بسم الله الرحمن الرحيم

BACTERIAL CONTAMINATION OF DRINKING WATER AND IT'S DEGREE OF SENSITIVITY TO SOME COMMERCIAL WATER DISINFECTANTS IN POULTRY FARMS AT KHARTOUM STATE

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A Dissertation Submitted to University of Khartoum in Partial Fulfillment for Requirement of the Degree of Master of Tropical Animal Health

(M.T.A.H)

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December 2007

PREFACE

This study was carried out at the Department of Preventive Medicine and Public health, Faculty of Veterinary Medicine, University of Khartoum under supervision of ***Dr. Tawfig Eltigani Mohammed***

DEDICATION

This work is dedicated

To

My mother's blessed soul in heaven.

My father, sisters & brothers.

My big family and my sincere friends.

To those who are concerned with poultry industry and want it to improve.

ACKNOWLEDGEMENT

First of all my thanks are due to Allah who gave me the strength to make this study.

My faithful and deep thanks to my supervisor Dr. Tawfig Eltigani Mohammed for his complete supervision, his great help, advice, cooperation and encouragement through my work.

I would thank the staff of the Department of Preventive Medicine and Public Health especially Mr. Hussain Abdel Rahim and Miss. Zainab Eltayeb for their cooperation during this work.

My thanks are extended to the UNISCO chair for water and WHO library for allowing me to use their libraries.

My great thankfulness to Mr. Elzain Bashir for helping me in data analysis, my friends and my colleagues Marwa A/Allah, Mona Taha, Ayman Albeelie and Ikrima Isam for their cooperation.

My special regards to my father and my sister Hiba for their encouragement and support during this study.

Abstract

This study was conducted to evaluate the bacterial contamination of drinking-water in poultry farms and to determine the degree of sensitivity of bacteria from water to some commercial disinfectants which are used for poultry drinking-water.

A total of ten farms were selected, eight farms from Khartoum North for open system and two farms from Elbagair, Butri for closed and semi closed systems. Three samples from each farm were taken, the first one was from the main source of water, the second was from the storage place and the third was from the drinkers, making the total numbers of samples up to thirty.

All water samples were examined for bacterial viable count, Multiple Tube fermentation Technique for total coli forms bacteria, and cultured the samples after using disinfectants (yodor & vircon's) to isolate the bacteria which resisted disinfectants.

The mean viable count for the samples from the main source was 442.6×10^3, and for the samples from the storage place was 317.9×10^5, and for the samples from the drinkers was 664.6×10^5. 93% of the samples were positive to the Multiple Tube fermentation technique for total coli forms bacteria.

The bacteria which resisted yodor disinfectant were (Flavobacterium, Enterobacter, Acinetobacter, staphylococcus, Micrococcus, Bacillus) and the bacteria which resisted vircon's disinfectant were (Acinetobacter, Micrococcus, Bacillus) from the same farms.

ملخص الاطروحة

أجريت هذه الدراسه بغرض تقييم التلوث البكتيري لمياه الشرب في مزارع الدواجن ومعرفة درجة إستجابة البكتريا الموجوده في مياه الشرب لعدد من مطهرات المياه المستعمله تجارياً.

أختيرت ١٠ مزارع دواجن لعمل الدراسه، ٨ منها في منطقة الخرطوم بحري للنظام المفتوح في مزارع الدواجن و٢ منها في منطقتي الباقير وبتري للنظامين المغلق وشبه المغلق.

تم أخذ ٣ عينات من كل مزرعه، الأولي من مصدر المياه الرئيسي في المزرعه والثانيه من مكان تخزين المياه والثالثه من الشرابات، ليصبح مجموع العينات ٣٠ عينه.

كل عينات مياه الشرب تم إختبارها في العد الكلي للخلايا البكتيرية الحية، وإختبار التخمر في أنابيب متعدده للكشف عن البكتريا المعوية المخمره لسكر اللاكتوز (Total coli forms) ومعاملة كل عينات المياه بكل من المطهرين Yodor و virconˈs.

متوسط العدد الكلي للخلايا البكتيرية الحيه لعينات مياه المصدر الاساسي هو 442.6×10^{3} ولعينات المياه المأخوذه من مكان تخزين المياه هو 317.9×10^{5} ولمياه الشرابات هو 664.6×10^{5}. ٩٣% من العينات كانت موجبه لإختبار التخمر في أنابيب متعدده.

البكتريا المقاومة لمطهر ال Yodor في مياه الشرب هي:

(Flavobacterium,Micrococcus,staphylococcus, Acinetobacter, Enterobacter Bacillus)

والبكتريا المقاومة لل virconˈs في مياه الشرب هي :

(Bacillus, Micrococcus, Acinetobacter) من نفس المزارع.

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INTRODUCTION

The need for understanding health aspects in the tropical developing country a large amount of misery, sickness and death due to infectious diseases related to water supplies.

Good quality water is odourless, colourless, tasteless, and free from faecal pollution and chemicals in harmful amounts. The World Health Organization (WHO) has estimated that up to 80% of all sickness and disease in the world is caused by inadequate sanitation, polluted water, or unavailability of water (Cheesbrough, 1984).

The provision of an adequate supply of safe water was one of the eight components of the Primary Health Care identified by the international conference on Alma-Ata in 1978 (Primary Health Care-Geneva, WHO,1978).

Safe water is essential to life and health .People and animals can survive longer without food than without water .Thus the provision of water demands immediate attention .The aim is to assure availability of enough water to allow sufficient distribution and to ensure that it is safe to drink.

Water quality is always difficult to assess .Always assume that all water available during an emergency is contaminated ,especially if available sources are surface water bodies .Immediate action must be taken to stop further pollution and to reduce contamination.

Protection of water supplies from contamination is the first line of defense .Source protection is almost invariably the best method ensuring safe, drinking-water and is to be preferred to treating a contaminated

water supply to render it suitable for consumption .Once a potentially hazardous situation has been recognized, the availability of alternative sources, and the availability of suitable remedial measures must be considered.

As far as possible, water sources must be protected from contamination by human and animals waste, which may contain a variety of bacterial, viral, protozoan pathogens and helminthes parasites .Failure to provide adequate protection and effective treatment will expose the community to the risk of water –borne diseases.

The acceptable quality of water is defined by WHO guide lines as that which is suitable for all usual domestic purposes ,including personal hygiene (WHO ,2006).It should be palatable ,wholesome ,be attractive to sense of sight and hygienically safe .There is an urgent need for simple ,effective and low-cost methods for the production of water free of pathogenic and chemical substances (John ,1977).

Full examination of water supply embodies several lines of investigations including topographical ,chemical ,biological and microbiological .Each line of investigation has it's uses and indication .The first routine microbiological (bacteriological) examination of a public water supply was commenced by Frank Land in London in 1885 (Hammerton,1967) .

Faecal pollution of water leads to the spread of microbial infection. Faeces contain large numbers of organisms including E.coli ,Stryptococcus faecalis and Clostridium perfringens .These organisms from part of the normal bacterial flora of the intestinal tract of human and other warm blooded animals .The faecally polluted water contains the above mentioned

microorganisms and may also contain pathogenic microorganisms that are found in the faeces of diseased human or animals.

Water Disinfection:

Disinfection is unquestionable importance in the supply of safe drinking-water .The destruction of microbial pathogens is essential and very commonly involves the use of reactive chemical agents such as chlorine. Disinfection is an effective barrier to many pathogens (especially bacteria) during drinking-water treatment and should be used for surface water and for ground water subjected to faecal contamination .All disinfectants are effective against the vegetative forms of organisms but not necessarily against their spores.

Objectives:

The general objective of this study is to evaluate the drinking-water quality of poultry farms in the city of Khartoum North.

The specific objectives are:

1- To determine the degree of water contamination in terms of colony forming units per 100 ml water samples.

2- To compare the quality of water samples in the same farm (from the main source of water to the drinkers).

3- To determine the degree of sensitivity of bacteria from water to some commercial disinfectants (Yodor &Vircon's) which are used for poultry drinking-water.

CHAPTER ONE

LITERATURE REVIEW

1.1 Water

1.1.1 Water types

For purposes of simplicity, scientists classify water into two major types; ground water and surface water. Ground water originates from deep wells and subterranean springs and because of the filtering action of soil, deep sand, and rocks, it's virtually free of microorganisms. As water flows up along channels, may enter it and alter it is quality (Alcamo, 1997).

Microbial contamination may occur when a well is situated within 200 feet of the source of contamination (Smith, 1981). Surface water is found in lakes, streams and shallow wells. It is microbial population may reflect the air through which rain has passed, or the sewage treatment facility located along rivers bank (Alcamo, 1997).

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 nonpathogenic microbes from soil, and in the vicinity of cities it is often contaminated with sewage bacteria (Smith, 1981).

1.1.2 Water resources in Sudan:

A – Surface water: surface water either permanent or temporary (Pagot, 1992)

i – The permanent surface water origins include:

1- Main course of the Nile from El Mogran till Sudanese –Egyptian boarder.

2- Nile main tributaries which include White Nile River, Blue Nile River and Sobat River.

3- Nile sub tributaries which include Dinder river , Rahad river ,Bahar Algabal , Bahar Algazal , Bahar Alarab , Algoor river and Bahar Alzaraf .

4- Lakes which include Lake Alobaid, lake No, Lake Kailak and Lake Alrahad.

5- Natural pools (Foolas).

ii- The temporary surface water origins which include :

1- Seasonal streams and rivers pouring in the Nile or it's tributaries as Toker, Algash and Atbara.
2- Wadis pouring in the Nile valley as wadi Almalek and wadi Almogadam.
3- Seasonal streams not pouring in the Nile as khor Baraka, khor Arbaat and khor Abohabil.
4- Artificial pools (Hafirs).

B- Ground water

The ground water resources include:-

1-Shallow sources of ground water which their depth varies from several meters to around 30 meters (Pagot, 1992).

They are exploited by:

- Traditional wells.
- Water holes.

2- Deep sources of ground water and by this expression we mean those whose water level is more than 40 meters below ground level (Pagot, 1992). Among this group we can distinguish:

* Free sources which are exploited by deep wells up to 80 meters deep.

* Captive sources which are exploited by artesian and semi artesian – wells. Deep water resources are usually distant from the Nile.

* Valleys.

1.2 Water needs:

Water is usually demanded to meet the following needs: A- drinking B- irrigation for Agriculture, industry and trade. Water needed for use in animal industry should have the characteristics and criteria of water needed in category (A) (Hosny, 1981).

Drinking – water must contain no impurity that would offend sight, taste or smell and substances with physiologic effects must be eliminated or not introduced (Smith, 1981).

1.3 Health aspects of water supplies in tropics:

The diseases related to water supplies are more numerous, more important and more diverse in the tropics than in temperate lands and effects of improved supplies are more complex.

In most countries the principal risks to health associated with the consumption of polluted water are microbiological in nature (although the importance of chemical contamination should not be underestimated).

The way in which water supplies affect health , some sort of relation ship between water and health has been recognized from the time of Hippocrates,

if not early earlier , in the association of marshy places with fevers , and many un sophisticated communities in the tropics have similar views and are very discriminating in their choice and use of water .

Snow (1855) was the first to show a precise relation of a disease to water in well-known studies of cholera, and he was closely followed by Budd who demonstrated the spread of typhoid through water supplies. Later in the nineteenth century a different type of relationship to water was shown by Manson in (1877) for Filariasis and Ross for Malaria. Both infections were shown to be transmitted by Mosquitoes, whose larvae live in and are dependent on surface water. Before that, guinea worm, and subsequently Schistosomiasis or bilharziasis, were shown to depend on fresh water invertebrates for spread (WHO, 1997).

Classification of water related infections between 20 and 30 different infective diseases may be affected by changes in water supply. They are usually classified by the microbe causing them, into viral, bacterial, protozoal and helminthic diseases. But this is not very helpful in considering effects of improved water supplies. What affects them is the mode of spread, and it is more useful to have four main categories:

A- Infections spread through water supplies –water-borne diseases.

B- Infections transmitted through aquatic invertebrate animal-water-based diseases.

C- Diseases due to the lack of water for personal hygiene –water- washed diseases.

D- Infections spread by insects that depend on water- water- related insect vectors (Bradley, 1974, White, Bradley and White, 1972).

1.3.1 Chemical Aspects:

In rural areas of developing countries, the great majority of health – related water- quality problems are the result of bacteriological or other biological contamination. Nevertheless, a significant number of very serious problems may occur as a result of the chemical contamination of water resources.

Some potentially chronic effects may occur in rural areas where over use of agrochemicals leads to significant levels of pesticides in water sources. The excessive application of fertilizers or from leaching of waste water or other organic waste into surface water and ground water. Although effects may be difficult to detect, such contamination may pose a risk to health (WHO, 1998).

Table (1) Chemical Parameters According to Sudanese Standards and Metrology Organization

Parameters	Levels likely to Give Rise to Consumer Complaints
Inorganic Constituents	
Aluminum	0.2 mg/l
Ammonia	1.5 mg/l
Chloride	250 mg/l
Hydrogen sulfide	0.05 mg/l
Iron	0.3 mg/l
Sodium	200 mg/l
Sulfate	250 mg/l
Total Dissolved Solids (TDS)	1000 mg/l
Zinc	3 mg/l
Organic Constituents	
2-Chlorophenol	5 µg/l
2,4-Dichlorophenol	2µg/l

1.3.2 Physical and aesthetic aspects:

The chemical and physical quality of water may affect it is acceptability to consumers. Turbidity, colour, taste, and odour, whether of natural or other origin, affect consumer perceptions and behaviour.

- Turbidity in excess of 5 NTU (5JJU) may be noticeable and consequently objectionable to consumers.
- Colour in drinking water may be due to the presence of organic matter such as humic substances, metals such as Iron, and Manganese, or highly coloured industrial wastes. Displeasing levels of colour typically exceeding 15TCU. Drinking water should ideally be colourless.
- Odour in water is due mainly to the presence of organic substances (WHO, 1998).

Table (2) Physical Parameters According to Sudanese Standards and Metrology Organization

Parameters	Levels likely to Give Rise to Consumer Complaints
Colour	15 TCU
Taste & Odour	Acceptable
Temperature	Acceptable
Turbidity	5 NTU
pH	6.5 – 8.5

1.3.3 Microbial Aspects:

Securing the microbial safety of drinking –water supplies is based on the use of multiple barriers, from catchment to consumer, to prevent the contamination of drinking-water or to reduce contamination to levels not injurious to health.

In general terms, the greatest microbial risks are associated with ingestion of water that is contaminated with human or animal (including bird) faeces. Faeces can be a source of pathogenic bacteria, viruses, protzoa and helminthes.

Faecally derived pathogens are the principal concerns in setting health-based targets for microbial safety.

Microbial water quality often varies rapidly and over a wide range. Short – term peaks in pathogen concentration may increase disease risks considerably and may trigger outbreaks of water-borne disease (WHO, 2006).

Table (3) Microbial Parameters (Guideline value) According to Sudanese Standards and Metrology Organization

Organisms	Guideline value
1. All water intended for drinking: A. E. coli or thermo tolerant coli form bacteria. B. Pathogenic intestinal protozoa	Must not be detectable in any 100 ml sample
2. Treated water entering the distribution system A. E. coli or thermo tolerant coli form bacteria. B. Total coli form bacteria C. Pathogenic intestinal protozoa	Must not be detectable in any 100 ml sample
3. Treated water in the distribution system: A. E. coli or thermo tolerant coli form bacteria. B. Total coli form bacteria C. Pathogenic intestinal protozoa	 Must not be detectable in any 100 ml sample Must not be detectable in any 100 ml sample. In the case of large supplies where sufficient samples are examined, must not be detectable in 95% of samples examined throughout any consecutive 12- months period. Must not be detectable in any 100 ml sample.

1.3.4 Agents of significance:

1.3.4.1 Agents of high significance:

Include most of the ingested pathogens, present a serious risk of disease. Their elimination should be given high priority. e.g.:- E- coli, Salmonella, Shigella, Vibrio cholera, Yersinia enterocolitica, Campylobacter jejuni , Giardia, Cryptosporidium , Entamoeba histolyica and Darcunuculus.

1.3.4.2 Opportunistic pathogens:

Naturally present in the environment and not normally regarded as pathogens may cause disease opportunistically. Cause disease among animals and people whose local or general natural defense mechanisms are impaired those most likely to be at risk include the very old, the very young and the patients. Water contain excessive numbers of these agents may produce a variety of infections involving the skin and mucous membranes of the eye, ears, nose and throat. e.g.:- Pseudomonas, Flavobacterium, Acinetobacteria, Klebsiella and Serratia.

1.3.4.3 Nuisance organisms:

Nuisance organisms by definition, have no public health significance, however they produce problems of turbidity, taste and odour or appear as visible animal life in the water. As well as being aesthetically objectionable, they indicate that both water treatment and the maintenance and repair of the distribution system are defective (WHO, 1997).

1.3.5 Route of exposure:

For the Faeco-oral pathogens, drinking water is the only vehicle of transmission.

Contamination of food, hands, utensils and other material can also play a role particularly when domestic hygiene is poor (WHO, 1997).

1.3.6 Resistance in water:

The persistence of a pathogen in water is a measure of how quickly it dies after leaving the body. In practice, the numbers of a pathogen introduced on given occasion will tend to decline expotentially with time, reaching significant and undetectable levels after a period.

The persistence of most pathogens in water is affected by various factors, of which sunlight and temperature are among most important (WHO, 1997).

1.3.7 Infective dose:

For several intestinal pathogens, attempts have been made to determine the number of organisms needed to produce either a clinically apparent infection or intestinal colonization (WHO, 1997).

1.4 Microbial indicators of water quality:

1.4.1 Rationale:

Examination for faecal indicator bacteria in drinking-water provides a very sensitive method of quality assessment. It is also important to determine the quality of the raw water, not only to assess the degree of pollution but also to enable the best form of treatment to be chosen.

1.4.2 Indicators of faecal contamination :

Methods for detection and enumeration are not yet available. Full identification of these indicator organisms would require such as extensive series of tests as to be impracticable in routine monitoring.

1- Escherichia coli

2- Thermo tolerant (faecal) coli form organisms.

These are defined as the group of coli form organisms that are able to ferment lactose at 44˚-45˚ C. They comprise the genus E.coli and, to lesser extent spp of Klebsiella, Enterobacter and Citrobacter.

3- Coli form organisms (total coli forms)

4- Faecal streptococci.

5- Sulfite – reducing Clostridia.

6- Bacteriophages (WHO, 1997).

1.5 Principal techniques used to isolate indicators organisms:

Routinely the following techniques are used in the isolation of indicator organisms from water:

1- Membrane filtration.

2- Multiple tube or Most probable number (MPN)

1.5.1 Membrane filtration test:

This technique is not suitable for semi-solid samples (sludge) or natural water with turbidity. When volume of samples is low, sterile diluents must be used to disperse the sample. Usually a volume of the samples or it's

dilutions is introduced into a sterile device containing sterile membrane filter with special pore size (Bartram and Wheeler, 1993). The sample is then drawn through the filter. Indicator organisms are retained and transferred to culture medium and then incubated for a period of time.

Identifiable colonies are then counted and results are expressed in cfu/100ml of sample, where cfu=colony forming unit.

1.5.2 Multiple fermentation tube technique (Most Probable Number MPN):

This technique has been used for the analysis of drinking –water for many years with satisfactory results. Separation analysis is usually conducted on five portions of each of three serial dilutions of water sample. The individual portion is used to incubate tubes of culture medium that are then incubated at standard temperature for a standard period of time. The presence of coli forms is indicated by turbidity in the culture medium, by a PH change and /or by presence of gas. The MPN index is determined by comparing the pattern of positive results (the number of tubes showing growth at each dilution) with standard statistical table. The tabulated value is reported as MPN per 100ml of sample (Bartram and Pedley, 1996).

1.6 Isolation of faecal coli form from drinking- water:

Several studies were conducted to isolate the faecal coli form bacteria to indicate the sanitary quality of drinking water.

In Sudan Mahagoub (1984) investigated the coli form bacteria in the Nile at Khartoum , he found that the Blue and the White Niles contained coli form bacteria that ranged from 33 to 9200 cells/100 ml and from 17 to 2400 cells/ 100 ml respectively . Faecal coli form counts ranged from 9 to

490 cells/100 ml and 2 to 1600 cells/100 ml respectively. Where as El-Hassan et al, (1984) evaluated the water sources in Khartoum, Nile, wells and tap water. They isolated 93-460 cells/100 ml either coli form or faecal coli form in the Nile and 3-2400 cells/100 ml also either coli forms or faecal coli forms in wells. Tap water contained only 3CFU/ 100 ml.

1.7 Water sources and the level of faecal coli form count:

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 faecal bacteria 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 faecal matter in unhygienic and inadequate sanitary conditions.

In Sudan, Abdel Mageid et al, (1984) evaluated two water sources, surface and deep bores in rural areas around Khartoum state; they found that coli forms and faecal coli forms count reach up to 2400 cells/ 100 ml in surface wells, and equal or less contamination in deep bores.

1.8 Water safety:

Safety is increased if multiple barriers are in place, including protection of water resources, proper selection and operation of a series of treatment steps and management of distribution systems (pipe or other wise) to maintain and protect treated water quality. The preferred strategy is a management approach that places the primary emphasis on preventing or reducing the entry of pathogens into water sources and reducing reliance on treatment processes for removal of pathogens (WHO, 2006).

1.8.1 Disinfection:

Chemical disinfection of drinking –water supply that is faecally contaminated will reduce the over all risk of disease but may not necessarily render the supply safe. For example, chlorine disinfection of drinking –water has limitations against the protozoan pathogens – in particular Cryptosporidium and some viruses. Disinfection efficacy may also be unsatisfactory against pathogens within flocks or particles, which protect them from disinfectant action.

High levels of turbidity can protect microorganisms from the effects of disinfectant action, stimulate the growth of bacteria and give rise to a significant chlorine demand. The use of chemical disinfectants in water treatment usually results in the formation of chemical by-products. However the risks to health from these by-products are extremely small in comparison with the risks associated with inadequate disinfection, and it is important that disinfection not to be compromised in attempting to control such by-products (WHO, 2006).

Table (4) Disinfectants (Max permissible level) According to Sudanese Standards and Metrology Organization

Parameters	Max. Permissible level in µg/l
Disinfectants	
Monochloramine	2000
Chlorine	3400
Disinfectants by Products	
Bromate	17
Chlorite	150
2,4,6-Trichlorophenol	150
Formaldehyde	600
Bromoform	75
Dibromochlormethane	75
Bromodichloromethane	40
Chloroform	150
Dichloroacetic acid	35
Trichloroacetic acid	75
Chloral Hydrate (Trichloroacetaldehyde)	7
Dichloroacetonitrile	60
Dibromoacetonitrile	75
Trichloroacteonitrile	0.7
Cyanogens Chlorides (CN)	50

1.9 Drinking-water for poultry farms:

The importance of good drinking-water is often underestimated. Good water helps the digestive process, the transport of nutrients in the body, regulation of the body temperature and the elimination of waste. Under normal climatic conditions, chickens drink about twice as much as they eat. The intake of clean water has a big impact on both the health status and the production performance of birds.

Chickens obtain water through ingestion of drinking- water, from moisture in their food and from biochemical reactions during utilization of carbohydrates, fats and proteins. Chickens require a constant supply of high quality water for optimum growth, production and efficiency of feed utilization (Scott et al., 1982). It is usually concluded that the best water is pure water, however, the best tasting water for birds, man and other animals often contains small amounts of carbon dioxide dissolved in the water (Dawes, 1968).

Early drinking-water research utilizing poultry (reviewed by National Academy of Sciences, 1974; Council for Agricultural Science and Technology, 1974; Coulston and Mark, 1977; Roland, 1977) was concerned with inadvertent contamination, total dissolved solids (TDS), PH and nitrate. Salinity and conductivity are used synonymously with TDS.

Natural waters have a PH of 4 to 9. Water intake of poultry is not influenced in a PH range of 2 to 10 (Fuerst and Kare, 1962). However, waters beyond a PH range of 6 to 9 can be corrosive for metallic equipment.

Water and feed consumption patterns are closely related with a much reduced feed intake during water abstinence (Sykes, 1983; Savory, 1978;

Duke, 1986). This relationship deteriorates during high ambient temperature when additional water needed for thermal regulation (Scott, 1982; Duke, 1986).

1.9.1 Drinking – water systems:

Drinking water systems are of great interest because they deliver the most important nutrient, water, to birds. Water systems have evolved from jugs to troughs to hanging bell –shaped drinkers to cup drinkers to enclosed nipple drinking systems.

May et al. (1997) reported water consumption from nipples was often similar to that from bells waterers during low temperature but was less during the periods of high temperature largely because panting broilers have difficult from high nipple waterers . All systems are subject to clogging, dripping, and microbial contamination, all of great economic importance.

1.9.2 Regular water testing:

Before any water is used for poultry it should be tested for microbiological and chemical content (calcium / hardness, iron, salinity, nitrates and other harmful chemical components). The shallower the source of water from farm wells, the more likely it's to be contaminated by bacteria from human or animal wastes.

Microbiological testing will show whether these pollutants are present. Water for poultry should meet the same microbiological standards set for human water supplies. Research demonstrated that a high bacterial load in the drinking water supplies to the young chickens will increase leg problems, femoral head, and necrosis and associated Staphylococcus aureus

infections. This bacterial contamination can often increase downgrading of broilers at factory due to septicaemia (Wiebe, 2002).

Zimmermann and Douglas (1988) suggested that water quality information from many regions, when compiled into a single large data base could make it be possible to identify mutual properties in water inclusion profiles. Thus would enable prediction of broiler performance potential in regard to water quality.

1.9.3 Decontamination:

Testing for total aerobic plate count identifies arrange of potential pathogens, which should be absent from water used for poultry. Where significant bacterial numbers are found and no alternative source of water is available, the water must be treated to kill the bacteria.

There are a few possible treatment methods to deal with bacteria. The three most commonly known are:

- Halogens: (usually chlorine, but also iodine and fluorine). Chlorine is the most frequent treatment for municipal and many agricultural water supplies. It's relatively cheap and effective, but has long –term residual down stream effects, which are causing people to question continuing use. Nevertheless it remains an important and useful treatment.
- Hydrogen peroxide: Hydrogen peroxide has been used a replacement for chlorine over the past decade. In it is natural form, relatively unstable, but it is breakdown product is water, which creates no risk for further pollution, in contrast to chlorine, which tends to persist in the environment.

- Ultra violet light: ultra violet light treatment is the most environmentally benign system for bacterial control in water supplies. Since nothing is added to the water and there are no residues. Ultra violet treatment must be free of even the smallest particles, and is usually filtered before passing through the system. Modern ultra violet systems have monitors which indicate when lamps be replaced, and very efficient at purifying water from microbiological perspective (Wiebe, 2002).

There is an increasing interest in disinfection of drinking –water delivered to chickens. Disinfection of drinking –water would be a critical control point for control of human pathogens in an on –farm Hazard Analysis Critical Control Point (HACCP) food safety program. Drinking –water is readily disinfected by chlorination, iodination, ultra violet light and ozone treatments (Wagenet et al., 1995).

1.9.4 Safety requirements of a good water sanitizer:

There are several different products available on the market for sanitizing drinking –water, but all must conform to high standards of safety. Firstly, the products must not be harmful to birds. There have been problems in some cases with toxicity due to over –use of sanitizing products, especially those that are chlorine based. Therefore, care must always be taken to follow the instructions given by the manufacturer, and the user should never be tempted to believe that adding a little more will increase efficacy. The water system should always be cleaned between flocks. The product used should be tasteless, colourless and have no odour.

The product used should also be safe for the user handle, and also should be safe to be released into the environment via the sewage systems. Formation of halogen hydrocarbons through reactions between the product and bio film should not occur.

From the point of view of the system it self, the product used should also cause minimal corrosion. It's pointless to improve the quality of drinking- water only to find that the life of the system is reduced as a result of corrosion. In particular, galvanized steel pipelines should not subject to corrosion, as zinc will be released into the water (Wiebe, 2002).

CHAPTER TWO

MATERIALS & METHODS

2.1 The study area:

Khartoum North was selected as study area for open system and Elbagair, Butri for closed and semi closed systems for poultry production.

2.2 Sample size:

A total of ten poultry farms were examined for drinking – water contamination and to measure the sensitivity of the bacteria from drinking – water to some commercial disinfectants (Yodor &vircon's).

Three samples from each farm were taken, making the total number of samples up to thirty.

Sample 1:- From the main source of water (network or wells).

Sample 2:- From the storage place (barrel almost).

Sample 3 :- From the drinkers

The farms were distributed in five areas namely Shambat, Elhalfaia, Elsamrab, Elkadaro and Elsagai for open system and two areas Elbagair, Butri for closed and semi closed systems.

2.2.1 Collection of samples:

Sterile glass bottles 500 ml, 1000 ml volume were used for the collection of the water samples, with known code number, date and time.

Water samples were taken in the period between March 2007 to July 2007. Twenty nine samples of water were collected (one sample was not collected) during the hole period. Water samples were collected aseptically from each of the selected locations.

2.2.1.1 Treated water:

Four farms their samples were taken from the network as fallows:

- Samples from farm number 1 taken from Shambat.
- Samples from farm number 3 taken from Elhalfaia.
- Samples from farm number 4 taken from Elsamrab.
- Samples from farm number 8 taken from Shambat.

2.2.1.2 Untreated water:

Six farms their samples were taken from wells.

- Samples from farm number 2 taken from Elhalfaia.
- Samples from farm number 5 taken from Elkadaro.
- Samples from farm number 6 taken from Elsagai.
- Samples from farm number 7 taken from Elsagai.
- Samples from farm number 9 taken from Elbagair.
- Samples from farm number 10 taken from Butri.

2.2.2.1 Collection of tap water sample:

Collection done as fallows:

1- The outside nozzle of the tap was cleaned carefully.

2- The tap was turned on fully, 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 neck or the inside of the cap.

5- The cap of the sample was then replaced.

6- Label the bottle with the sample code number.

2.2.2.2 Collecting water sample from well:

1- Continuously operate the hand pump for 5 minutes.

2- Aseptically collect a sample of water by allowing the water from the pump to flow directly into the sterile bottle, carefully replace the bottle cap and cover.

3- Label the bottle with the sample code number.

2.2.2.3 Collecting a sample from a storage place (the barrel):

1- The cap was removed and the mouth of the bottle was faced up.

2- The bottle was pushed forward horizontally until it was filled; carefully replace the bottle cap and cover.

3- Label the bottle with the sample code number.

2.2.2.4 Collecting water sample from a drinker:

The cap was removed and the bottle immediately and quickly was filled with water and covered and then Labeled with the sample code number.

.

2.2.3 Transportation of samples:

All precautions were taken to prevent accidental contamination of the water during it is transportation. Immediately after collection the glass bottles transported to the laboratory and the test was done immediately after the arrival of the sample to the laboratory.

2.3 Bacterial Examination:

2.3.1 Culture media:

2.3.1.1 MacConkey's broth (purple) [Oxoid]

Constituents of this medium include:

Peptone	20 grams
Lactose	10 grams
Bile salt	5 grams
Sodium chloride	5 grams
Bromo cresol purple	0.07 grams

Forty grams of powder were dissolved in one liter of distilled water, the PH was adjusted to 7.4, and then the bromo cresol purple was added.

The medium was distributed in tubes containing an inverted Durham tube, in each tube poured 10 ml of medium then the medium was sterilized by autoclaving at 121˚C under pressure 15 Ib / inch² for 15 minutes.

2.3.1.2 Nutrient agar [Oxoid]

Dehydrated nutrient agar was prepared according to the manufacturer instruction. 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 autoclaving (121° C for 15 minutes) cooled to 50ºC -55°C and then distributed into Petri dishes , 20 ml in each Petri dish .

2.3.1.3 Hugh & Leifson's (O/F) medium

Contents:

Peptone	20 grams
Sodium chloride	5 grams
Agar	3 grams
K_2hPO_2	0.3 grams
Distilled water	1000 ml
Bromothyol 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 55ºC except bromothyol blue solution which was added after adjusted of the PH to 7.1 . Ten sterile solutions of the appropriate carbon hydrate were added 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, and then the medium was mixed and distributed aseptically in 10 ml amounts into sterile test tubes. The prepared medium was kept at 4ºC until use.

2.3.1.4 Tryptone water [Oxoid]

This medium consists of:

Tryptone	10 grams
Sodium chloride	5 grams

Fifteen grams of medium were dissolved in one liter of distilled water, and then the medium was distributed into test tubes and sterilized by autoclaving at 121°C for 15 minutes.

2.3.1.5 Motility medium:

Contents

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

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

2.3.2 Reagents & Buffers :

2.3.2.1 Kovac's Reagent :

This reagent contains:-

Para dimethyl amino benzalaldahyde	5 grams
Amylalcohol	75 grams
Concentrated hydro chloric acid	25 grams

The aldahyde was dissolved in alcohol by gentle warming in water bath (50ºC - 55°C). It was then cooled and the acid was added with care. The reagent was protected from light and stored at 4ºC for indol test.

2.3.3 Sterilization:

2.3.3.1 Flaming:

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

2.3.3.2 Red Heat:

It was used to sterilize wires and pointed edges of forceps by holding them over Bunsen burner flame until became red- hot.

2.3.3.3 Hot air oven:

It was used to sterilize glass Petri dishes and pipettes by keeping them at 161°C for one hour.

2.3.3.4 Moist Heat (Autoclave):

It was used to sterilize media, bottles and cultures at 121ºC under pressure 15 Ib/ $inch^2$ for 15 minutes.

2.3.4 Disinfectants:

They were used to disinfect laboratory surfaces, pipette discard containers and filtration apparatus.

2.4 Bacterial viable count

The viable bacterial count was done according to Quinn P. et al, (2000) and the method is called Miles – Misra (1938).

2.4.1Preparation of the dilutions :

The serial dilution was prepared according to Harrigan and Maccance, (1976). A micro pipette with sterile tip was held vertically and introduce 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, (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}. Afresh 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 9ml normal saline), also without contact the diluting fluid then the tip was discarded and second dilution tube was labeled as second dilution tube 1/100, or 10^{-2}. Further dilution of 1/1000, or 10^{-3}, 1/10000, or 10^{-4}, 1/100000, or 10^{-5} were prepared in the same manner.

2.4.2Preparation of the Nutrient agar plates:

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

Afresh sterile tip was used to mix the content of the each dilution by sucking up and down ten times, 0.2 ml of each dilution were withdrawn and transferred to nutrient agar, and the plates were labeled by the number of the dilution.

2.4.3 Colony count :

Colonies were counted according to Miles and Misra surface 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 × dilution factor × 50).

2.5 Bacterial procedures for drinking –water:

2.5.1. Multiple Fermentation tube technique (most probable Number MPN)

Materials and Methods:-

The test was done according to the method described by Bartram and Pedley (1996), three volumes of a sample were prepared 10, 1, and 0.1 ml, cultured and incubated at 37℃.

1- Required number of Maconkey broth (purple) tubes was prepared.

2- The appropriate volumes of sample were pipetted into the tubes containing medium.

The sample and the medium were shaken gently to mix.

3- The tubes were labeled with the sample numbers and volumes added.

4- The tubes were incubated at 37℃ for 48 hours in incubator.

5- After 24 hours the tubes which showed growth (turbidity and gas production on colour change) were regarded as positive, the number of positive tubes at each dilution were recorded, then the tubes were returned to the incubator and examined after a total of 48 hours of incubation.

6- The pattern of positive results was compared with a most probable number (MPN) table.

2.5.2 Confirmatory test:-

2.5.2.1 Indol test:

Tryptone water tubes were inoculated with cultures from positive MPN tubes and incubated at 37℃ for 48 hours.

0.2 ml of Kovac's reagent was dropped along the slide of the culture tube. Development of a pink ring on the reagent layer within a minute indicated appositive reaction Feltham (1993).

2.5.3 Completed test

1. From any tube showed 10% gas production or more, one loop full of the broth inoculated onto an EMB plate using the isolation streaking technique. The plate was incubated at 37℃.

2. The plate was examined, looking for well-isolated coli form colonies. Typically, E.coli colonies appeared with a metallic green sheen on EMB.

Table (2): MPN index and 95% confidences limits for various combinations of positive results when five tubes are used per dilution (10ml, 1.0ml, o.1ml portion of sample) (Bartram and Pedley,1996)

Combination of positive	MPN index per 100 ml	95% confidences limits		Combination of positive	MPN index per 100 ml	95% confidence limits	
		Upper	lower			Upper	lower
0-0-0	<2	-	-	4-2-0	22	9.0	56
0-0-1	2	1.0	1.0	4-2-1	26	12	65
0-1-0	2	1.0	1.0	4-3-0	27	12	67
0-2-0	4	1.0	13	4-3-1 4-4-0	33 34	15 16	77 80
1-0-0	2	1.0	11	5-0-0	23	9.0	86
1-0-1	4	1.0	15	5-0-1	30	10	110
1-1-0	4	1.0	15	5-0-2	40	20	140
1-1-1	6	2.0	18	5-1-0	30	10	120
1-2-0	6	2.0	18	5-1-1 5-1-2	50 60	20 30	150 180
2-0-0	4	1.0	17	5-2-0	50	20	170
2-0-1	7	2.0	20	5-2-1	70	30	210
2-1-0	7	2.0	21	5-2-2	90	40	250
2-1-1	9	3.0	24	5-3-0	80	30	250
2-2-0	9	3.0	25	5-3-1	110	40	300
2-3-0	12	5.0	29	5-3-2	140	60	360
3-0-0	8	3.0	24	5-3-3	170	80	410

3-0-1	11	4.0	29	5-4-0	130	50	390
3-1-0	11	4.0	29	5-4-1	170	70	480
3-1-1	14	6.0	35	5-4-2	220	100	580
3-2-0	14	6.0	35	5-4-3	280	120	690
3-2-1	17	7.0	40	5-4-4	350	160	820
4-0-0	13	5.0	38	5-5-0	240	100	940
4-0-1	17	7.0	45	5-5-1	300	100	1.300
4-1-0	17	7.0	46	5-5-2	500	200	2.000
4-1-1	21	9.0	55	5-5-3	900	300	2.900
4-1-2	26	12.0	63	5-5-4 5-5-5	1.600 >1,600	600 _	5.300 _

Table (6): The degree of bacterial risk in drinking –water by using MPN determination:

According to WHO guidelines for drinking – water:

Count per 100 ml	Remarks
0	In conforming with WHO guidelines
1 -10	Low risk
10 -100	Intermediate risk
100 -1000	High risk
> 1000	Very high risk

Treated water in the distribution system the total coli forms bacteria must not be detectable in any 100 ml sample. In the case of large supplies, where sufficient samples are examined, must not be present in 95% of samples taken throughout any 12 month period (WHO, 1997).

2.6 Disinfectants Application

2.6.1 Yodor – vex (commercial disinfectant)

Solution for hygienic application

Composition:

Nonylphenoxypoly (ethyenoxy)

Ethanol iodide complex 8.75 ml

Diluent to 100 ml

Spp for which intended and indications:

In general: disinfection/cleaning of wounds in poultry and livestock facilities for the disinfection of premises, surgical material, water and means of transport.

Mean of administration and dosage:

Disinfection of contaminated water (its action remain even in water containing organic matter).

- 0.6 – 0.8 ml / Lt of water.

- Withdrawal time:

Not necessary

Manufacturing by:

S.p. veterinaria, S.a

- The method :-

0.8 ml yodor to 1 liter water (recommended by manufacturer instruction).

1- By adding 0.2 yodor to 125 ml water. The sample was culture onto nutrient agar and incubated at 37℃ for 24 hours.

2- The plate was examined, looking for bacterial growth.

* Farm No 2 was selected randomly to make serial concentration of yodor disinfectant for the drinkers' sample

A- 1- by adding 0.2 ml yodor to 125 ml water.

2- by adding 0.4 ml yodor to 125 ml water.

3- by adding 0.8 ml yodor to 125 ml water.

4- by adding 1.6 ml yodor to 125 ml water.

5- by adding 2.3 ml yodor to 125 ml water.

The samples were cultured onto nutrient agar plates and incubated at 37℃ for 24 hours.

B- The plates were examined, looking for bacterial growth.

2.6.2 Vircon's (commercial disinfectant)

Instruction for use:-

Because of it’s high level of safety and exceptionally broad spectrum, of biocidal activity vircon's can be used in a wide variety of ways.

Water sanitation:-

Terminal clean out

1:200 - 1:100

(0.5 – 1%)

Continuous water sanitation

1:1000 (0.1%)

Liters of water to be sanitation	Terminal cleanout		Continuous water sanitation
	1: 200	1: 100	1: 1000
1000 L	5kg	10kg	1 kg

- Chemical and physical properties:

+ Composition: A balanced, established blend of peroxygen compounds, surfactant, organic acids and inorganic buffer system.

+ Appearance: pink/grey powder (yellow in some markets including USA). Odour: faint lemon odour.

+ Activity: strong oxidizing system.

+ Stability: powder: 2.3% average loss of initial activity after 36 months at 20℃. 1% solution: 10% loss of initial activity after 7 days in 350 ppm hard water at 20℃.

+ Solubility: Readily soluble in tepid water giving a clear pink solution (yellow in some markets including USA).

+ Corrosivity: No corrosive effect on mild or stainless steel when used as directed.

+ Hydrogen ion concentration: 1% solution – PH 2.6

Manufacturing by: Antec international A Dupont Company.

- The method :-

10 kg vircon's to 1000 L water (recommended by manufacturer struction).

1- By adding 1.25 mg to 125 ml water. The water sample was cultured onto Nutrient agar and incubated at 37℃ for 24 hours.

2- The plate was examined, looking for bacterial growth.

2.7 Culturing:

2.7.1 Primary culture:

Primary cultures for all water with disinfectant were done as before onto Nutrients agar.

2.7.2 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 Nutrient agar. Pure cultures were obtained by replating the subcultures.

2.8 Identification of isolates:

The identification was based mainly on the colony characteristics, staining, and motility and biochemical reactions.

2.8.1 Microscopic examination:

Smears were made from purified colonies, fixed by gentle heating and stained by Gram stain method (Barrow and Feltham, 1993). Then examined microscopically for purity and shape of bacteria.

2.8.2 Biochemical tests:-

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

2.8.2.1 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.

2.8.2.2 Catalase test:

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

2.8.2.3 O/F test:

Duplicate tubes were 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℃ for 24 hours and examined.

CHAPTER THREE

RESULTS

3.1 Bacterial total viable counts:

The results of the total viable counts per ml in all samples were found between 2000 to 20.000.000 cfu/ml. The lowest viable count recorded in farm 1 from Shambat. Where the highest one was recorded in farm 9 from Elbagair.

3.1.1 From the main source of water:

From the nine farms examined viable counts were (1×10^4, 2×10^4, 1×10^4, 17×10^4, 10×10^4, 10×10^4, 3×10^3, 3×10^6, 57×10^4) from farms 1 to 9 respectively. The mean viable count was 442.6×10^3 colony forming units/ml.

3.1.1.1 Treated water from the main source

Water samples taken directly from the tap (network system) showed bacterial count that ranged between 3×10^3 to 2×10^4 cfu/ml.

3.1.1.2 Untreated water from the main source:

Water samples taken directly from the wells showed bacterial count ranged between 1×10^4 to 3×10^6 cfu/ml.

3.1.2 From the storage place:

From the ten farms examined viable counts were (3×10^3, 4×10^4, 6×10^5, 12×10^4, 3×10^4, 50×10^4, 4×10^5, 30×10^5, 16×10^6, 11×10^6) from farm 1 to 10 respectively. The mean viable count was 317.2×10^4 cfu/ml.

3.1.2.1 Treated water from the storage place:

Water samples taken from the storage place showed bacterial count that ranged between 2×10^3 to 30×10^5 cfu/ml.

3.1.2.2 Untreated water from the storage place:

Water samples taken from the storage place showed bacterial count that ranged between 3×10^4 to 16×10^6 cfu/ml.

3.1.3 From the drinkers

From the ten farms examined viable counts were (3.7×10^5, 1.9×10^5, 6.9×10^6, 14.8×10^6, 6×10^6, 2×10^6, 10.3×10^6, 4.5×10^6, 20×10^6, 1.4×10^6) from farms 1 to 10 respectively. The mean viable count was 664.6×10^6 cfu/ml.

3.1.3.1 Treated water from the drinkers:

Water samples taken from the drinkers showed bacterial count that ranged between 37×10^4 to 148×10^5 cfu/ml.

3.1.3.2 Untreated water from the drinkers:

Water samples taken from the drinkers showed bacterial count that ranged between19×10^4 to 20×10^6 cfu/ml.

3.2 Total coli forms MPN determination:

3.2.1 The results of total coli forms MPN/100ml water for the main source samples.

Table (7): Samples from the main source of water

Number of positive tubes and MPN Determination

Samples No.	Numbers of tubes giving positive reactions out of			MPN
	10 ml tubes	1.0 ml tubes	0.1 ml tubes	
1	-	-	-	-
2	3	0	0	8
3	5	2	0	50
4	5	4	1	170
5	4	2	0	22
6	5	2	0	50
7	5	2	0	50
8	5	2	1	70
9	4	2	0	22
10	0	0	0	<2

The lowest total coli from MPN/100ml water recorded was zero obtained from Butri. The highest total coli forms MPN/100ml water recorded was 170 MPN/100ml obtained from farm (4) Elsamrab.

3.2.2 The results of total coli forms MPN/100ml water for the storage place sample.

Table (8) Samples from the storage place of water

Number of positive tubes and MPN Determination

Samples No.	Numbers of tubes giving positive reactions out of			MPN
	10 ml tubes	1.0 ml tubes	0.1 ml tubes	
1	5	5	2	500
2	5	5	5	>1.600
3	5	5	5	>1.600
4	5	5	5	>1.600
5	5	5	0	50
6	5	5	5	>1.600
7	5	5	5	>1.600
8	5	5	5	>1.600
9	5	4	0	130
10	5	5	5	>1.600

The lowest total coli forms MPN/100ml water recorded was 50 MPN/100ml obtained from farm (5) from Elkadaro.

3.2.3 The result of total coli forms MPN/100ml water for the drinker's samples. All samples shown highest total coli form MPN/100ml.

Table (9) Samples from the Drinkers

Number of positive tubes and MPN Determination

Samples No.	Numbers of tubes giving positive reactions out of			MPN
	10 ml tubes	1.0 ml tubes	0.1 ml tubes	
1	5	5	5	>1.600
2	5	5	5	>1.600
3	5	5	5	>1.600
4	5	5	5	>1.600
5	5	5	5	>1.600
6	5	5	5	>1.600
7	5	5	5	>1.600
8	5	5	5	>1.600
9	5	5	5	>1.600
10	5	5	5	>1.600

Table (10) shows the level of risk in water samples by using MPN determination

		Frequency	Percent	Valid percent	Cumulative percent
Valid	No risk	1	3.3	3.4	3.4
	Low risk	1	3.3	3.4	6.9
	Intermediate risk	7	23.3	24.1	31.0
	High risk	3	10.0	10.3	41.4
	Very high risk	17	56.7	85.6	100.0
	Total	29	96.7	100.0	
	System	1	3.3		
Missing total		30	100.0		

3.3 Bacterial isolation from water after using yodor and vircon's disinfectants:

3.3.1 from the main source of water 9 samples were examined, all samples negative for bacterial growth.

3.3.2 From the storage place 10 samples were examined, one sample from Elbagair was positive for both yodor and vircon's.

3.3.3 From the drinkers 10 samples were examined, the positive results for yodor from farm 2 to 10, the positive results for vircon's in farms 4, 7, 9. The isolated genera were (Flavobacterium, Enterobacter, Acinetobacter, bacillus, Staphylococcus, Micrococcus).

Table (11) Bacterial Genera Isolated from water after adding disinfectants (Yodor and Vircon's)

Farms	Bacterial isolates
1/ Yodor disinfectant	
1	-
2	Flavobacterium , Enterobacter, Bacillus
3	Acinetobacter
4	Bacillus
5	Acinetobacter
6	Flavobacterium
7	Acinetobacter , Flavobacterium, Staphylococcus, Micrococcus
8	Enterobacter
9	Bacillus
10	Flavobacterium
(2) Vircon's disinfectant	
4	Bacillus
7	Micrococcus, Acinetobacter
9	Bacillus

Table (13) Genera of bacterial isolated after using serial concentration of yodor disinfectants for the sample 2 (from drinkers) from farm 2

Concentration /ml	Bacterial isolates
0.2 ⟶ 125 ml water	Flavobacterium , Enterobacter, Bacillus
0.4 ⟶ 125 ml water	Enterobacter, Bacillus
0.8 ⟶ 125 ml water	Bacillus
1.6 ⟶ 125 ml water	Bacillus
2.3 ⟶ 125 ml water	-

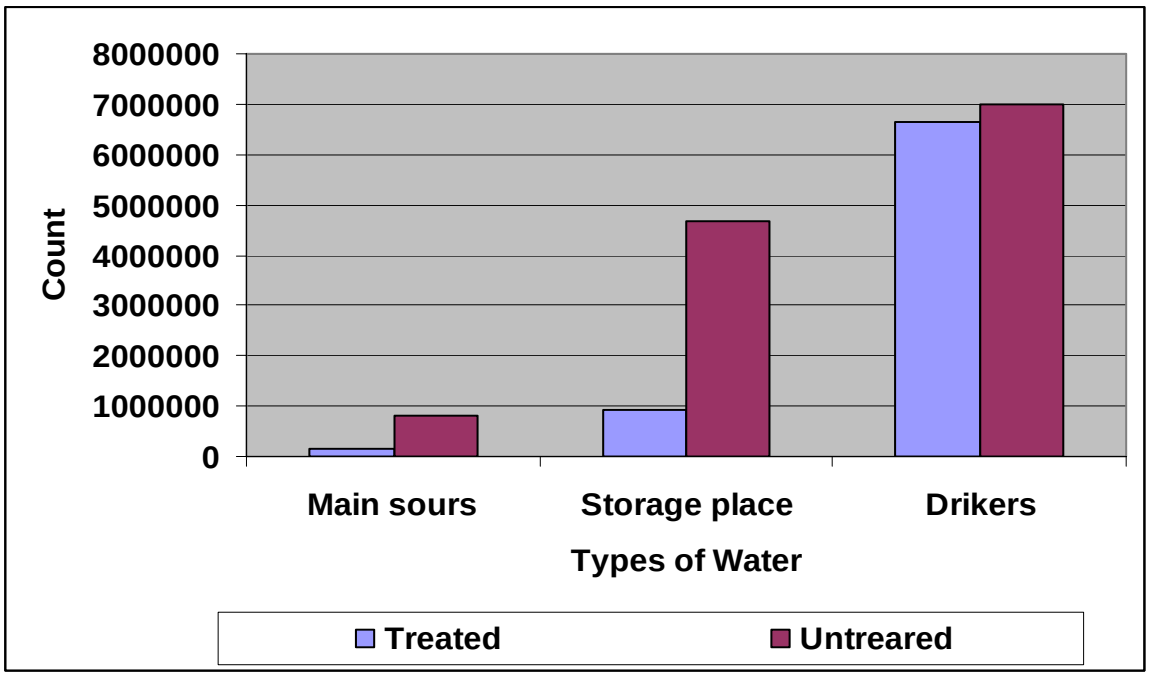

Figure (1) the average of bacterial count in treated and untreated water sources

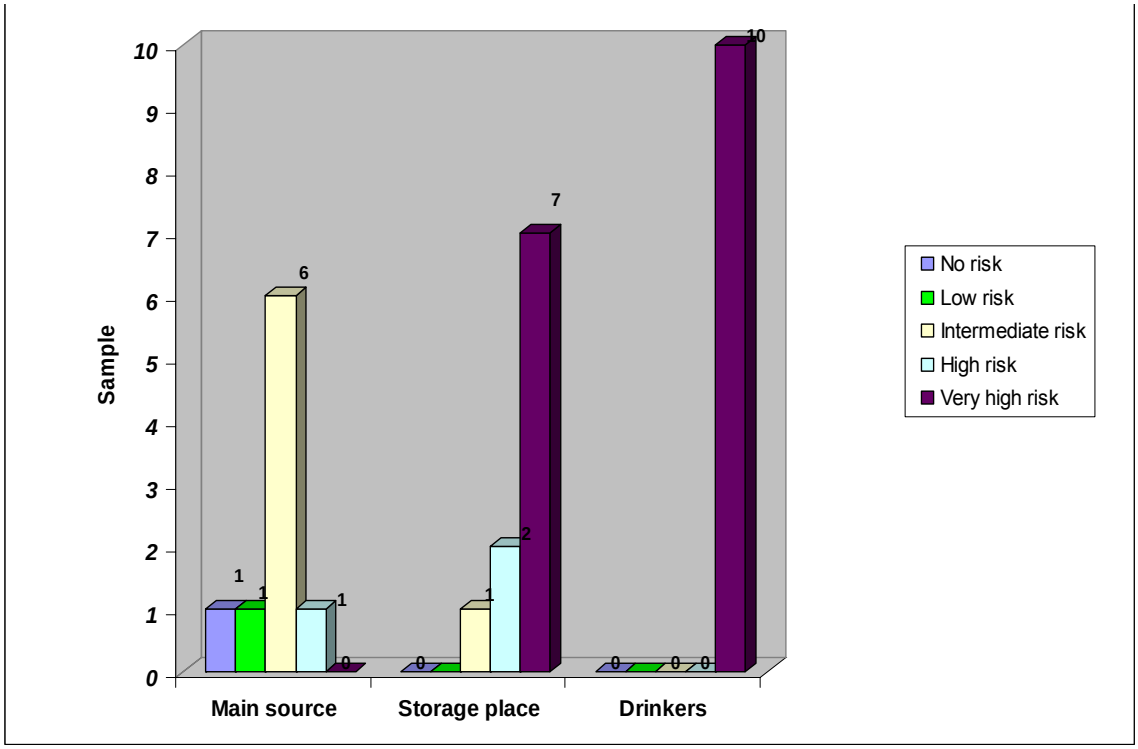

Figure (2) the level of risk in different water sources for the ten farms

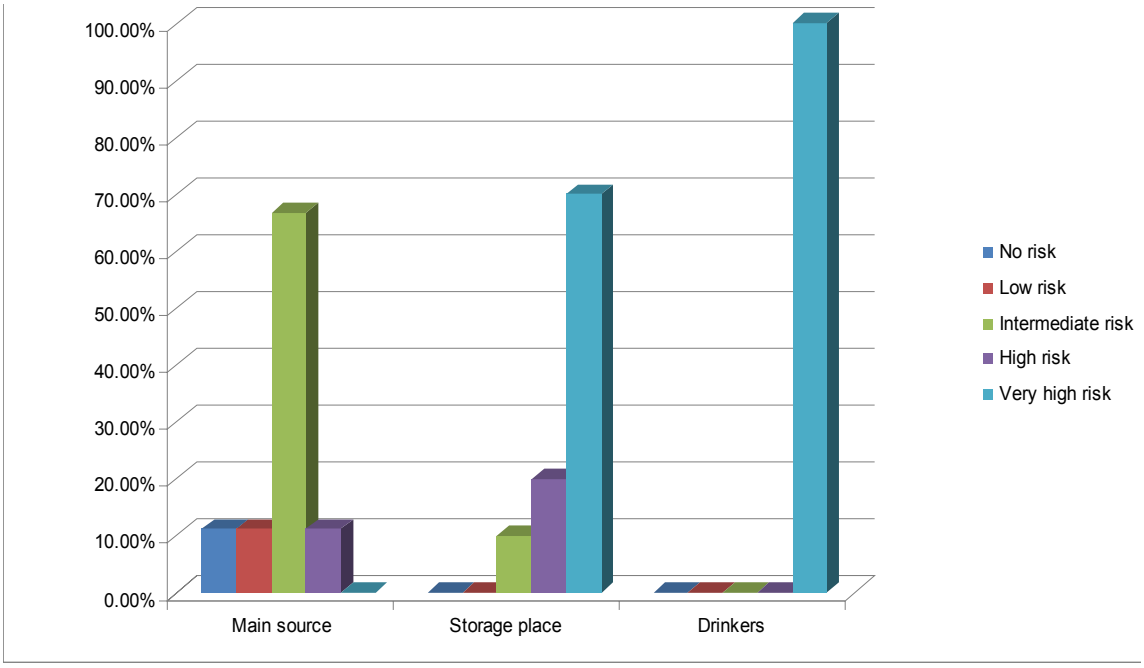

Figure (3) the risk of bacterial contamination in different sources in percentage

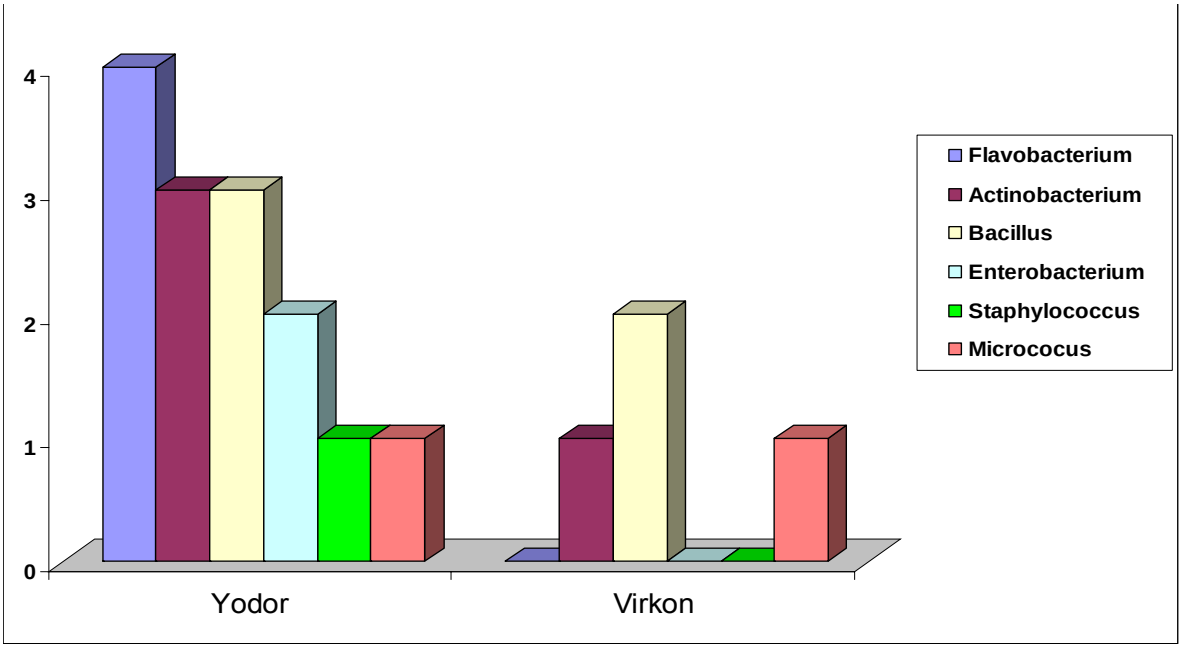

Figure (4) Bacterial recovered after treatment with different disinfectant (yodor and Vircon's)

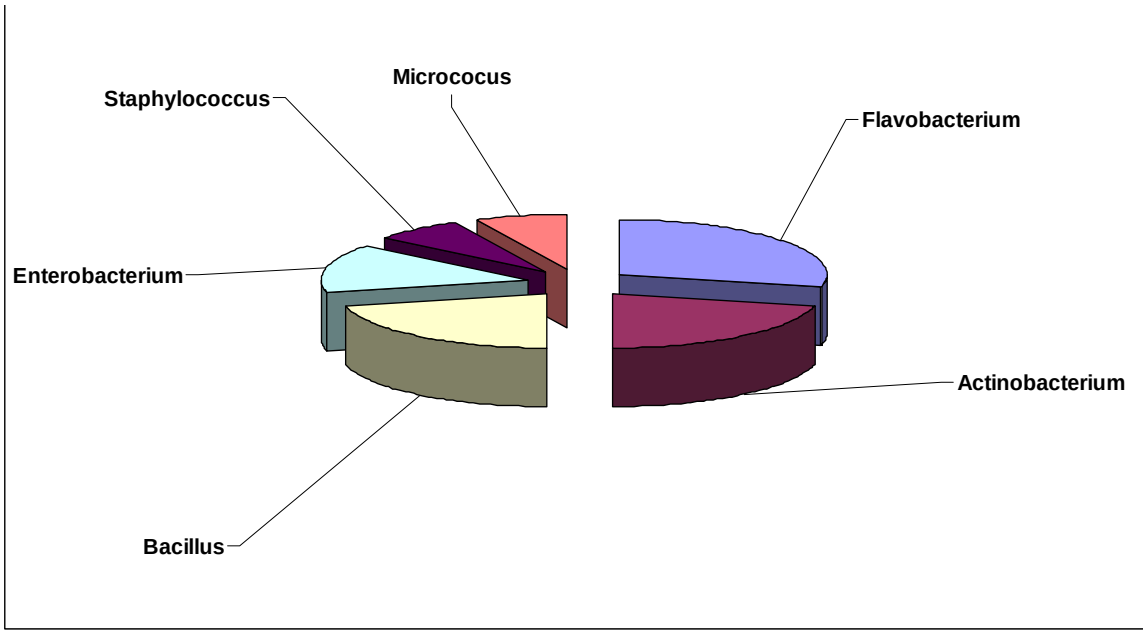

Figure (5) Bacterial genera isolated after using disinfectants

CHAPTER FOUR

DISCUSSION

Water is essential to sustain life; therefore, a satisfactory supply must be made available to consumers. Every effort should be made to maintain drinking-water quality as high as practicable.

In tropical developing countries we face difficult choices in finding good water quality.

All attention is directed for human drinking water but for animals drinking-water few researches are done to illustrate the contamination of animal drinking water without any solution yet.

Imad (2001) studied drinking –water in western Omdurman for faecal coli form MPN determination. He was the first one in Sudan who made a research in this area for animal's drinking water beside human. He found all the samples were higher than WHO recommendations.

Ibrahim (2006) isolated twelve genera from cattle drinking-water in Khartoum North. He was the first one who investigated drinking-water for animal in this area.

Animal's drinking-water needs real attention because it is contamination can affect animal's production or even their life.

In Khartoum North, the present study area, the productions of chickens are regarded as an important industry. The production types are layers and broilers. The production system is an open system, so the problem associated with environmental contamination is an important one that can affect this industry.

Most owners of these farms have no medical information to deal with chicken diseases and their labourers are mostly illiterate or weakly educated. This situation plus open system problems make drinking water contamination very easy. But the real problem is not the contamination itself, it's how they can deal with it. Most of them neglect this contamination and few are using disinfectants. Therefore, the question is: how can they use it?. Is it in a recommended dose or not? Decreasing or increasing the dose may expose the chickens to new problems which may result in chicken's toxicity in case of overdose or help the microorganisms to be resistant to the disinfectant in case of decreasing the dose.

In this study, the treated and untreated water showed higher results than WHO recommendations. The total coli forms count and total viable count for bacteria showed considerable difference between the main source of water, the storage place and the drinkers. The highest bacterial count and MPN determination found in the drinkers and the lowest in the main source of water samples. The high bacterial count and MPN determination of the drinkers could be due to the direct contact to environmental contamination, the bird behaviour in drinking and because the labourer change the water after along time. Where as the main source of water is protected from direct contact and treating with disinfectant. Bacterial contamination in the storage place (almost Barrels) due to environmental contamination and bad storage of water.

In comparison between these three types of samples we find that the degree of contamination is increasing from the main source of water to the drinkers for reasons shown above.

One sample is taken from closed system and another one from semi closed system. The degree of contamination is the same in open system, increasing from the main source of water to the drinkers. In comparison between open and closed and semi closed system we found that in the main source of water closed and semi closed systems showed less contaminant than open system. But in the storage place and drinkers are the same and that is may be due to bad storage of water and environmental contamination in semi closed system.

Some farms are making local drinker (plastic container) which is cut in the middle of two faces in square shape. They make it because it is cost effective. As my own observation the water in local drinker is less contaminated than in the drinkers because it is elevation level from the floor is higher than in the drinkers so the contamination will be less.

In poultry farms drinking-water contamination is regarded as a factor which exposes chickens to colibacillosis infection especially E.coli strain and other infections which are transmitted through water. The best solution for stopping water contamination is to use water disinfectants.

There are many factors which can affect disinfectants viability and reduce it:

1- According to WHO guidelines:

   a- High water turbidity.

   b- Water hardness.

   c- Bacterial sporing (which can protect microorganism from disinfectants).

2- Creation of poly saccharide layer in the water due to the administration of additives will protect microorganisms from disinfectants (Wiebe, 2002).

Turbidity of water exists due to high load of bacteria and other environmental factors. The hardness of water is always higher in ground water than in surface water.

Yodor and vircon's are common water disinfectants which are used in animal's farm especially for poultry farms.

Yodor is a chemical product which mainly consists of iodine. It's manufactured by S.P. veterinarian, s.a.

Vircon's is a chemical product too which mainly consist of peroxygen. It's manufactured by Antec international ADu Pont Company.

These two commercial chemical products are effective against microbial pathogens.

In this study the two chemical products yodor and vircon's are

effective against the bacteria from the main source of water samples

and storage place samples but in the drinkers samples the two products are not effective 100% and that is due to high water turbidity of the drinkers and may also be due to biofilm creation because in some farms they add additives to the water. One of these bacteria is sporing bacteria Bacillus).

After using serial concentrations of Yodor disinfectant to the drinkers water sample in farm two, we find that the bacteria which resist the recommended dose are dying and that means increasing the dose is effective but it's hazardous to poultry health.

Water disinfection for poultry farms is an effective barrier for microbial contamination which affects poultry industry.

An effective over all management strategy in corporate multiple barriers, including source water protection and appropriate treatment processes, as well as protection during storage and distribution in conjunction with disinfection to prevent or remove microbial contamination.

An objective of surveillance and quality control surveillance is an investigation activity under taken to identify and evaluate factors associated with drinking-water which could pose a risk to health.

Surveillance contributes to the protection of public health by promoting improvement of the quality, quantity, coverage, cost and continuity of water supplies. It's also both preventive detecting risks so that action may be taken before public health problems occur – and remedial – identifying the sources of outbreaks of water borne disease so the corrective action may be taken promptly (WHO, 1997).

CONCULSIONS

1- High viable count was obtain from all farms in different areas for the samples from the drinkers and the storage place and lesser to the samples from the main source of water.

2- 93% of samples were positive to MPN determination for total coli forms bacteria.

3- 34% of samples were positive to bacterial growth after using yodor disinfectant, 14% of samples were positive to bacterial growth after using vircon's disinfectant.

4- High viable counts and high MPN determination for total coli forms bacteria represent a real risk for poultry health may affect poultry industry.

RECOMMENDATIONS

1- The Ministry of Animal Resources and Fishers should make great attention for water contamination and it is hazards to health and production level.

2- Making a good water quality control by the owners and their labourers by:

A- Cleaning the storage place regularly.

B- Cleaning the drinker properly at least one time every day.

C- Closing the open well and the storage place of water.

3- Health education programs should be conducted aiming at increasing awareness on the importance of clean water for animal's health especially in poultry industry.

4- The Ministry of Health should be aware to renewal and repair the damage parts of the network pipelines.

5- The use of drinking-water disinfectants should be in a recommended dose and limited to the veterinarian's prescription.

6- They should not used disinfectant for water mixed with additives.

7- The disinfectants should be removed from the drinkers before the use of water vaccines or medications.

8- Making more researches in this field including season’s variations and other types of disinfectants.

9- I hope that the people who work in poultry industry will make use of this study.

9- Making more researches in this field including season's variations and other types of disinfectants.

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Appendix(1)Bacterial count comparing between different sources of water

Descriptives

	Water source			Statistic	Std. Error
Bacterial count	Main source	Mean		775555.6	424280.8
		95% Confidence Interval for Mean	Lower Bound	-202838	
			Upper Bound	1753949	
		5% Trimmed Mean		694506.2	
		Median		100000.0	
		Variance		1.6E+12	
		Std. Deviation		1272842	
		Minimum		10000.00	
		Maximum		3000000	
		Range		2990000	
		Interquartile Range		1770000	
		Skewness		1.546	.717
		Kurtosis		.585	1.400
	Storage place	Mean		3169200	1783718
		95% Confidence Interval for Mean	Lower Bound	-865851	
			Upper Bound	7204251	
		5% Trimmed Mean		2632333	
		Median		450000.0	
		Variance		3.2E+13	
		Std. Deviation		5640613	
		Minimum		2000.00	
		Maximum		16000000	
		Range		15998000	
		Interquartile Range		4962500	
		Skewness		1.868	.687
		Kurtosis		2.430	1.334
	Drinkers	Mean		1.6E+07	9487711
		95% Confidence Interval for Mean	Lower Bound	-5636695	
			Upper Bound	3.7E+07	
		5% Trimmed Mean		1.2E+07	
		Median		6450000	
		Variance		9.0E+14	
		Std. Deviation		3.0E+07	
		Minimum		190000.0	
		Maximum		1.00E+08	
		Range		99810000	
		Interquartile Range		1.2E+07	
		Skewness		2.998	.687
		Kurtosis		9.233	1.334

Appendix (2) Bacterial count comparing between levels of risk

Descriptives[a,b]

	Level of risk			Statistic	Std. Error
Bacterial count	Intermediate risk	Mean		917142.9	538121.6
		95% Confidence Interval for Mean	Lower Bound	-399593	
			Upper Bound	2233879	
		5% Trimmed Mean		851269.8	
		Median		100000.0	
		Variance		2.0E+12	
		Std. Deviation		1423736	
		Minimum		20000.00	
		Maximum		3000000	
		Range		2980000	
		Interquartile Range		2970000	
		Skewness		1.224	.794
		Kurtosis		-.844	1.587
	High risk	Mean		5337333	5331334
		95% Confidence Interval for Mean	Lower Bound	-1.8E+07	
			Upper Bound	2.8E+07	
		5% Trimmed Mean		.	
		Median		10000.00	
		Variance		8.5E+13	
		Std. Deviation		9234141	
		Minimum		2000.00	
		Maximum		16000000	
		Range		15998000	
		Interquartile Range		.	
		Skewness		1.732	1.225
		Kurtosis		.	.
	Very high risk	Mean		1.0E+07	5738673
		95% Confidence Interval for Mean	Lower Bound	-1934856	
			Upper Bound	2.2E+07	
		5% Trimmed Mean		5809542	
		Median		3000000	
		Variance		5.6E+14	
		Std. Deviation		2.4E+07	
		Minimum		40000.00	
		Maximum		1.00E+08	
		Range		99960000	
		Interquartile Range		1.0E+07	
		Skewness		3.826	.550
		Kurtosis		15.234	1.063

a. Bacterial count is constant when Level of risk = No risk. It has been omitted.

b. Bacterial count is constant when Level of risk = Low risk. It has been omitted.

Appendix (3) The main source of water (open well)

Appendix (4) Different types of storage places

Appendix (5) Different types of drinkers