannon, 1991).



Direct sunlight and temperature and competition with other microorganisms may influence the survival rate of bacteria in water (Barcina, 1995). The lower *E. coli* densities in the troughs exposed to direct sunlight was found consistent with reported deleterious effect of visible light on *E. coli* survival in other aquatic systems (Barcina *et al.*, 1989). Bacteria in aquatic system are more likely to proliferate as water temperature increases, especially above 15 °C (Lechevallier *et al.*, 1996). Salmonella tended to be isolated more frequently in the less recently cleaned troughs.

### **1.3 Bacterial water-borne diseases and agents**

Water-borne infectious diseases are those in which the pathogen, or causative agent, is present in water and ingested when the water is consumed (Meybeck *et al.*, 1996). Snow (1855) was the first to show a precise relation of a disease to water in well known studies of cholera. It was observed that nearly everyone who becomes ill obtained his drinking water from a specific well into which a cesspool was leaking. Those who became ill either drank water from the well, or come into contact with faecally contaminated material, while tending those already sick. Water may contain much kind of bacteria, both harmless and pathogenic (Meybeck *et al.*, 1996). Some of the better known water-borne diseases, caused by bacteria, are: cholera, bacillary dysentery, shigellosis, and typhoid fever.

#### **1.3.1 *Escherichia coli***

Observational studies have shown an association between the presence of *E. coli* in cattle water troughs and the infection status of cattle drinking from these troughs (Jeffrey *et al.*, 2001). Livestock water troughs contaminated with *E. coli* and left without regular cleaning may serve as a reservoir of the

agent on the farm for extended period of time. *E. coli* can cause significant disease problem to cattle, it can be found on most dairies, however; only a few animals will be shedding the bacteria in their manure at any one time. It is most easily found in weaned calves. Adult cattle are the least common shedders of all cattle types, but market cattle can shed significant amounts in their feces. On the dairy cattle, *E. coli* are often found in water troughs and wet feeds (Jeffrey *et al.*, 2001). It is more commonly found on dairies with flush alleys, during warmer months and in improperly cured silages. Few control measures have been tested on dairies. Some measures that might reduce the amount of *E. coli* on the dairy are: to frequently clean water troughs, to chlorinate water troughs and to insure proper silage preparation.

### **1.3.2 *Salmonella* spp.**

Bacteria of *Salmonella* spp. can be found in many cattle as well as humans, birds and reptiles. In cattle, *Salmonella* causes diarrhea, decreased milk production, abortions and sometimes death. Some *Salmonella* spp. such as *Salmonella dublin* affect primarily calves while others like *Salmonella typhimurium* attack adult animals. Carrier cows, especially during time of stress like around calving, can spread *Salmonella* in their manure wherever they go on the dairy. Typhoid fever disease, caused by *Salmonella typhi*, is usually contracted by ingestion of food or water contaminated by human faeces or urine (Twort *et al.*, 1985).

### **1.3.3 *Corynebacterium* spp.**

*Corynebacterium* spp. are commonly found on mucous membranes and skin of animals and gastrointestinal tract of normal dairy cattle and sheep, soil

and manure of the animals can contaminate water troughs (Hirsch and Yuan, 1999).

#### **1.3.4 *Shigella* spp.**

Dysentery caused by *Shigella* spp. infection is occasionally contracted via water contaminated by human feces, the disease is characterized by severe bloody diarrhea accompanied by abdominal pain (Cairncross *et al.*, 1980).

#### **1.3.5 *Bacillus* spp.**

Soil is the main source of *Bacillus* spp. The infection can be transmitted to animals by ingestion of contaminated water (Hirsch and Yuan, 1999).

#### **1.3.6 *Aeromonas* spp.**

These bacteria have been isolated from feces, soil, bedding and drinking water of healthy cows, and the mode of transmission via fecal-oral route is possible (Nayduch *et al.*, 2001). They have been associated with diarrheal diseases in both humans and livestock animals.

#### **1.3.7 *Staphylococcus* spp.**

They are a part of normal bacterial flora of the skin and mucus membranes of animals. They have been isolated from food, dairy products, soil and water. Some species are pathogenic, some opportunistic pathogens and many are harmless for animals (Ryan, 2004). *Staphylococcus* spp. are the predominant pathogens in sub-clinical and chronic bovine mastitis (Guidry *et al.*, 1998).

### **1.3.8 *Streptococcus* spp.**

They have been isolated from soil, plants, dust, water and manure (Ryan, 2004).

### **1.3.9 *Proteus* spp.**

They belong to the family enterobacteriaceae, forming a part of the normal intestinal flora of animals and it was found in manure, soil and polluted water (Guentezel, 1996).

### **1.3.10 *Klebsiella* spp.**

Some species were isolated from sewage, soil, drinking water, dairy products and feces. *Klebsiella pneumoniae* has become an important cause of clinical mastitis in dairy cows (Hogan and Smith, 2003)

## **1.4 How water transfers bacterial diseases?**

The mode of transmission of bacterial pathogens includes ingestion of contaminated water. The significance of the water means in spread of intestinal bacterial infections varies considerably, both with the disease and with the local circumstances. Among the various water-borne pathogens, there exists a wide range of minimum infectious dose levels necessary to cause animal infection. With *Salmonella typhi*, ingestion of relatively few organisms can cause disease, whereas many millions of cells of other *Salmonella* spp. are usually required to cause gastroenteritis. Similarly, with toxigenic organisms such as enteropathogenic *E.coli* and *Vibrio cholerae*, many organisms may be necessary to cause illness. The size of the infective

dose also varies in different individuals with age, nutritional status, and general health at the time of exposure (Van der, 1992).

### **1.5 Prevention of bacterial water –borne diseases in dairy cattle farms**

Water quality control devotes regular sampling and analyzing of water samples as well as recording of results obtained. However, it also involves assessing how good method is and how well is operating in practice (WHO, 1984).

Treating water or remove or reduce contaminants can be expensive and may require significant equipment maintenance. Therefore, before making a decision to treat, laboratory analysis of the drinking water, must be cost effective and bring about known health or production benefits for the cattle. The best treatment option for livestock drinking water depends on the target contamination. Elimination of disease-causing microorganisms involves disinfecting the water. The most common chemical disinfectant used in surface water treatment is chlorine. Bacterial contamination is much more likely to occur in the drinking vessel, so keeping water troughs clean is a must (FAO, 2000).

#### **1.5.1 General control measures**

1. Complete composting and deep stacking of manure may reduce bacterial numbers.
2. Minimize re-cycling of water from lagoon into cattle housing areas.
3. Avoid re-cycling lagoon water through the sprinkler pens.
4. Protect water troughs from manure contamination.
5. Clean and sanitize water troughs often and regularly.

## **Chapter Two**

### **Materials and Methods**

#### **2.1 The study area**

Khartoum North (Bahri) was selected as the study area. The selection was based on the high number of dairy cattle farms in the area. The dairy farms were located in six locations; namely, Shambat, Elhalfaia, Elsamrab, Eldoroshab, Elhag Yosif and Helat Khogaly.

#### **2.2 Samples**

Water samples were collected from dairy farms during the period from March to June 2009.

##### **2.2.1 Size of samples**

A total of 33 dairy farms were examined for drinking water contamination. From each farm, three samples were collected, one from the main source of water (network or wells), the second from the surface of water in troughs and the third from the wall of water troughs. In addition, five samples were taken from the storage place of five farms, making the total numbers of samples as 104.

##### **2.2.2 Collection method**

Sterile glass bottles, 250 ml previously sterilized by autoclaving at 121°C for 15 minutes were used for collection of water samples. Samples were labeled with farm location, water source and date.

### **2.2.2.1 Collection of samples from the main sources of water**

The main sources of water were either tap water (network surface water) or water of wells made inside farms.

#### **2.2.2.1.1 Collection of samples from tap water**

Collection was done according to WHO (1996) as follows:

1. The outside nozzle of the tap was cleaned carefully.
2. The tap was turned on full, and the water was allowed to run to waste for one minute.
3. The sample bottle was then filled from the gentle flow of water
4. Contamination was avoided by not allowing any surface to touch the bottle mouth or the inside of the cap.
5. The bottle cap was then replaced.
6. The bottle was then labeled.

#### **2.2.2.1.2 Collection of samples from wells**

1. The hand pump was operated for 5 minutes.
2. A sample of water was collected by allowing the water from the pump to flow directly into the sterile bottle, the bottle cap was carefully replaced and firmly tied.
3. The bottle was then labeled.

#### **2.2.2.2 Collection of samples from storage places**

1. The cap was removed and the mouth of bottle was faced up.
2. The bottle was pushed forward horizontally until it was filled; the bottle cap was carefully replaced and firmly tied.

3. The bottle was then labeled.

#### **2.2.2.3 Collection of samples from surface of water in troughs**

The cap was removed and the bottle was immediately and quickly filled with surface water, covered and then labeled.

#### **2.2.2.4 Collection of samples from walls of water troughs**

1. The cap was removed and the wall of the trough was scratched by the mouth of bottle; then the bottle cap was carefully replaced and firmly tied.
2. The bottle was then labeled.

#### **2.2.3 Transportation of samples**

All precautions were taken to prevent accidental contamination of the water during its transportation. Immediately after collection, the glass bottles were transported to the laboratory and the examination started as soon as possible after arrival.

### **2.3 Sterilization**

#### **2.3.1 Flaming**

It was used to fix smears on glass slides and prevent contamination during cultivation of different media.



### **2.3.2 Hot air oven**

This method was used for sterilization of clean glass containers which were wrapped in paper or put in stainless steel cans, and the temperature was 160°C for one hour (Stainer *et al.*, 1986).

### **2.3.3 Red heat**

The method was used for sterilizing wire loops, straight wires and tissue forceps. It was done by holding the object over flame as near and vertical as possible until it became red (Cruickshank *et al.*, 1975).

### **2.3.4 Autoclaving (Moist heat)**

This method was used for sterilizing culture media and for materials that could not withstand the dry heat. The temperature was 115-121°C under 10-15 pounds pressure for 15-20 minutes (Barrow and Feltham, 1993).

## **2.4 Disinfection**

Alcohol (70%) was used to disinfect work benches and phenol was used to disinfect floors.

## **2.5. Culture media**

### **2.5.1 Solid culture media**

#### **2.5.1.1 Nutrient agar (Oxoid)**

This medium contained peptone (5g), lab-lemco powder (1g), yeast extract (2g), sodium chloride (5g) and agar No 3(15g). The medium was prepared by dissolving 28 grams of the dehydrated medium in one liter distilled water,

and the pH was adjusted to 7.4 and then sterilized by autoclaving for 15 minutes at 121°C. The medium was allowed to cool to 55°C and poured aseptically in 15- 20 ml amounts into sterile Petri-dishes.

#### **2.5.1.2 Blood agar (Oxoid)**

This is one of the enriched media that was composed of blood agar base and defibrinated sheep blood. The blood agar base contained proteose peptone (15g), liver digest (2.5g), yeast extract (5g), sodium chloride (5g) and agar (12g). It was prepared by dissolving 40 grams of the basal medium in one liter of distilled water. The mixture was then boiled until the powder dissolved completely. The solution was autoclaved at 121°C for 15 minutes. It was then cooled to 45-50°C. 7% of sterile blood was added with gentle rotation and then the medium was poured into Petri dishes as 15-20 ml amounts and left to solidify.

#### **2.5.1.3 MacConkey's agar (Oxoid)**

This medium contained peptone (20g), lactose (10g), bile salts (1.5g), sodium chloride (5g), neutral red (0.03g), crystal violet (0.001g) and agar No.3 (15g). The medium was prepared by dissolving 52 grams in one liter of distilled water by heating. The pH was adjusted to 7.4 and then autoclaved at 121°C for 15 minutes. Then it was allowed to cool to 55°C and poured gently in 15 ml amounts into sterile Petri dishes.

#### **2.5.1.4 Urea agar base**

This medium contained peptone (1.0g), dextrose (1.0g), sodium chloride (5.0g), phenol red (0.012g), di-sodium phosphate (1.2 g), potassium dihydrogen phosphate (0.8g) and agar (15.0g). The medium was prepared

according to manufacturer's instructions by dissolving 2.4g of the dehydrated powder in 95 ml of distilled water, and then dissolved by boiling. It was then sterilized by autoclaving at 121°C for 15 minutes. Then it was cooled to 50 °C and 5 ml of sterilized 40 % urea solution (Oxoid SR 20) were added under aseptic condition. The medium was then distributed into sterile Bijou bottles in 5 ml amounts and allowed to solidify in a slope position.

## **2.5.2 Semi-solid media**

### **2.5.2.1 Motility medium**

This medium was prepared according to Cruickshank *et al.*, (1975). New Zealand agar (0.2% w/v) was dissolved in nutrient broth and distributed in sterile test tubes containing Craigie tubes, and then the medium was autoclaved at 121°C for 15 minutes.

### **2.5.2.2 Hugh and Liefson's (O/F) medium**

Hugh and Liefson's (O/F) medium contained peptone (2g), NaCl (5g), KHPO<sub>4</sub> (0.3g), agar (3g), distilled water (1000 ml), and bromocrysol purple, 0.2% aqueous solution (15 ml). The solids were dissolved by heating in the water. The pH was adjusted to 7.1, then the medium was filtered and the indicator was added. Sterilization was done by autoclaving for 20 minutes at 115°C. Sterile glucose solution was aseptically added to the medium to give a final concentration of 1%, mixed and distributed aseptically in 10 ml volumes into sterile test tubes with cotton plugs.

## **2.5.3 Liquid media**

### **2.5.3.1 Peptone water**

Peptone water was prepared according to Cruickshank *et al.* (1975). Ten grams peptone and 5 grams NaCl were dissolved by heating in 1000 ml distilled water. The pH was adjusted to 7.2 and the medium was distributed in 5 ml amounts in test tubes and then sterilized by autoclaving at 115°C for 15 minutes. The medium was kept at 4 °C and used for indole test.

### **2.5.3.2 Nutrient broth**

Nutrient broth (Oxoid Lab) contained lab-lemco powder (1g), yeast extract (2g), peptone (5g) and sodium chloride (5g). An amount of 13g of the dehydrated medium was added to one liter of distilled water; mixed well and then the pH was adjusted to 7.4. The medium was distributed in 5 ml amounts in the test tubes and sterilized by autoclaving at 121°C for 15 minutes.

### **2.5.3.3 MR-VP medium (Glucose-phosphate medium)**

MR-VP medium (Oxoid Lab.) contained peptone (5g), glucose (5g) and  $K_2HPO_4$  (5g). An amount of 15g of the dehydrated medium was added to one liter of distilled water and mixed well. Then the pH was adjusted to 7.0 and the medium was distributed in test tubes in 5 ml amounts and sterilized by autoclaving at 121°C for 15 minutes.

### **2.5.3.4 Nitrate broth**

Nitrate broth (Cowan and Steel, 1985) contained  $\text{KNO}_3$  (1g) and nutrient broth dehydrated medium (13g), which were dissolved in 1000 ml distilled water. Then the medium was distributed in sterile test tubes with cotton plugs and then sterilized by autoclaving at  $121^\circ\text{C}$  for 15 minutes.

#### **2.5.3.5 Carbohydrate liquid medium**

Twenty grams of peptone water and (10g) of sugar were dissolved in (900 ml) of distilled water, (10 ml) of bromocrysol purple, 0.2% aqueous solution were added and then the medium was sterilized by autoclaving at  $115^\circ\text{C}$  for 10 minutes.

### **2.6 Chemical reagents, indicators and solutions**

#### **2.6.1 Oxidase test reagent**

Tetramethyl-p-phenylenediamine dihydrochloride was prepared as 1% aqueous solution. Filter papers of 50 x 50 millimeters in size were impregnated with the reagent and dried at  $50^\circ\text{C}$  (Barrow and Feltham, 1993).

#### **2.6.2 Hydrogen peroxide**

Hydrogen peroxide 30%, produced by British Drug House, London, was diluted to 3% aqueous solution for catalase test.

#### **2.6.3 Kovac's reagent**

This reagent was prepared as described by Barrow and Feltham (1993). Five grams of p-dimethylamino-benzaldehyde were dissolved in (75 ml) of amyl

alcohol by warming in water bath. After the mixture was cooled, (25 ml) of concentrated hydrochloric acid were added. It is used for indole test.

#### **2.6.4 Potassium hydroxide and alphanaphthol**

The reagent was prepared as 40% potassium hydroxide and 5% alphanaphthol for use in Voges-Proskauer (V.P) test.

#### **2.6.5 Methyl red solution**

This solution was prepared by dissolving (0.04 g) of methyl red in (40 ml) ethanol and the volume was made up to (100 ml) with distilled water.

#### **2.6.6 Nitrate test reagent**

According to Bio Merieux, it consists of two separate solutions. The first of them, the sulfanilic acid reagent, was prepared by dissolving (0.4g) of sulfanilic acid in (100 ml) acetic acid. The other solution, alpha-naphthylamine, was prepared by dissolving (0.6g) of dimethyl- $\alpha$ -naphthylamine in (100 ml) acetic acid.

#### **2.6.7 Bromocrysol purple indicator**

Bromocrysol purple was prepared as 0.2% aqueous solution for O/F & carbohydrate fermentation tests .

#### **2.6.8 Gram's stain reagents**

##### **2.6.8.1 Crystal violet solution**

This solution was prepared by dissolving (10g) crystal violet and ethanol (95%) (100ml). They were mixed together till dissolved

### **2.6.8.2 Lugol's iodine**

Lugol's iodine contained iodine (5g), potassium iodide (10g) and distilled water (100 ml). The potassium iodide was dissolved with the iodine and 10 ml of water and then the rest 90 ml distilled water were added and mixed.

### **2.6.8.3 Acid alcohol**

It contained concentrated HCl (3 ml), ethanol (97 ml) and were mixed well before use.

### **2.6.8.4 Weak carbol fuchsin**

Weak carbol fuchsin was prepared by diluting one volume of strong carbol fuchsin with 10 volumes of distilled water.

### **2.6.9 Normal saline**

Normal saline was prepared as described in Oxoid Manual by dissolving (8.5 g) of sodium chloride in one liter of distilled water to obtain (0.85%) concentration.

## **2.7 Culturing of samples**

### **2.7.1 Primary culture**

Primary culture for all water samples was done onto blood agar and MacConkey's agar media. Each water sample was centrifuged at 8000 rpm for 5 minutes and the sediment was cultured, then all Petri dishes were incubated at 37°C for 24 hours.

## **2.7.2 Purification**

Typical and well isolated colonies from the primary plates were picked with a wire loop and each one was streaked on the surface of a fresh plate of nutrient agar medium. Pure culture was obtained by replating the sub-culture on nutrient agar.

## **2.8 Identification of isolates**

The purified isolates were identified according to criteria described by Barrow and Feltham (1993). This included staining reaction, organism morphology, growth condition, colony characteristics on different media, motility and biochemical characteristics.

### **2.8.1 Microscopic examination**

A smear was made from each type of colony from primary culture and from purified colonies, fixed by heating and stained by Gram's method. Then the stained smears were examined microscopically under oil immersion lens. The smears were examined for cell morphology, arrangement, presence of capsule and staining reaction.

#### **2.8.1.1 Staining method**

##### **Gram's staining method**

1. Crystal violet was added to fixed smears for 30 sec.
2. Washed with distilled water.
3. Lugol's iodine was added for 30 sec.
4. Decolorized with acetone-alcohol for 2-3 seconds.
5. Washed with distilled water.
6. Counter stained with dilute carbol fuchsin for 30 sec.



7. Washed with distilled water.
8. Dried with filter paper and examined under microscope by oil immersion objective lens.

Gram-positive bacteria appeared purple, while Gram-negative bacteria appeared red.

### **2.8.2 Cultural characteristics**

The colony characteristics of all isolates (shape, size, consistency, opacity, pigments, and type of growth on different media) were observed, and used for identification.

### **2.8.3 Biochemical testing**

All the following biochemical tests were conducted and performed according to Barrow and Feltham (1993), unless otherwise stated.

#### **2.8.3.1 Primary tests**

##### **2.8.3.1.1 Oxidase test**

A sterile platinum loop was used to spread the isolated colony on oxidase paper. Color change to violet within 5-10 seconds indicated a positive reaction.

##### **2.8.3.1.2 Catalase test**

Using a sterile glass rod, a part of an isolated colony was emulsified in one drop of hydrogen peroxide on a clean slide. Appearance of gas bubbles indicated a positive reaction.

### **2.8.3.1.3 Motility test**

The isolates were studied for motility by Craigie technique according to Cruickshank *et al.* (1975), in which the bacteria was inoculated into a central tube containing semi-solid agar placed in test tube using a straight wire. After incubation at 37°C for 24 hours, the tubes were examined for migration of the bacteria outside the Craigie tube.

### **2.8.3.1.4 Hugh and Leifson's test**

Hugh and Leifson's test or oxidation fermentation test (O/F) was done as shown by Cruickshank *et al.* (1975). Duplicate tubes of freshly prepared medium were inoculated by stabbing with a straight wire. One of the inoculated media was immediately covered with a layer of sterile liquid paraffin to a depth of 1 ml and examined daily for up to 14 days. A color change from green to yellow in both tubes indicated a fermentative organism but change in the uncovered tube only indicated that the organism is oxidative.

### **2.8.3.2 Secondary tests**

#### **2.8.3.2.2 Nitrate reduction test**

Nitrate reduction test was carried out as described by Cowan and Steel (1985). Nitrate broth was inoculated and incubated for up to five days. One milliliter nitrate solution 1 was added followed by 1 ml of solution 2. A red color indicated a positive reaction. To the tubes not showing red coloration within five minutes, zinc powder was added to them and allowed to stand. Absence of red coloration in this case indicated absence of nitrate (positive reaction).

#### **2.8.3.2.3 MR & VP tests**

A tube containing glucose-phosphate peptone water medium was inoculated with a 24 h peptone water culture and then incubated at 37°C for 24 h. The culture was halved in to another tube two drops of methyl red reagent were added to one tube, shaken well and examined. Appearance of bright red color indicated a positive result whereas orange yellow color indicated a negative reaction. For VP test 0.6 ml 5% alpha-naphthol solution and 0.2 ml 40% KOH aqueous solution were added to the second tube and shaken well. The tube was sloped and examined after 15 min. A strong red color indicated a positive reaction.

#### **2.8.3.2.4 Urease test**

A slope of urea agar base medium was heavily inoculated with test organism, incubated at 37°C and examined daily for 5 days. If the color changed from yellow to red-pink, it was considered as a positive result.

#### **2.8.3.2.5 Indole test**

The test organism was cultured into peptone water which contains tryptophan and incubated at 37°C for 48 h. One milliliter of Kovac's reagent which contains 4-p-dimethylamine benzaldehyde was run down along side of the test tube. Appearance of pink color in the reagent layer within a minute indicated a positive reaction.

#### **2.8.3.2.6 Carbohydrate fermentation tests**

Carbohydrate fermentation tests were carried out as described by Cruickshank *et al.* (1975). Carbohydrate media containing 1% of any of

glucose, lactose, mannitol, sucrose, inositol, fructose, trehalose and maltose, were inoculated with peptone water culture by a sterile loop and incubated at 37 °C and examined daily for 4 days. A fermentation reaction was indicated by change of color of the medium to pink.

## **2.9 Bacterial viable count**

The bacterial count was done according to Miles and Misra (1938).

### **2.9.1 Preparation of the dilutions**

Ten- fold serial dilutions of water samples were prepared. Three test tubes containing 9 ml sterile normal saline were prepared. A micropipette with sterile tip was held vertically and introduced not more than 3 cm below the surface of the water sample and then 1 ml was taken to the first tube of the dilution series without touching the diluting fluid, the tip was discarded and the tube was labeled as the first dilution tube,  $10^{-1}$ . A fresh sterile tip was used to mix the content of the first dilution and 1 ml was transferred to the second tube of dilution series without touching the diluting fluid. The tip was discarded and the tube was labeled as the second dilution tube,  $10^{-2}$ . Further dilutions of  $10^{-3}$  were prepared similarly.

### **2.9.2 Preparation of the plates**

The surfaces of the Nutrient agar plates were dried for one hour at room temperature with the plate lid closed, followed by two hours at 37°C with lid and base separated. A fresh sterile tip was used to mix the content of each dilution by sucking up and down ten times, then 0.02 ml of each dilution was withdrawn and transferred to nutrient agar and evenly distributed on the surface using a sterile glass rod. Two replica of each dilution were made.

The plates were labeled by the number of the dilution, and incubated at 37 °C for 24 h.

### **2.9.3 Colony count**

An average colony count from the two replica of each dilution was obtained. The average was multiplied by 50 to obtain a figure for the bacteria/ ml in the original sample and by the reciprocal of dilution factor.

### **2.9.4 EPA Drinking Water Standards**

Environmental Protection Agency (EPA) standards say:-

“A total microbial (aerobic) count that may be used for source drinking water is 100 colony-forming units cfu/mL.”

### **2.10 Water sanitary measurements in dairy cattle farms**

Few simple data about water sanitary measurements of farms examined was collected using a questionnaire (appendix).

## Chapter Three

### Results

#### 3.1 Overall bacterial isolates and bacterial types

Out of 104 samples of drinking water from 33 dairy farms located in Khartoum North area, 188 bacterial isolates were recovered. According to their microscopic, cultural and biochemical properties, the isolates were identified to a total of 19 bacterial types (Table 1). The highest rate of isolation was of *Corynebacterium renale* (n=31, 29.8%) and the lowest rate of isolation was of *Klebsiella oxytoca* (n=1, 0.96%), (Fig. 1). The Gram-positive types were more than Gram-negative and the isolates of Gram-positive bacteria constituted 57% of the total number of isolates.

#### 3.2 Bacterial isolates according to the source of water

According to the source of drinking water, 81 (43.08%) isolates were recovered from water of troughs, 73 (38.83%) were isolated from wall of troughs, 20 (10.64%) were isolated from water of the main sources and 14 (7.45%) were isolated from water of the storage places. Details of these percentages and types of bacteria from each source of water are shown in Table 2 and Figs. 2-5.

The most contaminated source of water was the water in troughs; 18 bacterial types were isolated, which included all bacterial types isolated from all sources, except *Klebsiella oxytoca*. *Corynebacterium renale* was the most isolated bacterial species from this source as well as from other sources.

The second most contaminated source of water was the walls of troughs; 16 bacterial types were obtained.

Nine types of bacteria were isolated from the samples of storage places (n=5). Their frequency of isolation ranged from 1-3 (20-60%).

The least contaminated source of water was the main source, only four types of bacteria were isolated. The most isolated bacterium from this source was *Aeromonas salmonicida*, which was isolated from 30.3% (10/33) of samples from this source, followed by *Micrococcus luteus* (18.1%).

*Aeromonas salmonicida*, *Bacillus mycoides*, *Bacillus sphaericus* and *Micrococcus luteus* were found in all water sources, whereas *Micrococcus lylae* and *Kingella kingae* were found only in water from troughs, and *Klebsiella oxytoca* (one isolate) was found in a wall of a trough.

### **3.3 The total viable count of bacteria in water from different sources**

The mean viable count of bacteria in water samples from the four sources was  $4 \times 10^3$ ,  $4.4 \times 10^5$ ,  $1 \times 10^6$  and  $5.2 \times 10^6$  colony forming units/ ml for main sources, storage places, water in troughs and wall of troughs, respectively (Table 3 and Fig. 6). However, viable counts of some individual samples from the source of highest mean were lesser than some samples from other sources and vice versa.

### **3.4 Water sanitary measurements in dairy farms**

Most of farms examined were of bad sanitary measurements. Water troughs and water trough's material were poor and troughs were contaminated with faeces, feed and algae. Also, the storage places of water were not clear and

exposed to contamination. Pipes of main sources of water were of bad quality that might allow contamination from soil. Common bacterial diseases in these farms were mainly Brucellosis, Salmonellosis, *E. coli* infections, mastitis and respiratory tract infections, which, at least, some of them are water-borne diseases and some of their causes were isolated in this study. Cattle breeds were mainly cross breed that could averagely resist bacterial infections.

**Table 1: Isolation frequency and percentages of isolated bacterial from drinking water of dairy cattle farms in Khartoum North**

<b>Bacteria</b>	<b>Isolation frequency</b>	<b>Percentages from total number of samples</b>	<b>Percentages from total number of isolates</b>
<i>Corynebacterium renale</i>	31	29.8%	16.49%
<i>Aeromonas salmonicida</i>	18	17.31%	09.57%
<i>Micrococcus luteus</i>	17	16.34%	9.04%
<i>Klebsiella pneumoniae</i> ssp. <i>aerogenes</i>	15	14.42%	7.98%
<i>Aeromonas</i> spp.	14	13.46%	7.45%
<i>Bacillus mycoides</i>	13	12.5%	6.91%
<i>E. coli</i>	12	11.53%	6.38%
<i>Corynebacterium pseudodiphtheriticum</i>	11	10.57%	5.85%
<i>Staphylococcus epidermidis</i>	9	8.65%	4.79%
<i>Moraxella urethralis</i>	9	8.65%	4.79%
<i>Nocardia asteroides</i>	7	6.73%	3.72%
<i>Corynebacterium diphtheria</i>	6	5.76%	3.19%
<i>Proteus</i> spp.	6	5.76%	3.19%
<i>Bacillus sphaericus</i>	6	5.76%	3.19%
<i>Staphylococcus warneri</i>	5	4.8%	2.69%
<i>Kingella kingae</i>	3	2.88%	1.59%
<i>Aeromonas sobria</i>	3	2.88%	1.59%
<i>Micrococcus lylae</i>	2	1.92%	1.06%
<i>Klebsiella oxytoca</i>	1	0.96%	0.53%



**Table 2: Frequency and percentage of isolated bacteria according to the source of water samples**

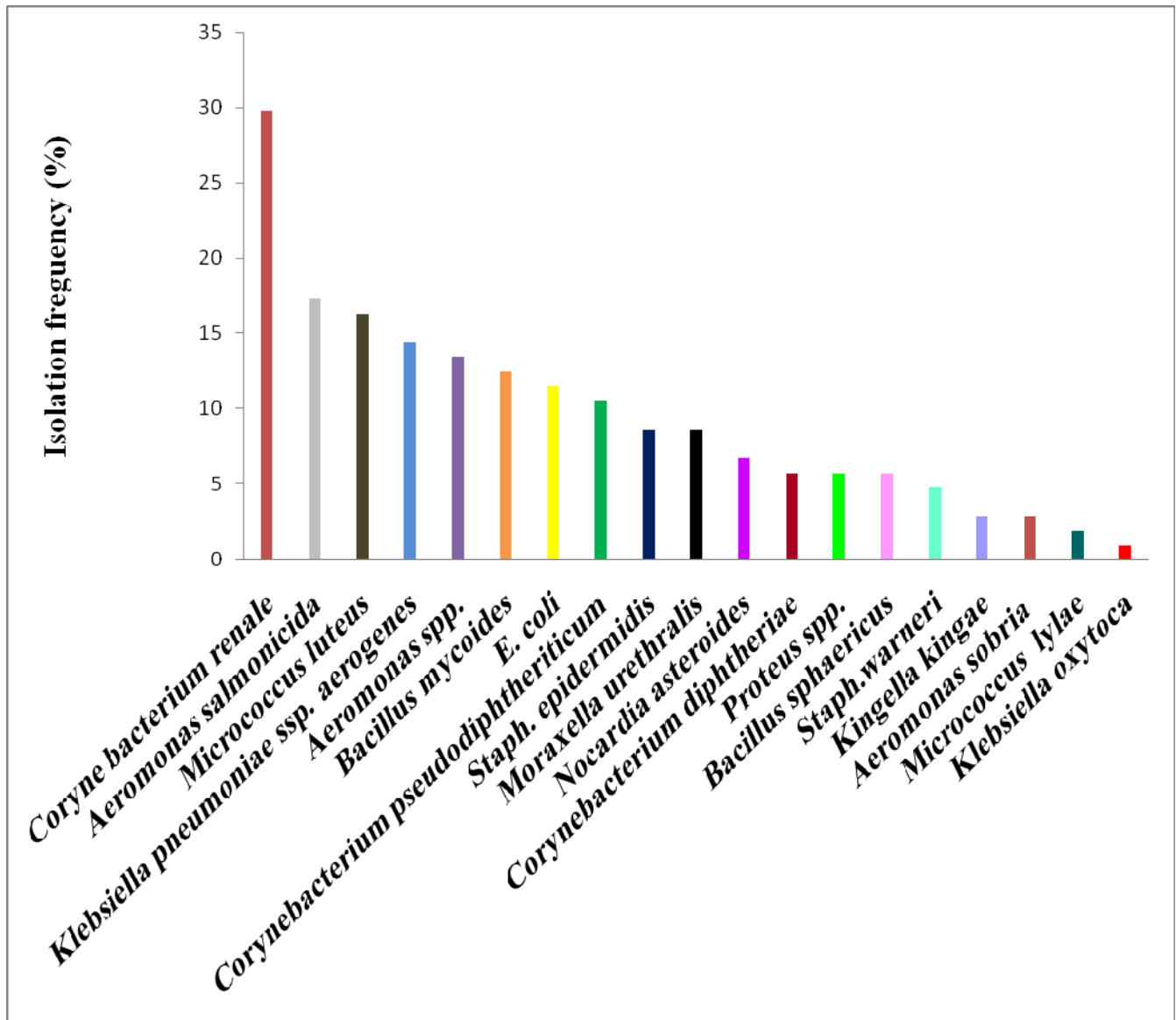
<b>Bacteria</b>	<b>Main sources of water</b>	<b>Storage places of water</b>	<b>Water in troughs</b>	<b>Wall of troughs</b>
<i>Aeromonas</i> spp.	0	2(40%)	4(12.12%)	8(24.24%)
<i>Aeromonas salmonicida</i>	10(30.3%)	1(20%)	2(6.06%)	5(15.15%)
<i>Aeromonas sobria</i>	0	0	2(6.06%)	1(3.03%)
<i>Corynebacterium pseudodiphtheriticum</i>	0	0	6(18.18%)	5(15.15%)
<i>Corynebacterium renale</i>	0	3(60%)	18(54.54%)	10(30.30%)
<i>Corynebacterium Diphtheriae</i>	0	1(20%)	3(9.09%)	2(6.06)
<i>Proteus</i> spp.	0	2(40%)	4(12.12%)	0
<i>Moraxella urethralis</i>	0	2(40%)	4(12.12%)	3(9.09%)
<i>E. coli</i>	0	0	5(15.15%)	7(21.21%)
<i>Klebsiella pneumonia</i> ssp. <i>aerogenes</i>	0	0	8(24.24%)	7(21.21%)
<i>Klebsiella oxytoca</i>	0	0	0	1(3.03%)
<i>Bacillus mycoides</i>	3(9.09%)	1(20%)	6(18.18%)	3(9.09%)
<i>Bacillus sphaericus</i>	1(3.03%)	1(20%)	2(6.06%)	3(9.09%)
<i>Micrococcus luteus</i>	6(18.18%)	1(20%)	3(9.09%)	6(18.18%)
<i>Micrococcus lylae</i>	0	0	2(6.06%)	0
<i>Staphylococcus warneri</i>	0	0	1(3.03%)	4(12.12%)
<i>Staphylococcus epidermidis</i>	0	0	5(15.15%)	4(12.12%)
<i>Nocardia asteroides</i>	0	0	3(9.09%)	4(12.12%)
<i>Kingella kingae</i>	0	0	3(9.09%)	0
<b>Total</b>	<b>20</b>	<b>14</b>	<b>81</b>	<b>73</b>

**Table 3: Reading of total and mean viable counts of bacteria in water from different sources**

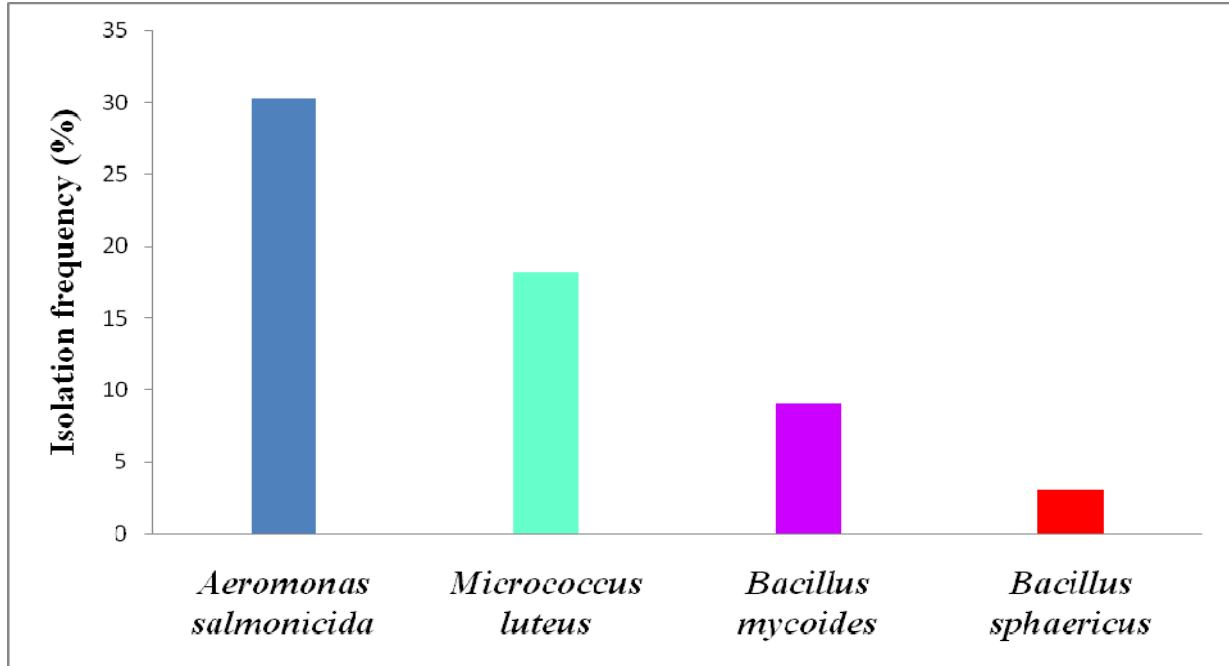
Farms	Main sources	Storage places	Water in troughs	Wall of troughs
1	0	-	$1.5 \times 10^6$	$1.4 \times 10^6$
2	0	-	$7 \times 10^3$	$9 \times 10^4$
3	0	$4 \times 10^4$	$6 \times 10^3$	$4.1 \times 10^6$
4	$5.2 \times 10^2$	-	$3 \times 10^6$	$1 \times 10^5$
5	$4 \times 10^2$	-	$9 \times 10^4$	$6 \times 10^4$
6	$1 \times 10^2$	$2 \times 10^5$	$1.6 \times 10^5$	$1.6 \times 10^4$
7	$6 \times 10^3$	-	$1.3 \times 10^4$	$1.3 \times 10^7$
8	0	-	$1 \times 10^5$	$1.1 \times 10^6$
9	0	-	$5 \times 10^5$	$1.2 \times 10^4$
10	$1.7 \times 10^3$	-	$2.3 \times 10^5$	$2.3 \times 10^3$
11	0	-	$9 \times 10^6$	$2.7 \times 10^6$
12	$4.1 \times 10^2$	-	$1.3 \times 10^6$	$7.5 \times 10^3$
13	0	-	$1.1 \times 10^5$	$6.6 \times 10^6$
14	0	-	$5.3 \times 10^3$	$3.7 \times 10^5$
15	0	-	$2.2 \times 10^5$	$2 \times 10^6$
16	$1.8 \times 10^3$	$1 \times 10^4$	$1 \times 10^6$	$3 \times 10^7$
17	0	-	$2 \times 10^6$	$3.1 \times 10^5$
18	0	-	$1.7 \times 10^5$	$3.5 \times 10^5$
19	$3.3 \times 10^4$	$3.4 \times 10^5$	$2.5 \times 10^4$	$2.5 \times 10^6$
20	$1 \times 10^3$	-	$3 \times 10^5$	$2 \times 10^6$
21	$2.6 \times 10^3$	-	$3.6 \times 10^6$	$2 \times 10^6$
22	$1.8 \times 10^3$	-	$3.3 \times 10^6$	$3 \times 10^7$
23	$2 \times 10^3$	-	$9 \times 10^4$	$2 \times 10^6$
24	0	-	$2.4 \times 10^5$	$3 \times 10^7$
25	$9 \times 10^2$	-	$3 \times 10^5$	$2 \times 10^4$
26	0	-	$2.8 \times 10^6$	$1.7 \times 10^5$
S	$1 \times 10^4$	-	$1.8 \times 10^4$	$3 \times 10^6$
28	0	-	$3.2 \times 10^5$	$1.4 \times 10^5$
29	0	$1.6 \times 10^6$	$6.2 \times 10^5$	$3.3 \times 10^6$
30	0	-	$3 \times 10^6$	$2 \times 10^6$
31	$1.4 \times 10^3$	-	$2.6 \times 10^5$	$1.5 \times 10^3$
32	$1.9 \times 10^2$	-	$2 \times 10^5$	$1.2 \times 10^4$
33	0	-	$2.6 \times 10^5$	$3.7 \times 10^7$
Mean viable counts	$4 \times 10^3$	$4.4 \times 10^5$	$1 \times 10^6$	$5.2 \times 10^6$

0 = Either no growth or number of colonies on plates was less than 30.

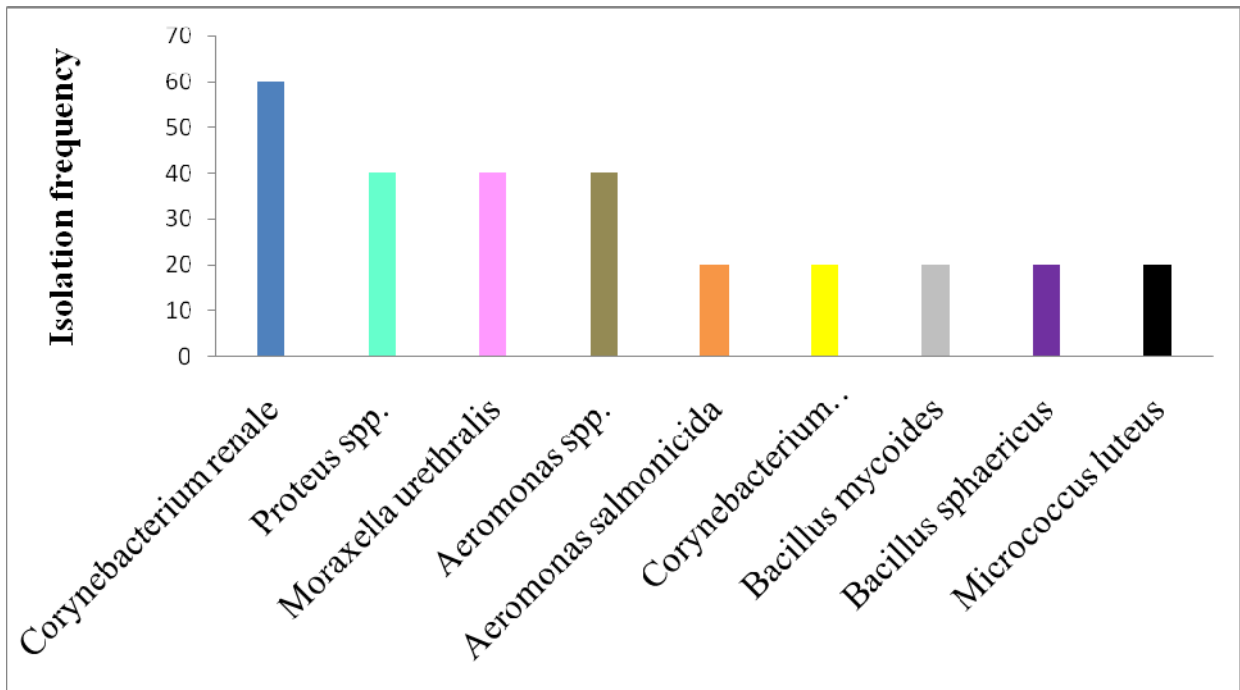
- = No storage place in these farms and hence no sample from this source.



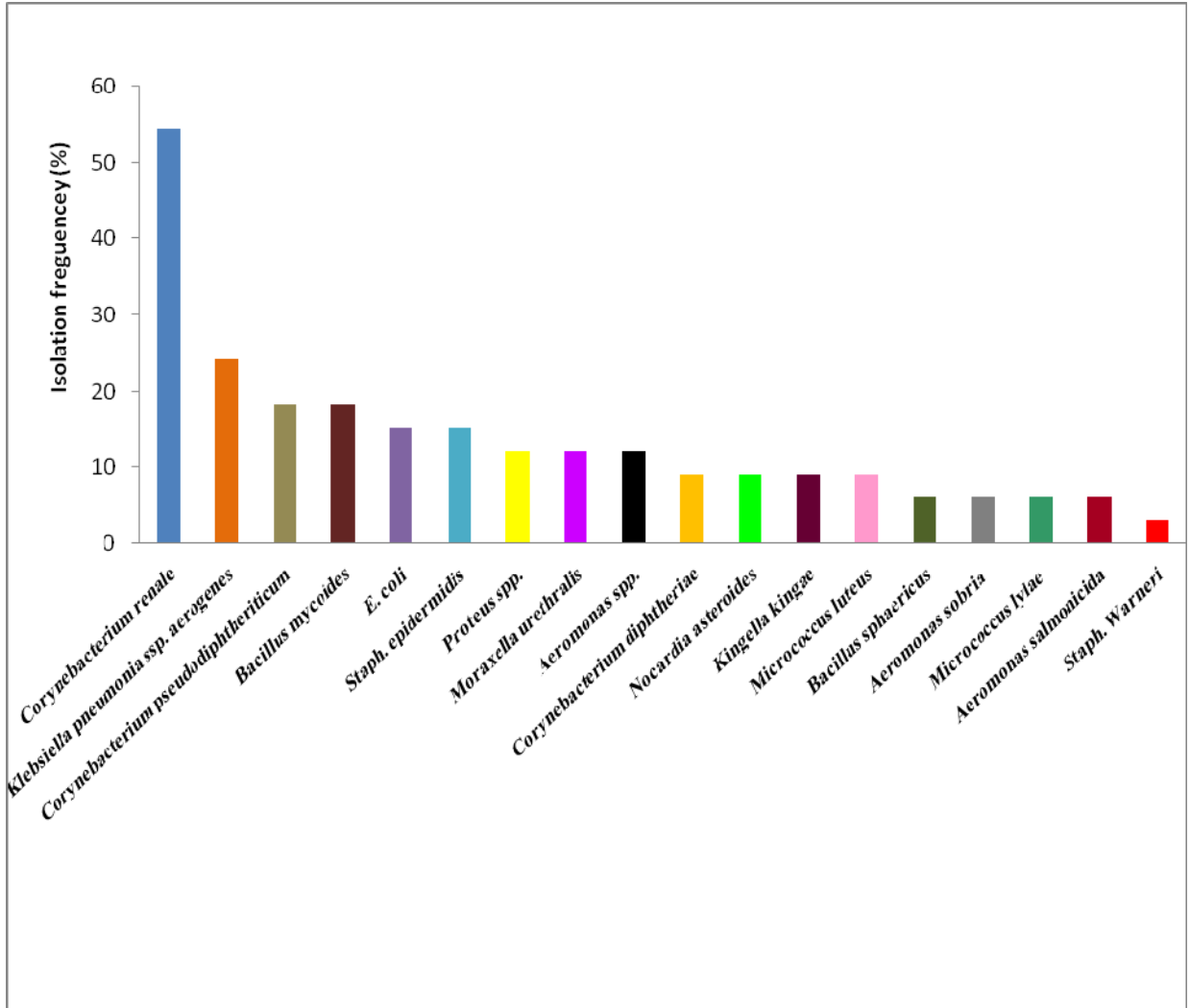
**Fig.1: Bacteria isolated from 104 samples collected from drinking water of dairy cattle farms in Khartoum North**



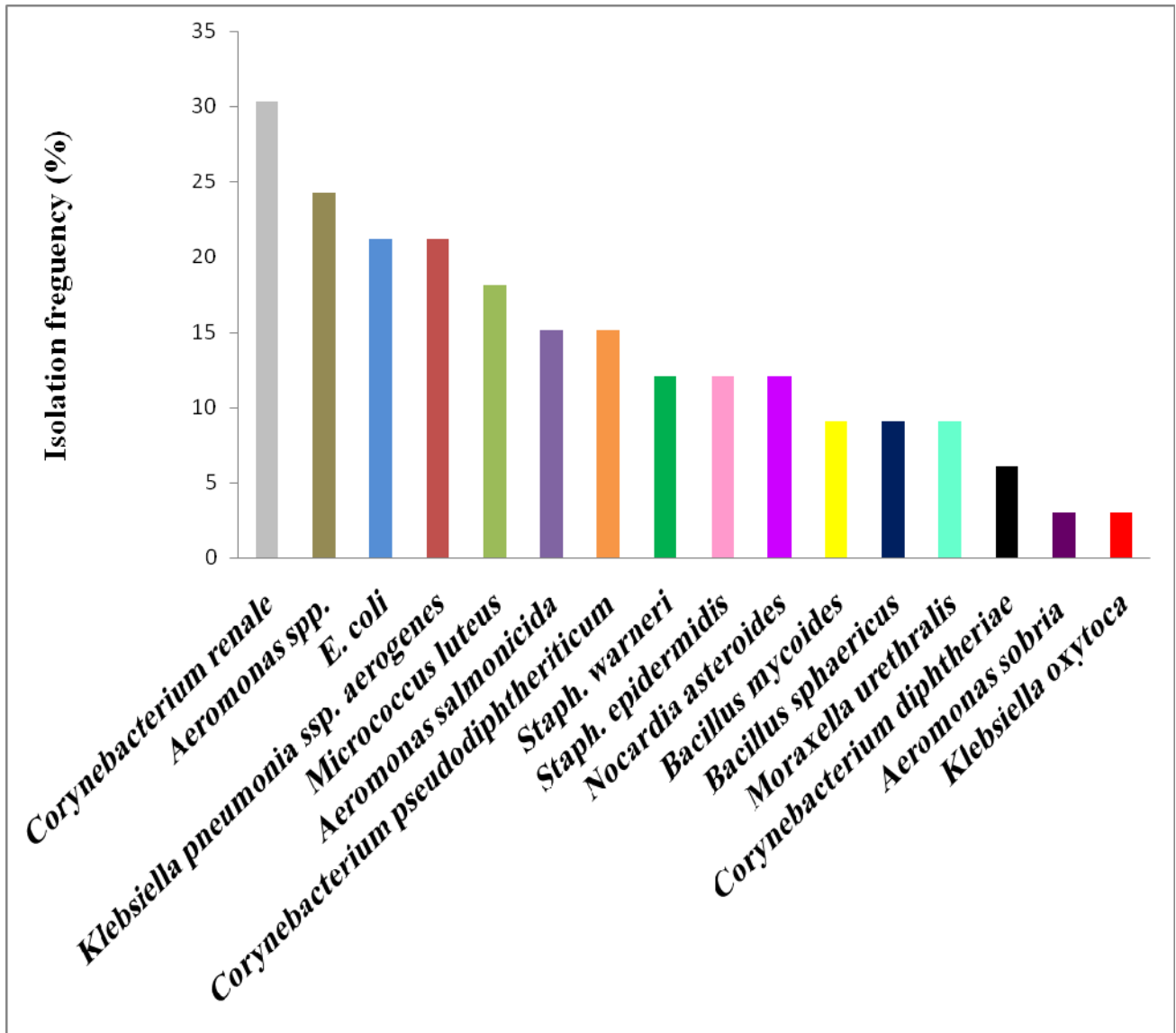
**Fig. 2: Bacterial species isolated from main sources of water**



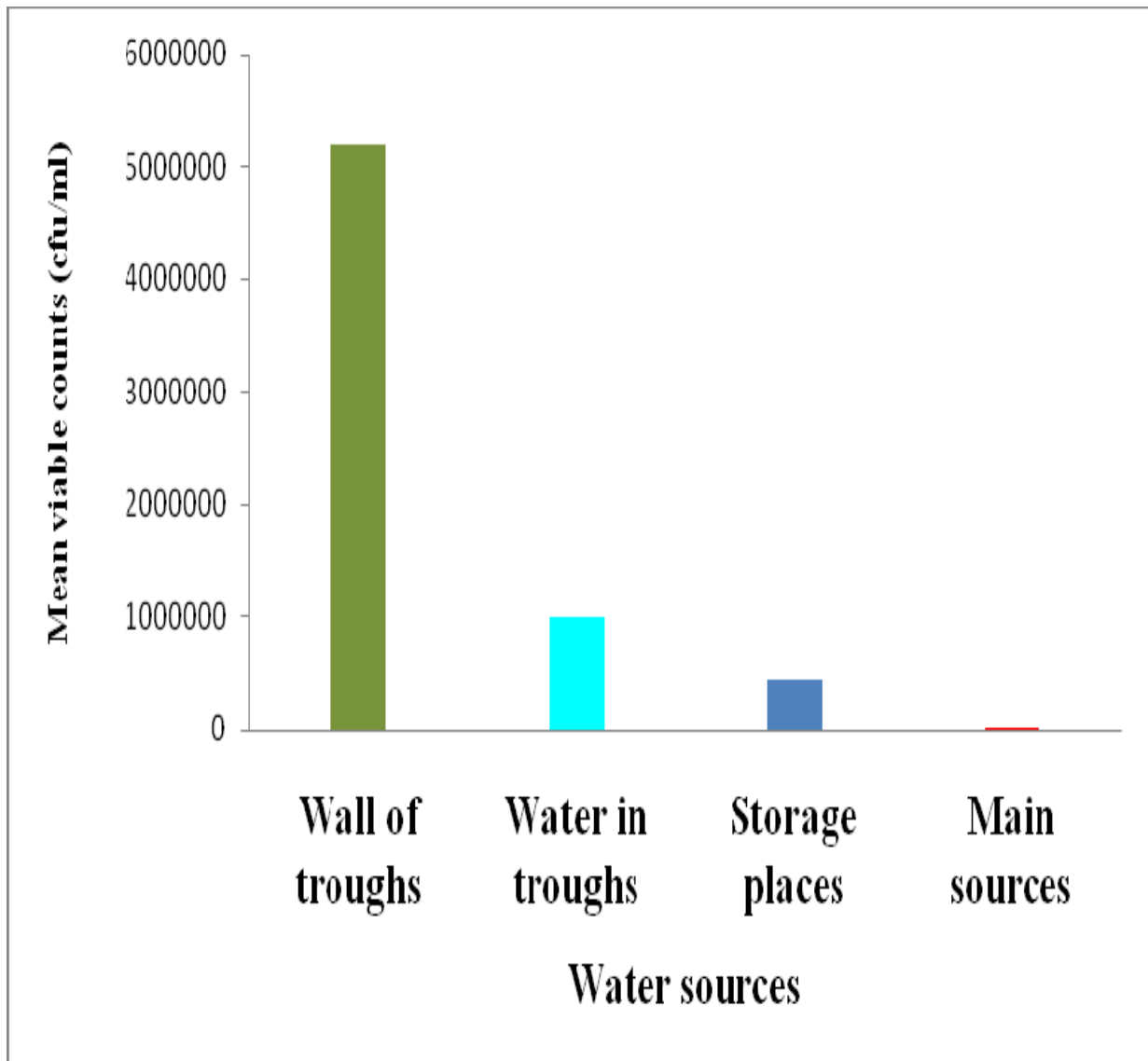
**Fig. 3: Bacterial species isolated from storage places**



**Fig. 4: Bacterial species isolated from water in troughs**



**Fig. 5: Bacterial species isolated from wall of troughs**



**Fig. 6: Mean viable counts of bacteria in water samples according to the source of water**

## Chapter Four

### Discussion

An adequate supply of a good quality water for dairy cattle is extremely important for optimal production (Kevin, 2007). Dairy cow will drink less if water quality is poor and that will limit its milk production and jeopardize its health (FAO, 2000). The present study was designed to assess bacterial quality of drinking water commonly present in dairy cattle farms, based on bacterial isolation and total viable count.

Out of 104 samples taken from 33 dairy farms, 188 isolates were recovered (1-3 organisms per sample). Isolation result showed that 57% of isolates were Gram-positive bacteria. This close isolation frequency of Gram-positive and negative bacteria indicated that they are almost equally present in the environment of dairy cattle farms and their sources may be the soil, feeds, animal skin and mucous membranes and feces as well as water.

In total, 19 bacterial types were identified; ten of them were Gram-positive bacteria. This finding showed that number of Gram-positive and negative is almost similar and this is in consistent with their number of isolates.

The most isolated bacterial types in this study were *Corynebacterium* and *Aeromonas* spp., which were amounted for 44% of the isolates. *Corynebacterium renale* and *Aeromonas salmonicida* were the most dominant species, isolated from 29.8% and 17.3% of samples, respectively. This high isolation rate may be due to contamination of water from various sources such as animal wastes, soil and feed stuffs (Hirsch and Yuan. 1999; Nayduch *et al.*, 2001). *Corynebacterium renale* is known bovine pathogen; it



causes cystitis, urethritis and pyelonephritis and it can be transmitted via water (Hirsh *et al.*, 2004).

*Klebsiella pneumoniae* spp. *aerogenes* was recovered from 14.4% of samples of this study. This bacterium has become an important cause of clinical mastitis (Saeed and El Sanousi, 2002; Hogan and Smith, 2003). Also *Nocardia asteroides* was isolated in this study and it can cause bovine mastitis (Cook and Holliman, 2004). So, systemic infection through drinking of contaminated water or direct introduction of the two organisms to healthy udder by contaminated hands through teat canal or skin abrasions is possible.

The presence of *E. coli* in water from troughs is especially important for health of weaned calves (Jeffrey *et al.*, 2001). The isolation could be due to adult cattle shed significant amounts of *E. coli* in their feces which in turn contaminated water.

Some *Bacillus*, *Staphylococcus* and *Micrococcus* spp. were isolated here and they are not known pathogens and expected to be normally found in the environment. However, at least some of them were reported as opportunistic pathogens (Smith *et al.*, 1999). So, their existent in water may cause infections during stress times such as time of calving.

According to source of water, samples from water in troughs, followed by samples from wall of the troughs were most contaminated with bacterial types. Eighteen and 16 bacterial types were recovered from the two sources, respectively, followed by storage places and the least contaminated source was the main source of water. Although number of samples from storage places was small (5 samples), nine bacterial types were obtained and high

viable counts were found. The result could be explained by the fact that storage places in these farms are exposed to contaminated air and dust and may rarely be cleaned. A similar observation was noted by Selma (2007). Likewise, the total viable count for bacteria showed that water samples from wall of troughs, followed by water in troughs were found most loaded, while samples of main sources of water were the least loaded. This is may be logic because troughs are exposed to contamination from many sources; cattle while drinking, animal feces, air, dust and feed stuffs. In addition, cleaning and change of water is not appropriates (bad hygienic measurements), walls of troughs are especially difficult to clean in case of poor trough material and detergents may not be used. Similarly, bacterial contamination in the storage places could be due to environmental contamination and bad storage of water. In contrast, the main sources of water are protected from direct contact, surface water usually treated with disinfectants and ground water (wells) is expected to be of minimum bacteria unless mixed with human sewage (Alcano, 1997). All samples from main sources did not grow on MacConkey's agar. This finding was in agreement with El Tom (1997) and Esrey *et al.* (1985), who reported that water samples from direct main source of water supply are completely free from coliform bacteria. Contrarily, Imad (2001) and Wright (1984) were able to detect coliform bacteria from main sources of water.

The overall result indicated that the degree of contamination is increasing from the main source of water to the troughs. This poor microbiological quality of drinking water of dairy cattle is expected to have adverse health and productivity effects (Jeffery *et al.*, 2001).

From the epidemiological data that were collected in this study there were cases of calf diarrhea, new borne calf deaths and mastitis. Some of the bacteria isolated here justify these disease cases.

Microbiological quality of drinking water in dairy farms is of paramount concern because of the possible acute risk to health caused by bacteria in drinking water. Therefore, regular monitoring and assessment of drinking water is primarily a health-based activity which helps to protect public health through ensuring provision of good quality water.

## Conclusions

It can be concluded that:

- Pathogenic bacteria were isolated from drinking water of dairy cattle; e.g., *Klebsiella pneumoniae* ssp. *aerogenes*, *Escherichia coli*, *Corynebacterium renale* and *Nocardia asteroides*. These organisms could be transmitted via drinking water and constitute a real hazard for dairy cattle health and consequently their productivity.
- The bacteriological quality of water in all farms was evaluated as very poor as it was reflected by the very high existence of bacterial types and loads. Water in troughs was especially dangerous.

## **Recommendations**

- Provision of good quality drinking water is crucial for life and optimum productivity of dairy cattle. So, practicing and application of appropriate hygienic measures are important. These should include good water source, appropriate selection of materials of storage places and troughs and be properly located to minimize bacterial contamination; in addition to regular proper cleaning. Health education programs aiming at increasing awareness on the importance of clean water for animal's health are helpful.
- As it was demonstrated here that drinking water of dairy cattle is not safe, regular monitoring of microbial quality may help in application of proper preventive measures that would help reducing the effect. This could be achieved by conduction of further researches to investigate the risk associated with microbial contamination of drinking water in dairy cattle farms in the Sudan.

## References

- Abd Mageid, H.M., Ibrahim, S. and Dirrar, H.A. (1984). Chemical and microbiological examination of well and Nile water. *Enviro. Internat.* pp. 259-263.
- Alcano, E.L. (1997). *Fund. Microbiol.* 4<sup>th</sup> ed. The Benjamin/ Cumming publishing company Inc, Red Wood city.
- Ashbolt, N.,G., Grohmannand, A.S., and Kuch, C. (1993). Significance of specific bacterial pathogens in the assessment of polluted receiving water of Sydeny. *Water Sci. Tech.* **27**:449-452.
- Barcina, I. (1995). Survival strategies of enteric bacteria in aquatic system. *Microbiologia*, **11**:389-392.
- Barcina, I., Gonzaez, J.M., Iriberry, J., and Egea, L. (1989). Effect of visible light on progressing dormancy of *Echerichia coli* cells during the survival process in natural fresh water. *Appl. Environ. Microbiol*, **55**:246-251.
- Barrow, W.G.L. and Feltham, R. K. A. (1993). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 3<sup>rd</sup> ed. Cambridge University press, U.K.
- Bray, D. R., Beede, D. K., Delorenzo, M. A., Wolfenson, D., Giesy R. G. and Bucklin, R. A. (1990). Environmental modification update.

*Proc. Florida Dairy Production Conf.*, Florida Coop. Ext. Serv., University of Florida, Gainesville.

Brendan, H. (1975). The dissemination of disease. In: *Pattern of Animal Disease*, The Williams & Wilkins Company, Baltimore, pp 56-87.

Cairncross, S., Carruthers, I., Curtis, D., Feachem, R., Breadley, D. and Baldwin, G. (1980). *Evaluation for village water supply planning*. Published in association with International Reference Center for Community Water Supply, by John Wiley and SONS. Chichester. New York.

Cook, J.G. and Holliman, A. (2004). Mastitis due to *Nocardia asteroides* in a UK dairy herd following restocking after FMD, *Vet. Rec.*, **28**: 154(9)- 267(8).

Cowan, S.J. and Steel, KJ. (1985). *Manual for Identification of Medical Bacteriology*, 2<sup>nd</sup> ed. Cambridge University Press, London.

Cruickshank, R., Duguid, J. P., Marmion, B.P. and Swan, R.H .A. (1975). *Medical Microbiology*, 12<sup>th</sup> ed. Longman group limited, London.

Davis, C. L., Grenwalt, D. A. and McCoy, G. C. (1983). Feeding value of pressed rewers grains for lactating dairy cows. *J. Dairy Sci.* **66**:73.

- Duerden, B.I., Reid, T.M.S., Jewsbury, J.M. and Turk, D.C. (1988). *A New Short Text Book of Microbial and Parasitic Infection*, 2<sup>nd</sup> ed. Edward Arnold, London.
- El Shazali, H. and Erwa, H. (1971). An over looked source of gastroenteritis in Sudanese children. *Sud. Med. J.* **9**: 46.
- El Tom, A.A.M. (1997). *Microbiology of Port-Sudan water supply*, M. Sc. Thesis, University of Khartoum.
- Esrey, S. A., Feachem, R. G., and Hughes J. M., (1985). Intervention for control of diarrhoeal diseases among young children by improving water supplies and excreta disposal facilities. *Bull. WHO.* **63**:752-757.
- FAO (1997). *Ground Water Pollution*, No. 31, FAO. Rome.
- FAO (2000). Livestock Numbers and Products. *FAO Bull. Stat*, Vol. **1**: pp 78-88. FAO, Rome.
- Forrest, B. (1956). *Rural Water Supply and Sanitation*. John Wiley and Sons, Inc. New York.
- Gonzalez, J.M., Iriberry, J., Egea, L., Barcina.1. (1992). Characterization of culture ability, grazing, and death of enteric bacteria in aquatic ecosystems. *Appl. Environ. Microbiol*, **59**:998-1004.
- Guentzel, M.N. (1996). *E. coli, Klebsiella, Enterobacteria, Serratia and Proteus*. In: *Barron's Medical Microbiology*, 4<sup>th</sup> ed, University of Texas, Medical Branch.



- Guidry, A., Fattom, A., Atul, P., Shepherd, S. and Lohuis, J. (1998), serotyping scheme for *staphylococcus aureus* isolated from cows with mastitis. *Am. J. Vet. Res*, **59**: 1537-1539.
- Hirsch, D. C. and Yuan, C. Z. (1999). *Veterinary Microbiology for School of Veterinary Medicine*. University of California.
- Hirsch, D. C, Maclachlan, N. J. and Walker, R. L., (2004). *Veterinary Microbiology*, 2<sup>nd</sup> ed. Black well.
- Hogan, J., and Smith, K. L. (2003). coliform mastitis. *Vet. Res*. **34**: 507-519.
- Hosny, A. (1981). Examination of water. In: *Notes on Animal Hygiene*. Assuit University Press, pp. 18-41.
- Imad Eldein, O. A. (2001). Bacteriological quality assurance study of drinking-water of western Omdurman. M. Sc thesis, University of Khartoum.
- Jeffrey, T., Thomas E. and Hancock, D. D. (2001). Cattle water troughs as reservoirs of *Escherichia coli* 0157. *Appl. Envir. Microbiol*, **67**:3053-3057.
- Kabler, P., Clark, H. and Geldreish, E. (1960). Coliforms in sanitary bacteriology, *J. Amer. Wt. Wks. Assoc*. **52**: 105.
- Keenan, T.W. and Patton, S. (1995). The structure of milk. In: *Handbook of Milk Composition*. Academic Press, USA; pp. 5-50.
- Kevin, M. (2007). *Water Requirements of Livestock*. OMAFRA

Factsheet, order No.86-053.

- Lechevallier, M.W., Welech, N.J., and Smith, D.B. (1996). Full-scale studies factors related to coliform re-growth in drinking water. *Appl. Environ. Microbiol.* **62**:2201-2211.
- Lewis, W. (1985). The significance of water management in relation to public and environmental health. *Supp. J. Appl. Bacteriol.* **59**: 1-13.
- Linn, J. G., Plegge, S.D., Otterby, D. E. and Hansen, S. A. (1987). *Water quality and Importance of it in Dairy and Beef Production*. Midwest PMS, Gering, NE.
- Linn, J.G. (1991). Water quality for dairy and beef cattle. Proceedings from the Fourth State Applied Nutrition Conference. Lacrosse, WI.
- Mahgoub, D.M. (1984). *Coliform bacteria in the Nile*. M.Sc. thesis, Faculty of Agriculture, University of Khartoum.
- Mallory, L.M., Yuk, C.S., Liang, L.N., and Alexander, M. (1983). Alternative prey: a mechanism for elimination of bacterial species by protozoa. *Appl. Environ. Microbiol.* **46**: 1073:1079.
- Marino, R.P., and Gannon, J.J. (1991). Survival of faecal coliforms and faecal streptococci in storm drain sediment. *Water Res.* **25**:1089-1098.
- Meybeck, M., Kuusisto, E., Makela, A. and Malkki, E. (1996). Water Quality. In: UNEP/WHO. *Water Quality Monitoring- A*

*Practical Guide to the Design and Implementation of Fresh water Quality Studies and Monitoring Programmes.*

Milam, K. Z., Coppock, C. E., West, J. W., Lanham, J. K., Nave, D. H.,

Labore, J. M., Stermer, R. A. and Brasington, C. F. (1986). Effects of drinking water temperature on production responses in lactating holstein cows in summer. *J. Dairy Sci.* **69**:1013.

Miles, A. A and Misra, S. S. (1938). In laboratory methods in food and dairy microbiology 1976. Academic Press. 31.

Murphy, M. R., Davis, C. L. and McCoy, G. C. (1983). Factors affecting water consumption by Holstein cows in early lactation. *J. Dairy Sci.* **66**:35.

Nayduch, D., Honko, A., Noblet, G. P. and Stutzenberger, F. (2001). Detection of *Aeromonas caviae* in the common housefly *Musca domestica* by culture and polymerase chain reaction. *Epidemiol. Infect.* **127**: 561-566.

Ryan, K. J. and Ray, C. G. (2004). *Sherris Medical Microbiology*, 4<sup>th</sup> ed, McGraw Hill.

Saeed, E. M. A., El Sanousi, S. M. (2002). Biochemical characteristics of *Klebsiella* strains isolated from man and animals. *Sud. J. Vet. Sci. Anim. Husb.* **41** (1&2): 56-62.

- Selma, K. A. (2007). *Bacterial Contamination of Drinking Water and its Degree of Sensitivity to Some Commercial Water Disinfectants in Poultry Farms at Khartoum state*. M. Sc. Thesis, University of Khartoum.
- Smith, A. L. (1981). *Principles of Microbiology*. 9<sup>th</sup> ed. C.V. Mosby Company, St. Louis Toronto, London.
- Smith, K., Neafie, R., Yeager, J. and Skelton, H. (1999). *Micrococcus folliculitis in HIV-1 disease*. *Br. J. Dermatol.* **141** (3): 558–561.
- Snow, J. (1855). *On the mode of communication of Cholera*. 2<sup>nd</sup> ed. John Churchill, London.
- Stanier, R. X.; Ingraham, J. L.; Weelis, M. L. and Painter, P. P. (1986). *The Microbial World*, 5<sup>th</sup> ed. New Jersey, Prentice Hall.
- Theroux, F.R. and Leroy, W. (1943). Purpose of bacteriological analysis. In: Theroux, F. R. and Eldridge, E. F. (eds.). *Laboratory Manual for Chemical and Bacteriological Analysis of Water and Sewage*. Michigan State College, Michigan, pp. 253-264. .
- Twort, A.C., Law, F.M. and Crowley, F.W. (1985). *Water Suppl*, 3<sup>rd</sup> ed. Edward Arnold, London, USA, Astustralia.
- Van der, V. (1992). *Microbial Contamination of Drinking Water in Troughs for Dairy Cows in Loose Housing*. M.Sc. Thesis. Institute fur Tiersucht, Tierarzthich Hochschule, Hanover, Germany.

- White, W.R. and Godfree, A.F. (1985). Pollution of fresh water and estuaries. *J. Appl. Bacteriol*, **8**: 77- 81.
- WHO (1984). *Guidelines for Drinking Water Quality*. World Health Organization Press, Geneva.
- WHO (1996). *Guidelines for drinking water quality*. World Health Organization Press, Geneva.
- WHO (2006). Guidelines for drinking-water quality (electronic resources): incorporation first addendum. Vol. **1**, Recommendations. 3<sup>ed</sup> ed.
- Wright, R. (1984). Water Quality Analysis. An integral component of water supply development in developing countries. *Wat. Qual. Bull.* **4**: 222-224.
- Wright, T. (2007). *Water Quality for Dairy Cattle*. OMAFRA Factsheet Order No. 03-085

**Questionnaire on Bacterial Contamination of Drinking Water in Dairy  
Farms in Khartoum North Area**

**Date of sampling:** .....

**Sample serial no.:** .....

**Sample source:** .....

**Farm no.:** .....

**Location:** .....

**Availability of a veterinarian:** .....

**Cattle breed:**

Local ( )      Cross ( )      Foreign ( )      Mixture ( )

**Cleanness of the farm in general:**

Bad ( )      Fair ( )      Good ( )

**Common bacterial diseases found in the farm** .....

.....

**State of main source of water in the farm:** .....

**State of storage place of water in the farm:** .....

**Type and state of the drinkers in the farm:** .....

**Level of troughs compared to feed level** .....

**Others**.....

.....



Water troughs put in cattle house floor surrounded by mud, feces and urine which may increase contamination of drinking water



Washing of human hands and face is an unhygienic practice, which may contribute to animal and human hazards





Water troughs are not cleaned regularly, disinfectants and detergents are not used for washing troughs and water trough material is poor. The photo compares between very dirty walls of cement troughs (lower) and semi-clean metal troughs (upper).