BACTERIAL CONTAMINATION OF DRINKING WATER IN
SELECTED DAIRY FARMS IN KHARTOUM NORTH, SUDAN

By

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The present study has been done at the Department of Preventive Medicine and Public Health, Faculty of Veterinary Medicine, University of Khartoum under supervision of Dr. Isam Mohamed Ali El-Jalii.
DEDICATION

This work was dedicated

To

The Soul of my mother Mahasine,

Kindly Father, and To all whom I love.
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ABSTRACT

This study was conducted to investigate contamination of drinking water in selected dairy farms in Bahri province based on bacterial isolation and total viable count.

A total of 50 farms were examined. The farms were distributed in 5 areas namely Alhalfaya, Alsamrab, Helat Koko, Helat Kogaly and Shambat. Ten farms from each area were examined. A total of 50 drinking water samples (one from each farm) were collected. All water samples were examined for bacterial viable count and cultured to isolate common bacteria present. 63 bacterial isolates were recovered from all samples. The isolated bacterial genera were Bacillus, Corynebacterium, Enterobacteria, Staphylococcus, Streptococcus, Actinobacillus, Campylobacter, Moraxella, Aeromonas, Cardiobacteria, Pseudomonas and Branhamella. All water samples examined showed moderately high viable count. The mean viable count was ($1.2 \times 10^6$, $2.2 \times 10^6$, $2.2 \times 10^5$, $2 \times 10^5$, $3.7 \times 10^5$) C.F.U/ ml for Shambat, Alsamrab, Alhalfaia, Helat Koko and Helat Kogaly areas respectively.

Most of the farms examined were with bad hygiene specially arround drinking water troughs. It could be concluded that drinking water in the selected dairy farms were bacteriologically contaminated.
ملخص الأطروحة

كان الغرض الأساسي من هذه الدراسة هو عمل فحص لدرجة تلوث مياه شرب أبقار الحليب بمنطقة بحرية وعزل البكتيرولوجي والعد الكلي للخلايا البكتيرية الحية هو مركز هذه الدراسة.

اختبرت 50 مزرعة أبقار للاختبار وكان جمع عينات مياه شرب الأبقار موزع على 5 مناطق بمعدل 10 مزارع لكل منطقة والمناطق هي كما يلي: (شمبات، السامر، الحلفايا، حلة خوجلي، حلة كوكو).

كل عينات مياه شرب الأبقار تم اختبارها في عزل البكتيرولوجي والعد الكلي للخلايا البكتيرية الحية وتم عزل 63 جنس من البكتيريا من أصل 50 عينة. والأجناس التي تم عزلها في هذه الدراسة هي كما يلي:

(Bacillus, Enterobacterium, Corynebacterium, Actinobacillus, Sterpetococcus, Staphylococcus, Branhamella, Pseudomonas, Cardiobacteria, Aremonas, Moraxella, Campylobacter).

وكل عينات مياه شرب الأبقار المختبرة أظهرت درجة عالية من التلوث بالخلايا البكتيرية الحية وكان متوسط العد الكلي كالآتي: (1.2x10^5, 2.2x10^5, 2.2x10^6, 2.2x10^5, 3.7x10^5) لكل من حلة خوجلي، حلة كوكو، الحلفايا، السامر، وشمبات على التوالي.

الغلافية العظمى لأبقار الحليب المختبرة أظهرت درجة عالية من التداني الصحي خصوصاً حول مناهل شرب الأبقار وهذا بالتأكيد إد إلى ارتفاع درجة التلوث البكتيري لمياه شرب الأبقار.
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INTRODUCTION

An adequate supply of clean and fresh drinking water is widely considered essential for optimal cow health and maximum milk production (Church, 1991; Ens-minger et al., 1990). Physico-chemical properties of water suitable for livestock have been documented but, despite the fact that waterborne transmission of pathogen among livestock has been long recognized. Little information is actually available concerning the microbiological quality of water offered to cattle (Reilly, 1981., Hanninen et al., 1998). Logically, livestock drinking water heavily contaminated with enteric bacteria could serve as a common source of exposure to potential pathogens to cattle that could result in infection of large numbers of animals during a relatively brief period. The extent to which water troughs serves as reservoirs for enteric microorganisms and the frequency that waterborne transmission of these pathogens occurs from water to cattle is not fully known. Clean hygienic water is a major factor contributing to good diary cattle.

Water is said to be bacteriologically contaminated or polluted either due to presence of certain pathogens or due to high increase of total viable count or due to presence of what called indicator bacteria at certain levels (Therax and Leoroy, 1943). Many bacteria are used as indicator to demonstrate presence of bacterial pollution of drinking water. The microbiological examination of drinking water is normally conducted to
assess hygienic quality of water. Microbiological parameters as 37 c° viable count are used not for disease risk estimation but as indictor for treatment process.

Bacterial contamination of drinking water could be a problem for dairy cattle because pathogenic organism that found in drinking water may lead to intestinal infection, dysentery, hepatitis, typhoid fever, cholera and other illnesses.

Khartoum North (Bahri) area is known for its high population density of diary cattle; these cattle have local, cross and foreign breeds. There are different sources of drinking water in these diary farms like ground water and general net water sources.

No previous study was carried out in Bahri area to investigate diary farms drinking water contamination, although the problem is a health importance.

Hence the present study was designed to investigate this problem.

The main objective of the present study was to investigate the quality of drinking water commonly present in selected diary farms in Khartoum North (Bahri). The specific objectives were:-

1\ To investigate the degree of water contamination based on bacterial count.

2\ To isolate and identify the main bacteria found in the drinking water.
CHAPTER ONE
LITERATURE REVIEW

1.1 Water

Clean hygienic water denotes that it is free of pollution. Water is said to be polluted either due to presence of harmful chemical or bacterial contamination (WHO, 1984).

Unhygienic water contributes to many animal and human hazards like infections (Bartram and Wheeler, 1993).

1.1.1 Importance of water

The use of water by man, animals and plant is universal. It is known that water quantity and quality are ever controlling the size and the shape of human and animal's settlement. In addition to the direct use of water in our homes, there are many indirect ways which water affects lives. The use of water is increasing rabidly with growing population (Abdel Mageid et al, 1984). There are acute shortages in both surface and underground water in many localities in the world (Forreset, 1995).

1.1.2 Water sources and supply

In nature, water is constantly changing for one state to another. Rain water can be collected from outdoor water sheds and stored in cisterns or ponds for daily use. Other sources are surface water like rivers, stream, lakes, ect. A third source is the underground water stored in the earth's
crust. Ground water is usually good in quality and can be used for domestic purposes with much treatment, (Mohammed, 1982).

1.1.3 Drinking water quality

The nature of the water environment needs to be defined in relation to the use for which water is required (Cox, 1973). Drinking water therefore concerns both the quality and quantity of the water required to meet the need of man and animals (Mahgoub, 1984). It is necessary to determine the physical, chemical and biological parameters that affect the quality of water. Any change in water quality parameter will lead to the changes in nature and composition of the water environment (Abd Alla, 1997)

Surveillance of drinking water quality is a conditions vigilant public health assessment and over view of safety and acceptability of drinking water by world health organization (WHO, 1976).

Water quality control devotes regular sampling and analysis of water sample as well as recording of results obtained. However, it also involves assessing how good method is and how well is operating in practice (WHO, 1976).

WHO (1976) has issued guidelines for drinking water quality, a report in three volumes, Vol.1 deals with guidelines values, Vol.2 deals with each water contaminations and Vol.3 gives informations on how to handle water supplies in small rural countries.
WHO (1976) recognizes that very stringent standards can not be used universally and so arrange of guidelines values for more than 60 parameters have been elaborated. Most nations have their own guidelines or standards. The control exerted by local regulatory authorities may differ from place to place depending on the local situation. Qualitative and quantitative measurements are needed from time to time for constant monitor of the quality of water from the various sources of supply.

1.1.4 Characters of safe water

Water is usually demanded to meet different needs such as drinking, agriculture and industrial activities. When it is polluted it will becomes not acceptable for human or animal consumption. So good quality of water must fulfill the fowling requirements:-

- It must be acceptable with good taste not turbid, colorless and with no smell.
- Microbiologically safe, so it should not contain any pathogenic bacteria, viruses, fungi, protazoa and helminthes eggs.
- Chemically hygienic water content of toxic materials, organic or inorganic, must be zero or at minimum. It should contain appropriate content of iodine, fluoride and harmless Ions.
- It must be safe radiologically (White and Godfree, 1985).

The most common and widespread danger associated with drinking water is contamination, either directly or indirectly, by
sewage (Table 1), other wastes, or human or animal excretions. If such contamination is recent, and if among the contributors there are carriers of communicable enteric diseases, some of the living causal agents may be present. The drinking water will be contaminated or its use in the preparation of certain foods may result in further cases of infection. Natural and treated waters vary in microbiological quality. Ideally, drinking water should not contain any microorganisms known to be pathogenic to man and animals. In practice, this means that it should not be possible to demonstrate the presence of any coliform organism in any sample of 100 ml.

Pathogenic organisms found in contaminated water may be discharged by human beings or animal infected with disease or carriers of a particular disease.

1.2 Water needs

Water is usually demanded to meet the following needs like drinking, irrigation for agriculture, industry and trade. Water needed in each category of the previous ones has different quality criteria.

1.3 Water classification

Scientists classified water into two major types; ground water and surface water. Ground water originates from deep wells and because of
filtering action virtually free of microorganisms. As water flows up along channels, contaminants may enter and alter its quality (Alcano, 1997).

Surface water is found in lakes, stream and shallow well.

Generally, surface water contains more microbes than ground water and rain water since the majority of soil microorganisms are found in the upper crust (6 inches) of the earth. Surface water contains many non pathogenic microbes from soil, and in the vicinity of cities it is often contaminates with sewage bacteria (Smith, 1981).

A major type of bacteria in polluted water is coliform bacteria, a group of Gram negative non spore forming bacilli which inhabit human and animal intestines. They usually ferment lactose without acid and gas. The most important species of the group include *Escherishia coli*, *Klebsiella spp* and *Enterobacter spp*.

Non coliform bacteria are also found in polluted water and include *Sterptococcus, Proteus* and *Pseudomonas* species (Alcano, 1997).

1.4 Water pollution

1.4.1 Definition
Pollution was defined in the British Royal Commission Tenth Report (Jones and Walkins, 1985) on environmental pollution as: The introduction by man into the environment by the substances or energy liable to cause hazards to human health, harms to living resources and ecological systems damage to structure or amenity, or interfere with the legitimate use of environment. This definition can be applied to pollution of water since water is a part of the environment.

1.4.2 Water pollutant

The most common pollutants of water according to (Elrofaei, 2000) are biodegradable organic matters, suspended solids ammonia, nutrients, bacteria from industrial and agriculture sewage, waste effluents, detergents, phenols, cyanide, metals, acids, alkalis, pesticides, ails and industrial chemicals.

Industrial chemicals and microbial contaminants from sewage and waste constitute the major source of pollutants for drinking water (Alrofaei, 2000).

1.5 Water bacterial contamination
There are variety of sources which contaminate water including human and animals.

- Human and animal wastes which are a primary source of bacteria in water.
- Insects, rodent or animals that enter wells.
- Discharge from septic tanks and sewage treatment centers.
- Natural soil/plant bacteria. Bacteria from these sources can enter wells that open at the land surface.
- Infiltration by flood waters or by surface runs off. Flood water commonly contain high levels of bacteria.

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 that 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.
The modes of transmission of bacterial pathogens include ingestion of contaminated water. The significance of the water route in the spread of intestinal bacterial infections varies considerably, both with the disease and with local circumstances. Among the various waterborne 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 *Salmonella* serotypes are usually required to cause gastroenteritis. Similarly, with toxigenic organisms such as enteropathogenic *E. coli* and *V. cholerae* 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 veer, 1992).

### 1.5.1 General water contamination in aquatic system

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 contaminant in diary 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 the 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 bottom of the trough (Ashbolt et al., 1993).

In addition to the nutrient content of the water, several other factors may influence the survival rate of bacteria in water, including the exposure to direct sunlight and temperature and competition with other microorganisms (Barcina, 1995). The lower *E. coli* densities in the troughs exposed to direct sunlight observed was consistent with the reported deleterious effects of visible light on *E. coli* survival in other aquatic systems (Barcina et al., 1989). The observed seasonal fluctuations in *E. coli* counts in water parallel the seasonal trend in total bacterial counts reported in longitudinal study of troughs on a single farm (Van der Veer, 1992). Bacteria in aquatic systems are more likely to proliferate as water temperature increases, especially above 15 °C (Lechevallier et al., 1996). The reported increases in infection of cattle with *E. coli* 0157 during summer months may result, in part, from increased concentration of agent contaminated water troughs (Hancock et al., 1994).

*Salmonella* tended to be isolated more frequently in the less recently cleaned troughs. The ability of *E. coli* and *Salmonella* spp to survive in other aquatic environments suggests that, once introduced, these bacteria may persist and possibly proliferate as endogenous flora within the troughs, whereas recently cleaned troughs would be less likely to harbor
these particular strains of bacteria until they are recontaminated from an outside source (Burton et al., 1987; Marino and Gannon, 1991).

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 systems (Gonzalez et al., 1992; Mallory et al., 1983; Marino and Gannon, 1991).

The extent of bacterial contamination observed in the drinking water offered to cattle demonstrated that the animals are daily exposed to multiple types of bacteria from the source of drinking water. Multiple factors that influence the survival and persistence of bacteria in natural aquatic systems also appear to have an effect on the complex ecosystems present in diary cattle water troughs (Gonzalez et al., 1992; Mallory et al., 1983; Marino and Gannon, 1991).

1.5.2 Bacterial contamination associated with diary cattle water troughs

Water from troughs used by weaned dairy calves was sampled on California, USA dairies to determine the prevalence and associated risk factors for Salmonella contamination. Salmonella was found on 4 of 48 dairies (4/82 water samples) in fall and on 8 of the same 37 dairies (8/83 water samples) in summer. Primary risk factors associated with the increased prevalence of Salmonella in water offered to wean dairy calves
were continuous water tank-filling method compared to a valve (Adhikari. *et al*, 2004).

*E. coli* shedding in cattle populations is spatially and temporally clustered, consistent with point sources of exposure to the organism, such as periodically contaminated feed or water. Water offered to livestock is often of poor microbiological quality, and *E. coli* is present in as many as 10% of troughs. Although drinking water is recognized as an important vehicle in diary cattle for *E. coli* infections, it is not known whether cattle drinking from previously *E. coli* contaminated water troughs are prone to colonization with this agent. If *E. coli* persists and remains infectious in livestock water troughs, then farm management practices that target this environmental reservoir may ultimately aid in the control of *E. coli* in cattle (Jeffrey T. *et al*, 2001).

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. While it is very likely that infected cattle frequently contaminate their water troughs with feces or saliva containing *E. coli*, it was contributed that contaminated troughs can act as long-term reservoirs of the organism with a real potential for infection of cattle weeks or months later. 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 periods of time, such as during the cooler times of the year when *E. coli* typically occurs at a very low prevalence in cattle. Since *E. coli* can survive for extended periods within complex aquatic environments, caution should be used prior to the use of water for livestock or for human drinking and recreational purposes after a suspected contamination event (Jeffrey T. *et al*, 2001).

*Corynebacterium* commonly found on mucous membrane and the skin of the animal and gastrointestinal tract of normal diary cattle and sheep also found in the soil and the manure of the animals can contaminate water troughs. *Streptococci* live commensally in the upper respiratory tract, alimentary tract and lower genital tract can be transmitted to the water troughs by infected saliva. *Staphylococci* found in the upper respiratory tract and other epithelial surface of diary cattle. They present also on skin and mucous membrane but are rarely pathogenic. *Bacillus* in soil is the source of infection and infection can be transmitted to animals by ingestion of contaminated water (Dwight *et al* 1999).
**Table 1: List of infectious agents potentially present in drinking water contaminated by sewage**

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<tr>
<th>Bacteria</th>
<th>Diseases</th>
<th>Remarks</th>
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<td><em>Escherichicoli</em> (Entropathogenic)</td>
<td>Gastroenteritis</td>
<td>Diarrhoea</td>
</tr>
<tr>
<td><em>Legionella pneumophila</em></td>
<td>Legionellosis</td>
<td>Acute respiratory illness</td>
</tr>
<tr>
<td><em>Leptospira (150 spp.</em>)*</td>
<td>Leptospirosis</td>
<td>Jaundice, fever</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>Typhoid fever</td>
<td>High fever, diarrhoea</td>
</tr>
<tr>
<td><em>Salmonella (~1700 spp.</em>)*</td>
<td>Salmonellosis</td>
<td>Food poisoning</td>
</tr>
<tr>
<td><em>Shigella (4 spp.</em>)*</td>
<td>Shigellosis</td>
<td>Bacillary dysentery</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>Cholera</td>
<td>Extremely heavy diarrhoea, dehydration</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>Yersinosis</td>
<td>Diarrhoea</td>
</tr>
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1.6 Poor water quality in dairy cattle farms

An average dairy cow drink about 25 gallons of water each day, but it will drink less if water quality is poor and that will limit its milk production and jeopardize its health (FAO, 2000).

Livestock drinking water was analyzed for coliform bacteria and other microorganisms to determine its microbial quality. Highly contaminated water exposed cattle to disease causing organisms. Microorganisms can contaminate water in wells. However, bacterial contamination is much more likely to occur in the drinking vessel, so keeping water troughs clean is a must (FAO, 2000).

Treating water to remove or reduce contaminants can be expensive and may require significant equipment maintenance. Therefore, laboratory analysis of the drinking water before making a decision to treat, so treatment needs to 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 contaminant. Eliminating disease-causing microorganisms involves disinfecting the water. The most common chemical disinfectant used is chlorine, a powerful oxidizing agent that is expensive and effective at low concentrations. The effectiveness of chlorine disinfection is determined by the product of the chlorine concentration and contact time. Therefore, chlorine usage typically requires a contact tank that
allows the chlorine time to disinfect the water prior to consumption (FAO, 2000).
CHAPTER TWO
MATERIAL AND METHODS

2.1 Study area

Khartoum North (Bahri) was selected as a study area. The selection of the location of the study was based on the dairy cattle livestock density.

2.2 Farms

A total of 50 farms were examined for drinking water contamination. The farms were distributed in five areas namely Elhalfaia, Elsamrab, Helat koko, Helat Khogaly and Shambat. 10 farms from each location were examined.

2.3 Samples

2.3.1 Collection

A total of 50 water samples (one from each farm) were collected. 30 water samples were from farms with net water supply while 20 water samples were from farms with underground water supply. All samples were taken by sterile 10 ml syringe then put in sterile closed glass bottles (previously sterilized in autoclave at 120 °C under 15 lb atmospheric pressure for 15 minutes).
2.3.2 Transportation

Collected water samples were transported promptly to the laboratory within 30 minutes. Samples were examined immediately within one hour after collection.

All water samples were examined for bacterial viable count and cultured to isolate common bacteria present.

2.4 Bacteriological examination

2.4.1 Culture media

2.4.1.1 Nutrient agar

Dehydrated nutrient agar (Oxoid) was prepared according to the manufacturer instruction. This medium consists of yeast extract, sodium chloride and agar. Twenty eight grams of the powder was dissolved in a liter of distilled water by boiling. The pH was adjusted to 7.4 and then the medium was sterilized by autoclave (121 °C for 15 minutes) cooled to 50 °C-55 °C and then distributed into Petri dishes 20 ml in each dish.

2.4.1.2 Blood agar

This is the one of the enriched media that was composed of blood agar base (Oxoid) and defibrinated sheep blood. The blood agar base contain protease liver digested, yeast extract, sodium chloride and agar. It was prepared by dissolving 40 grams of the basal medium in 1 liter distilled water, then dissolved completely by boiling, sterilized at 121 °C for 15 minutes, cooled to 45-50 °C and 7% sterilized defibrinated sheep
blood was added aseptically. The media were gently mixed and poured in 15 ml amounts in sterile Petri dishes. The plates were allowed to solidify and kept at 4°C till use. The pH was within the range of 7.2-7.6.

2.4.1.3 Hugh and Leifson’s (O/F) medium

Contents:-

Peptone 20 grams
Sodium chloride 5 grams
Agar 3 grams
K$_2$HPO$_4$ 0.3 grams
Distilled water 1000 ml
Bromothyl blue 0.2% 15 ml

This medium was used to test ability of the organism to attack dextrose under aerobic and anaerobic conditions. This medium was prepared by dissolving all ingredients in one liter of distilled water by heating in water bath set at 55°C except bromothyl blue solution which was added after adjustment of the pH to 7.1. Then sterile solution of the appropriate carbon hydrate was added an aseptically to give a final concentration of 1% and the medium was sterilized at 115°C for 20 minutes.

A volume of 10 ml of sterile glucose solution was aseptically added to 90 ml of medium, then the medium was mixed and distributed aseptically in
10 ml amounts into sterile test tubes. The prepared medium was kept at 4°C until use.

**2.4.1.4 Motility medium**

**Contents:**

- Peptone: 10 grams
- Yeast extract: 3 grams
- Sodium chloride: 5 grams
- Agar: 4 grams
- Gelatin: 4 grams
- Distilled water: 1000 ml

The gelatin was soaked in water for 30 minutes, and then the other ingredients were added, heated to dissolve and sterilized at 115°C for 20 minutes.

**2.4.2 Sterilization**

**2.4.2.1 Sterilization of glasswares**

Glasswares such as test tubes, pipettes, flasks and Petri dishes were sterilized in the hot air oven at 160°C for an hour. Others like Bijou and universal bottles were sterilized in the autoclave at 15 lb for 15 minutes (121°C). Instruments such as forceps, spatulas, scissors
and scalpels were sterilized in the hot air oven at 160 °C for an hour or by flaming after dipping in 70% alcohol while used.

2.4.2.2 Sterilization of culture media

Unless otherwise stated, culture media were sterilized in the autoclave at 121 °C (15 lb/inch) for 15 minutes.

2.4.2.3 Sterilization of solutions

Normal saline and distilled water were sterilized by autoclaving at 115°C for 10 minutes.

2.4.3 Culturing

2.4.3.1 Primary culture

Primary culture for all water samples was done onto blood agar. The water sample was taken by disposable pipette and put into Ependorf tube then centrifuged at 8000 rounds for 5 minutes and the sediment was cultured onto blood agar. Then all cultured samples were incubated at 37°C for 24 hours.

2.4.3.2 Sub culture

Typical and well isolated colonies from the primary culture were picked with a wire loop and streaked on the surface of a fresh plate of the blood agar. Pure cultures were obtained by replating the subcultures on blood agar.

2.4.4 Identification of isolate

Purified isolates from the primary or from subcultured plates were identified to the genus level according to Barrow and Feltham (1993).
The identification was based mainly on the colony characteristics, staining, motility and biochemical reactions.

2.4.4.1 Colony characteristics

All well purified growth colonies were examined for shape, colour and consistency.

2.4.4.2 Staining

2.4.4.2.1 Preparing of smear from culture

Smears were prepared by emulsifying part of a typical and well isolated colony in a drop of sterile normal saline and spread in a clean slide. The smears were then allowed to dry by air then fixed by gentle flaming. All smears were examined by Gram stain.

2.4.4.2.2 Gram stain

Gram stain was done according to Barrow and Feltham (1993):

1. Crystal violet solution was added to a fixed smear for tow minutes.

2. Washed with water

3. Lugol’s iodine was added for one minute.

4. Washed with water.

5. Decolorized with alcohol for 30 seconds.

6. Washed with water.

7. Countered stain with diluted carbol fuchsin for one minutes.
8. Washed with water.

9. Dried with filter paper and examined microscopically under oil immersion lens.

Gram-negative organisms stained red while Gram-positive organisms stained violet.

2.4.4.3 Motility

Motility was determined by inoculation of the isolated organism into semi-solid motility media by means of a wire loop in a straight line then incubated at 37°C and examined daily for five consecutive days. A positive reaction was indicated by the bacterial growth towards the surface.

2.4.4.4 Biochemical tests

The biochemical tests were performed according to Barrow and Feltham (1993) and they included:

2.4.4.4.1 Oxidase test

The oxidase test was performed by removing a portion of freshly grown colonies with a sterile glass rod and rubbing it on a strip of filter paper, which had been impregnated, with 1% solution of oxidase reagent. The immediate development of a dark purple colour within 10 seconds indicated a positive reaction.
2.4.4.2 Catalase test

This test detects the enzyme catalase that converts hydrogen peroxide to water and gaseous oxygen. A loopful of bacteria grown was took from the top of the colonies avoiding the blood agar medium, and were put in a clean slide and dropped 3% hydrogen peroxide. Presence of oxygen gas within a few seconds indicates a positive reaction.

2.4.4.3 O/F test

Duplicate tubes were in cultured by stabbing with straight wire. To one of the tubes a layer of melted soft paraffin (petrolatum) was added to depth about 1 cm, then incubated at 37°C for 24 hours and examined.

2.5 Bacterial viable count

The viable count was done according to (Quinn P. et al, 2000) and the method called Miles-Misra.

2.5.1 Preparation of the dilutions

The serial dilution was prepared according to Harrigan and Maccance, (1976). A micropipette with sterile tip was held vertically and introduced not more than 3 cm below the surface of the water sample and then 1ml was taken to the first tube of the dilution, (which contain 9 ml sterile normal saline) series without contact the diluting fluid, the tip was discarded and the tube was labeled as the first dilution tube 1/10, or 10⁻¹.
A fresh sterile tip was used to mix the content of the first dilution and 1 ml of the first dilution was transferred to the second tube of dilution series (which contain 9 ml normal saline), also without contact the diluting fluid then the tip was discarded and the second dilution tube was labeled as second dilution tube 1/100, or 10^{-2}. Further dilutions of 1/1000, or 10^{-3}, 1/10000, or 10^{-4}, 1/100000, or 10^{-5} were prepared similarly.

2.5.2 Preparation of the plates

The plates were prepared according to Harrigan and Mccance, (1976). The surface of the nutrient agar plates were dried for one hours at 27 °C with the plate lid closed, followed by 2 hours at 37 °C with the lid and the base separated. This enables the medium to absorb the water of the inoculums quickly.

A fresh sterile tip was used to mix the content of the each dilution by sucking up and down ten times, then 0.02 ml of each dilution were withdrawn and transferred to nutrient agar, the plates were labeled by the number of the dilution.

2.5.3 Colony count

Colonies were counted according to Miles and Misra surface colony count (Miles and Misra, 1938). An average colony count from at least 4 drops of each dilution was obtained; the conversion factor was 50 to obtain a figure for the bacteria/ml in the original sample.
The formula used for counting was (The total number of bacteria = The average of colonies count × dilution factor × 50).

2.6 The epidemiological Data

Data about farms examined was collected. The data was about system, water sources, hygiene of water trough, water additives and common diseases in the farms.
CHAPTER THREE

RESULTS

3.1 Bacterial isolation

From the 50 drinking water samples examined from all farms, 63 bacterial isolates were recovered. The highest rate of the isolation was Bacillus (28.57%). The other isolated genera were (Corynebacterium, Enterobacteria, Staphylococcus, Sterptococcus, Actinobacillus, Campylobacter, Moraxella, Areomonas, Cardiobacteria, Pseudomonas and Branhamella). Table 2 shows the numbers and percentages of different isolated genera.
### Table 2: The Numbers and Percentages of isolated bacterial genera

<table>
<thead>
<tr>
<th>Genus</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus</em></td>
<td>18</td>
<td>28.57</td>
</tr>
<tr>
<td><em>Corynebacterium</em></td>
<td>16</td>
<td>25.39</td>
</tr>
<tr>
<td><em>Enterobacteria</em></td>
<td>11</td>
<td>17.46</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>3</td>
<td>4.76</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>3</td>
<td>4.76</td>
</tr>
<tr>
<td><em>Actinobacillus</em></td>
<td>3</td>
<td>4.76</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>3</td>
<td>4.76</td>
</tr>
<tr>
<td><em>Moraxella</em></td>
<td>2</td>
<td>3.17</td>
</tr>
<tr>
<td><em>Areomonas</em></td>
<td>1</td>
<td>1.58</td>
</tr>
<tr>
<td><em>Cardiobacteria</em></td>
<td>1</td>
<td>1.58</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>1</td>
<td>1.58</td>
</tr>
<tr>
<td><em>Branhamella</em></td>
<td>1</td>
<td>1.58</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>63</td>
<td>100%</td>
</tr>
</tbody>
</table>
3.2 Gram positive and Gram negative Bacteria

From the total of 63 isolates, 40 (63.5%) were Gram positive while 23 (36.58%) were Gram negative (Table 3).

Table 3: No, and Gram positive and Gram negative bacteria isolated

<table>
<thead>
<tr>
<th></th>
<th>NO</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram +ve</td>
<td>40</td>
<td>63.5</td>
</tr>
<tr>
<td>Gram -ve</td>
<td>23</td>
<td>36.5</td>
</tr>
</tbody>
</table>
3.3 Bacterial isolates according to the source of water

Fourty seven (74.6%) isolates were recovered from water of general net water sources while sixteen (25.4%) were isolated from water of underground source. *Pseudomonas* and *Aeromonas* were isolated only from general net water source while *Staphylococcus* and *Actinobacillus* were isolated only from underground water source, the distribution of different genera was shown in (Table 4).
<table>
<thead>
<tr>
<th>Genus</th>
<th>General net water (%)</th>
<th>Underground water (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corynebacterim</td>
<td>(38.3)</td>
<td>(32)</td>
</tr>
<tr>
<td>Bacillus</td>
<td>(21.26)</td>
<td>(24)</td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>(12.77)</td>
<td>(16)</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>(6.38)</td>
<td>(0)</td>
</tr>
<tr>
<td>Actinobacillus</td>
<td>(6.38)</td>
<td>(0)</td>
</tr>
<tr>
<td>Sreptococcus</td>
<td>(4.26)</td>
<td>(4)</td>
</tr>
<tr>
<td>Moraxella</td>
<td>(4.26)</td>
<td>(4)</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>(2.13)</td>
<td>(4)</td>
</tr>
<tr>
<td>Cardiobacteria</td>
<td>(2.13)</td>
<td>(4)</td>
</tr>
<tr>
<td>Branhamella</td>
<td>(2.13)</td>
<td>(4)</td>
</tr>
<tr>
<td>Areomonas</td>
<td>(0)</td>
<td>(4)</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>(0)</td>
<td>(4)</td>
</tr>
</tbody>
</table>

**source of water**

<table>
<thead>
<tr>
<th>Total</th>
<th>100%</th>
<th>100%</th>
</tr>
</thead>
</table>

**Table 4: The percentage of Bacterial Isolation according to the source of water**
3.4 Bacterial isolation according to the area

From total isolates, 16, 14, 12, 12, 12, 13 have been isolated from farms at Halfaia area, Shambat area, Helat Kogaly area, Helat Koko area and Alsamrab respectively. The distribution of different bacterial genera according to the area is shown in (Table 5).
Table 5: The percentage of Bacterial Isolation according to the area

<table>
<thead>
<tr>
<th>Genus</th>
<th>Alhafaia</th>
<th>Shambat</th>
<th>Helatkogaly</th>
<th>Helatkoko</th>
<th>Alsamrab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corynebacterium</td>
<td>37.5</td>
<td>52.63</td>
<td>16.67</td>
<td>16.67</td>
<td>30.78</td>
</tr>
<tr>
<td>Bacillus</td>
<td>31.25</td>
<td>21.07</td>
<td>16.67</td>
<td>0</td>
<td>38.46</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>12.5</td>
<td>5.26</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sterptococcus</td>
<td>6.25</td>
<td>5.26</td>
<td>0</td>
<td>0</td>
<td>7.69</td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>6.25</td>
<td>0</td>
<td>41.67</td>
<td>25</td>
<td>7.69</td>
</tr>
<tr>
<td>Actinobacillus</td>
<td>0</td>
<td>0</td>
<td>78.33</td>
<td>8.33</td>
<td>0</td>
</tr>
<tr>
<td>Moraxella</td>
<td>0</td>
<td>5.26</td>
<td>8.33</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>0</td>
<td>5.26</td>
<td>0</td>
<td>0</td>
<td>7.69</td>
</tr>
<tr>
<td>Areomonas</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8.33</td>
<td>0</td>
</tr>
<tr>
<td>Cardiobacteria</td>
<td>0</td>
<td>0</td>
<td>8.33</td>
<td>8.33</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8.33</td>
<td>0</td>
</tr>
<tr>
<td>Branhamella</td>
<td>0</td>
<td>5.26</td>
<td>0</td>
<td>0</td>
<td>7.69</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100%</strong></td>
<td><strong>100%</strong></td>
<td><strong>100%</strong></td>
<td><strong>100%</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>
3.5 Viable count

3.5.1 Alhalfaia area

From the ten farms examined in Alhalfaia area, viable count was 

\((1.5 \times 10^6, 6.1 \times 10^4, 1.7 \times 10^5, 1.4 \times 10^6, 9 \times 10^5, 8 \times 10^4, 7.8 \times 10^5, 9 \times 10^4, 1.3 \times 10^7, 4.1 \times 10^6 \text{ cell/ml})\) from farms 1 to 10 respectively. The mean viable count was \(2.2 \times 10^7 \text{ cell/ml}\) (Figure 1).
Figure 1: The Bacterial viable count for farms in Alhalfaia area
3.5.2 Shambat area

From the ten farms examined in Shambat area, viable count was 
(3.2X10^7, 2.1X10^4, 2.5X10^7, 1.5X10^5, 3.3X10^7, 7.8X10^6, 7X10^4, 
5.4X10^4, 2.6X10^6, 1.4X10^7) from farms 11 to 20 respectively. The mean 
viable count was 1.2X10^8 cell/ml (Figure 2)
Figure 2: The Bacterial viable count for farms in Shambat area
3.5.3 Helat Kogaly area

From the ten farms examined in Helat Kogaly area, viable count was (1.4X10^6, 5.6X10^4, 5.1X10^6, 1.5X10^4, 1.6X10^5, 6.9X10^6, 7.3X10^5, 2.1X10^7, 1.9X10^5, 8.9X10^5) from farms 21 to 30 respectively. The mean viable count was 3.7X10^7 cell/ml (Figure 3).
Figure 3: The Bacterial viable count for farms in Helat Kogaly area
3.5.4 Alsamrab area

From the ten farms examined in Alsamrab area, viable count was (1.1X10^6, 1.6X10^5, 7.3X10^5, 6.8X10^5, 9X10^6, 2.3X10^6, 1.7X10^6, 2.3X10^6, 1.3X10^6, 2.7X10^6) from farms 31 to 40 respectively. The mean viable count was 1.2X10^8 cell/ml (Figure 4).
Figure 4: The Bacterial viable count for farms in Alsamrab area
3.5.5 Helat Koko area

From the ten farms examined in Helat Koko area, viable count was (1.2x10^6, 6.6x10^6, 8.4x10^5, 1.2x10^6, 1.1x10^5, 6.8x10^5, 7.5x10^6, 4.8x10^5, 5.3x10^5, 1.3x10^6) from farms 41 to 50 respectively. The mean viable count was 2x10^7 cell/ml (Figure 5).
Figure 5: The Bacterial viable count for farms in Helat Koko area
3.5.6 The viable count according to the area

The mean viable count was (1.2 X 10^6, 2.2X 10^6, 2.2X 10^5, 2X 10^5, 3.7X 10^5) cell/ml for Shambat, Alsamrab, Alhalfaia, Helat Koko, Helat Kogaly areas respectively (Figure 6).
Figure 6: Mean Bacterial viable count according to the area
3.5.7 The viable count according to the source of water

The mean viable count was $7.1 \times 10^6$ Cell/ml for underground while $2.2 \times 10^6$ Cell/ml for general net water (Figure 7).
Figure 7: Bacterial Viable count according to source of water
3.6 The Epidemiological data

From 50 diary farms were examined 38 farms were with bad environment surrounding water troughs, 50 not using disinfectant or detergents for washing troughs, 42 do not exchange water troughs regularly and 35 troughs contaminated with faeces, algae and feeds.
CHAPTER FOUR

DISCUSSION

Little information is actually available concerning the microbiological quality of water offered to cattle (Bitting 1898; Hanninen et al., 1988; Reilhy, 1981).

The present study was designed to describe the microbiological quality of water commonly present in diary cattle drinking water troughs based on bacteriological isolation and total viable count.

The mean viable count was found ($1.2 \times 10^6$, $2.2 \times 10^6$, $2.2 \times 10^5$, $2 \times 10^5$, $3.7 \times 10^5$) cell/ml for Shambat, Alsamrab, Alhalfaia, Helat Koko, Helat Kogala areas respectively.

The presence of high viable bacteria in drinking troughs was an indication of the contamination at these sites, this may agree with (Jeffrey T. et al 2001) who reported that water offered to diary cattle is often of poor microbiological quality.

The extent of bacterial contamination observed in the drinking water troughs may demonstrates animals' daily exposure to bacterial infection from water source (Lejene T et al 2001).

The association between water quality and water contamination factors that influence the survival and proliferation of the bacteria in the system of drinking water troughs is very close (Hancock, et al 1994). All of the dairy farms examined in this study were in open system so
chances of water troughs contaminations with feeds, faeces, urine, dust and any other factor. Also water troughs material is poor, and troughs were put directly on cattle house floor closely to the mud and faeces. Also outer troughs environment was very humid with cattle urine which increases contamination chances.

Water sample from direct source of underground water supply is completely free from coliform bacteria (El Tom, 1997). So water troughs will be contaminated after being poured in troughs for flowing reasons:-
- Bad hygiene measures in the farms.
- Persistence water for long time in troughs.
- Water troughs are not cleaned regularly.
- Disinfectant or detergents are not used for washing troughs.

Accordingly, all of the above reasons were observed in the examined farms which more explain the high viable count.

Abdalmagid et al, 1994 reported the contamination of underground water in Sudan.

Viable count technique which was used in this study named Miles-Misra. This method has advantages of being economical and sensitive also not required much glasswares and equipments comparing with other technique (Quinn P, et al 2000).

Waterborne infectious diseases are diseases caused by a number of different bacteria, which spread through contaminated drinking water;
Examples of these diseases include diarrheas, dysenteries, salmonellosis, hepatitis, and giardiasis.

According to bacterial isolation in this study the highest isolated bacteria which found in all water troughs samples were *Bacillus* (28.57%), *Corynebacterium* (25.39%) and *Enterobacteria* (17.46%). These genera are pathogenic and isolation may be of importance due to their contribution to infection.

Although *Campylobacter* was isolated, larger outbreaks of *Campylobacteriosis* are not usually associated with drinking water troughs and unpasteurized milk (Jones *et al* 1985).

From the epidemiological data which were collected in this study there were cases of calf diarrhea and new borne calves deaths. The highest results of the viable count obtained in these farms may indicate poor hygiene practices which justify these diseases cases.
CONCLUSIONS

1\ Many Gam +ve and Gram –ve bacteria were isolated from drinking water troughs.

2\ High viable count was obtained from most farms in different areas.

3\ All examined farms were of bad hygiene.
RECOMMENDATIONS

1. Drinking water hygiene should be practiced in dairy farms to avoid bacterial contamination.

2. More research is needed to investigate the risk factor associated with drinking water contamination in dairy farms.

3. The different types of dairy water troughs (cement, metal, gar ect...) may have effect in water contamination in dairy farms so more studies needing in future including this factor.

3. Further water troughs hygiene analysis are needed in all diary farms in Sudan.


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Appendix (1): Different Types of Water Troughs
Appendix (3): Environmental Condition Surrounded Diary Water Troughs