An-Najah National University
Faculty of Graduate Studies

Water-Borne Pathogens With Relation To Gastroenteritis in Tubas District: An Epidemiological Study

By

Husam Hasan Odeh Abu Alian

Supervised by

Dr. Yahya Faidy

Submitted in Partial Fulfillment of the Requirements for the Degree of Masters of Environmental Science, Faculty of Graduate Studies at An-Najah National University.

Nablus – Palestine

December 2001

II

WATER- BORNE PATHOGENS WITH RELATION TO GASTROENTERITIS IN TUBAS DISTRICT: AN EPIDEMIOLOGICAL STUDY

BY
Husam Hasan Odeh Abu Alian

This Thesis was successfully defended on 2/12/2001 **and Approved by:**

Committee Members	Signature
1. **Dr. Yahya Faidy (Supervisor)** **Assistant Professor of microbiology.**	
2. **Dr. Suleiman Al-Khalil (Internal examiner)** **Associated professor of medicinal chemistry.**	
3. **Dr. Issam A. Al- Khatib (External examiner)** **Assistant professor of environmental engineering .**	

TO

MY DEAR MOTHER, BROTHER, SISTERS AND TO MY LOVELY WIFE FOR THEIR ENCOURAGEMENT WITH LOVE AND RESPECT.

ACKNOWLEGEMENT

I would like to express my sincere special thanks and gratitude to my supervisor Dr. Yahya Rashed Faydi for his supervision, encouragement, guidance and help throughout this project.

I also like to express my gratitude and thanks to the mayor of Tubas Municipality Mr.Diab Abu Kiazuran and all members of Tubas Municipality for their support and encouragement during my study.

Thanks are expressed to the Environmental Science Department at An- Najah National University and to Medical staff in Red Cresent Society and The Union of Health Work Committees for their help during the course of the study.

I am deeply grateful to Dr. Ghassan Al-Khatib (Director of Jerusalem Media and Communication Center), to Dr. Othman Karami (from WHO office-Jerusalem) and to Dr. Rita Giacaman (Director of Institute of Community and Public Health -Birziet University) for their tremendous efforts and encouragement during my research and preparing for this thesis.

Thanks are expressed to the Palestinian Hydrology Group (PHG) for allowing me to use their research facilities.

Finally, I wish to thank my family and my wife for their patience and encouragement throughout my graduate study.

TABLE OF CONTENT

Subject	Page
Committee decision	II
Dedication	III
Acknowledgments	IV
Table of contents	V
List of tables	VIII
List of figures	X
List of symbols	XII
List of annexes	XIII
Abstract	XIV
CHAPTER ONE: INTRODUCTION	1-37
1.1 Introduction	1
1.2 Water resources in the West Bank and Gaza Strip	2
1.2.1Water supply systems in the West Bank	3
1.2.2 Water supply systems in Tubas district	4
1.2.3 Water supply systems in Tubas, Tammun, Tayasir Aqqaba and El-Fara villages	4
1.3 Water storage systems	5
1.3.1 Rainfed cisterns	6
1.3.2 Roof storage tanks	7
1.4 Persistence of water pathogens	8
1.5 Infective dose of water pathogens	9
1.6 Water – related modes of transmission of infectious agents	9
1.6.1 Water – borne infection	10
1.6.2 Water – washed infections	10
1.6.3 Water – based infections	11
1.6.4 Water – vectored infections	12
1.7 Water borne pathogens	12
1.7.1 Agents of significance	13
1.7.2 Epidemic water borne disease	15
1. Bacteria	15
2. Viruses	21
3. Protozoa	25
1.8 Microbial indicators of water quality	27
1.8.1 Total coliform	28
1.8.2 Thermotolerant coliforms bacteria	29
1.8.3 Faecal Streptococci	30
1.8.4 Sulfite-reducing Clostridia	31
1.8.5 Bacreriophages	31
1.8.6 Miscellaneous indicators	32

1.9 Current status of water supply and quality in Palestine 32
1.10 Aims of the study 33

CHAPTER TWO: MATERIALS&METHODS 38-52
2.1 Water sample collection 38
2.2 Water sample transport 38
3.3 Water sample filtration and culture media 39
2.4 Identification of bacteria 40
2.4.1 Identification of coliform bacteria (lactose fermenter) 40
2.4.2 Isolation and Identification of E.coli O157:H7 44
2.4.3 Isolation of Salmonella 45
2.4.4 Identification of Salmonella 46
2.5 Stool sample collection 48
2.5.1 Collection and transport of stool specimens 48
2.5.2 Microscopic examination 48
2.5.3 Isolation of Samonella & Shigella *48*
2.5.4 Identification of Salmonella & Shigella 49
2.5.5 Isolation and Identification of E.coli O157:H7 49

CHAPTER THREE: RESULTS 53-71
3.1 Bacterial quality of drinking – water according to water sources 53
3.2 Bacterial quality of drinking – water in Tubas, Tammun, El-Fara, Tayasir and Aqqaba villages. 54
3.3 Degree of contamination of drinking – water with respect to water sources. 56
3.4 Contamination of water sources as potential risk factors for human health. 57
3.5 Prevalence and distribution of intestinal parasites according to water sources. 60
3.6 Factors that affect water quality in storage systems (cisterns and roof storage tanks): 63
3.6.1 Distance between the cesspits and cistern 63
3.6.2 Animal raising 64
3.6.3 Cistern location 65
3.6.4 Type of storage systems 66
3.6.5 Usage of disinfectant (chlorine). 68
3.6.6 Storage time 69

CHAPTER FOUR: DISCUSSION 72-80
4.1 Average counts of total coliform and faecal coliform in water sources (Municipality, rain, and spring water) 72
4.2 Bacterial quality of water storage systems in Tubas, Tammun, El-Fara, Aqqaba and Tayasir villages. 73
4.3 Degree of contamination in storage systems 74
4.4 Contaminated water sources as potential risk factor for human health 74
4.5 Bacterial quality of water sources 75
4.6 Association between intestinal parasites and water sources 75
4.7 Factors that affect water quality in cisterns and roof storage tanks 75
4.7.1 Distance between cesspits and cisterns 75
4.7.2 Animal raising 76
4.7.3 Cisterns location 76
4.7.4 Storage time 77
4.7.5 Disinfection of water 77

Conclusions and recommendations 78
References 81
Annexes 85
Arabic summary 87

LIST OF TABLES

Table	*Subject*	*Page NO.*
1.1	Cities and villages with piped and unpiped water systems.	*3*
1.2	Population, buildings, cisterns, cesspits, and other services in the five villages.	*5*
1.3	Average of precipitation in Tubas area	*6*
1.4	The usual persistence time of excreted pathogens in fresh water at 20-30°C.	*8*
1.5	Water – borne pathogens and their significance in water supplies.	*13*
1.6	Viruses pathogenic to humans which can occur in polluted water and diseases attributed to them.	*22*
1.7	WHO classification for contamination degree and treatment methods.	*29*
1.8	WHO classification for *E.coli* counts and risk.	*30*
2.1	Basic reactions of Enterobacteriaceae on TSI agar.	*42*
3.1	Association between average counts for bacterial indicators and water sources.	*53*
3.2	Average counts of bacterial indicators in the five studied villages.	*54*
3.3	Distribution of municipality water, rainwater and spring water according to contamination degree.	*56*
3.4	Distribution of rainwater and spring water with respect to *E.coli* count and risk levels.	*57*
3.5	Number (N) and percentage occurrence (%) of bacteria in water sources.	*58*
3.6	Biochemical reactions for identification of bacteria in water	*60*
3.7	Number (N) and percentage occurrence (%) of parasite in stool samples.	*61*
3.8	Biochemical reaction for identification of bacteria in stool	*62*
3.9	Association between average count for bacterial indicators and distance between cesspits and cisterns.	*63*

Table	*Subject*	*Page No.*
3.10	Numbers, percentages and computed F-test of rainwater cisterns according to animals raising in households.	*64*
3.11	Numbers, percentages and computed F-test of cisterns according to cisterns location in every house.	*65*
3.12	Numbers, percentages of samples according to the type of storage systems.	*67*
3.13	Numbers, percentages and computed F-test of samples according to use of chlorine as disinfectant.	*68*
3.14	Numbers, percentages and computed F-test of cisterns according to storage time.	*69*
3.15	Numbers, percentages and computed F-test of roof storage tanks according to storage time	*71*

LIST OF FIGURES

Fig	Subject	Page No.
1.1	Palestine map including the study area (Tubas district).	35
1.2	Tubas district map including the study villages.	36
1.3	Main elements in the rainfed cistern collection system.	37
2.1	Checklist for collecting water sample from tap.	50
2.2	Checklist for collecting water sample from dug wells.	51
2.3	Membrane filter technique for determining coliform content of water.	52
3.1	Average count of bacterial indicators in municipality water, rainwater and spring water.	54
3.2	Average counts of bacterial indicators in storage system for Tubas, Tammun, El-Fara, Tayasir and Aqqaba villages.	55
3.3	Distribution of municipality water, rainwater and spring water according to contamination degree.	56
3.4	Distribution of rainwater and spring water with respect to *E.coli* count and risk level.	57
3.5	Distribution of bacteria in rainwater, spring water and municipality water (examined samples = 87).	59
3.6	Distribution of intestinal parasites in persons, depending on rainwater, spring water and municipality water for drinking.	61

Fig	Subject	Page No.
3.7	Average counts of bacterial indicators according to cesspits distance.	64
3.8	Average counts of bacterial indicators according to animal raising in house hold.	65
3.9	Average counts of bacterial indicators according to cistern location in every house.	66
3.10	Average counts of bacterial indicators according to the type of storage systems.	67
3.11	Average counts of bacterial indicators according to usage of chlorine as disinfectent.	68
3.12	Average counts of bacterial indicators according to storage time in cisterns.	70
3.13	Average counts of bacterial indicators according to storage time in roof storage tanks.	71

ANNEXES

No.	SUBJECT	Page no.
1-	*Questionnaire.*	*85*
2-	*Water borne diseases in Palestine in 1998*	*86*

LIST OF SYMBOLS

WHO: World Health Organization.

CFU: Colony Forming Unit.

TC: Total coliform.

FC: Faecal coliform.

X^2: Chi-quare.

P –value: Probability (Significant Level).

PVC: Poly venyl chloride.

C.I: Cast iron.

MCM: Million Cubic Meter.

ABSRTACT

Two hundred drinking - water samples were collected randomly from two storage systems (cisterns and roof storage tanks). The samples were collected during the summer season from March, 15 to June, 20/2000, from five villages (Tubas, Tammun, El-Fara, Aqqaba and Tayasir) in Tubas district. Both the average total coliform count and faecal coliform count were used as indicators for water quality using the membrane filtration technique. Water samples that were positive for coliform were further cultured for the isolation of *Salmonella, Shigella* and *E.coli* O157: H7.

Both indicators gave average counts higher than that recommended as safe drinking - water by WHO. The average counts of total coliform were 2.056, 20.73, and 16.79 CFU/100ml water in municipality water, rain water, and spring water respectively.

Based on the average count for both indicators, no significant variations were observed on the quality of drinking – water in Tammun, Tayasir and 'Aqqaba villages, but there is significant variation on the quality of drinking-water between these villages (Tammun, Tayasir and 'Aqqaba) on one side and (Tubas and El-Fara') on the other side.

The degree of contamination based on total coliform count in both rain water and spring water were similar and the majority were with first degree of contamination according to WHO classification, while the majority of municipality water samples were with zero degree of contamination (free of contamination).

Based on thermotolerent coliform *E.coli* with respect to the risk levels, both rain water and spring water were within no risk, low risk, and intermediate risk levels, according to WHO classification, while all municipality water samples were with no risk levels.

A noticeable decrease in both indicators is clear up to a distance of 20m and above between cesspits and cisterns, with respect to total coliform count and faecal coliform counts.

The households with history of animal raising, not cleaned cisterns, and no disinfectants were used in storage systems seem to have further effect on the contamination and risk levels compared to finding on households with no history of animal raising, cistern cleaning every year, and use of disinfectants.

During the period from May, 26 to July, 21/2000, one hundred stool specimens were collected from patients with diarrhoea at four Public Medical Units located in Tubas, Tammun, and El-Fara villages.

Prevalence rates of encountered parasites were as follows: *Giardia lambia* (12%), *E.histolytica* (16%), and *H.nana* (4%).

The findings indicated very weak association between drinking water source and intestinal parasites.

Chapter 1

Introduction

1. 1 Introduction

Water is the most important natural resource in the world, since without it life cant exist [1]. Efforts should be made to achieve a drinking water quality as high as practicable. Protection of water supplies from contamination is the first line of defence, and is the best method of ensuring safe drinking-water [2] .

Water sources must be protected from contamination by human and animal waste, which can contain a variety of bacterial, viral, protozoan, and helminthes parasites. Failure to provide adequate protection and effective treatment will expose the community to the risk of outbreaks of intestinal and other infecious diseases. The greatest microbial risks are associated with ingestion of water that is contaminated with human and animal excreta [3].

Potable water is water that can be used safely for domestic purposes, it must have the following characteristics : -

1-Its turbidity, colour and taste must be within acceptable and permissible limits .

2- It must be free of pathogens .

3- Its content of hazardous chemicals must be within permissible limits .

4- It must be neither corrossive nor containing dyeing substances[4].

Microbial risk can never be entirely eliminated, because the diseases that are waterborne may also be transmitted by person –to person contact, aerosols and food intake; thus a reservoir of cases and carriers are maintained [3].

Drinking-water should be suitable for human consumption and for all usual domestic purposes. All people, what ever their stage of development and social and economic condition, have the right to have access to drinking-water in quantities, and of quality equal to their basic needs. Drinking-water should not contain any microorganisms known to be pathogenic–capable of causing disease– or any bacteria indicative of faecal pollution [5].

The recognition that feacally polluted water can lead to spread of microbial infections has lead to the development of sensitive methods for routine examination to ensure that water intended for human consumption is free from faecal contamination, although it is now possible to detect the presence of many pathogens in water, but the method of isolation and enumeration are often complex and time consuming, it is therefore

impracticable to monitor drinking-water, for every possible microbial pathogens[2]. The detection of *Escherichia coli* provides definite evidence of bacterial pollution; in practice, the detection of thermotolerent (faecal) coliform bacteria is an acceptable alternative[5]. The years 1981-1990 has been declared the " *International Drinking Water and Sanitation Decade*" by the united nations. The aim of the decade is the promotion of safe drinking-water and adequate sanitation for the world's population [6].

1 . 2 Water Sources in the West Bank and Gaza Strip.

The Occupied Territories and Israel are dependent on the same water resources, and current use and control of the water is based on Israel's territorial control and political objectives. During the years of Israel's occupation of the West Bank and Gaza Strip, Israel has pursued a policy of taking control of Palestinian water resources for use inside its territory [7].

Since Israeli occupation of the West Bank and Gaza Strip, Israel has exploiting natural Palestinian resources, especially water. Israel has issued several military rules for the water in the West Bank, to diminish the Palestinian control on these resources in order to prevent them from exploiting such resources, for example prohibit drilling of new groundwater wells[8].

Renewable water sources in the Occupied Territories and Israel are all rain fed and comprise groundwater and surfacewater. The ground water sources consist of underground aquifers and subaquifers which are recharged by rainwater and underground flows between the basins. The surfacewater sources consist of perenial and seasonal river and lakes, the Jordan River and its tributaries, and lake Tiberies being the major ones [7].

It is well documented that the total annual water consumption of the Palestinians in the West Bank and Gaza Strip (with a population of nearly 2.5 million) is 250 million m^3 (mcm), which gives an average of 100 m^3 / capita / year. While the anual Israeli consumption (with a population of nearly 5 million) is about 2 milliard m^3, which gives an average of 400 m^3 / capita / year, which is four times as much as the Palestinian indivisual consumers [9].

1. 2 . 1 Water Supply System in the West Bank

After the Israeli occupation in 1967, the West Bank has been administratively divided into eight districts, namely: Jenin, Tulkarm, Ramallah, Nablus, Jerusalem, Bethlehem, Hebron and Jerico [8]. Four main types of water resources are utilized in the West Bank for drinking purposes: piped municipal supplies, rainfed cisterns, springs, and canals [6].

These districts depend on water – net work supply systems (piped water) and household rainwater; cisterns, and communal sources (unpiped water) [9]. Table 1.1 shows supply systems in various districts.

Table 1.1 cities and villages with piped and unpiped water systems. *

No	District	No . of Cities and Villages	Cities & Villages with piped water	Cities & Villages with unpiped water
1	Jenin	76	34	42
2	Tulkarm	78	41	37
3	Nablus	65	34	31
4	Ramallah	99	87	12
5	Bethlehem	59	39	20
6	Hebron	114	46	68
7	Jericho	13	9	4
	Total	504	290	214

*After : Al – Kahah [9].

Out of 504 listed cities and villages with the various districts, 214 villages are without piped water supply system, which comprize 42% of the total number of cities and villages which contain about 30 – 35 % of the total population [9]. Most of these villages rely on the following systems for their water supply: House hold rain water (cistern), and communal sources as wells, springs and canals. Where communal sources are used, water is transported to homes by tankers and animals [8].

At present most of out-water network supply depend on, private wells (35.8%), Mekorote systems (27.6%) and West Bank department wells or springs (36.6%) [9].

The use of water in the West Bank is mainly for domestic, agriculture and industrial purposes. The total pumped quantities of water among these, from wells, springs and Mekorote resources are bout 30 – 31 mcm / year, 22 mcm from wells, 4.5 mcm from springs and 4–5 mcm from Mekorote [8].

The average daily consumption of water for domestic and industrial sector in the West Bank was estimated as follows: 100 – 120 liters / capita / day in cities and towns and 40 – 60 liters / capita / day in villages [9].

1.2.2 Water Supply Systems in Tubas District

In 1997, the Palestinial National Authority devided the West Bank area into eleven districts, by considering Qalqillia, Salfeet and Tubas as new districts. Based on this new division, several villages, that were belonged to Jenin and Nablus districts were transferred and connected to Tubas district. According to this, the number of villages around Tubas city was 23 with a total population of 36609. Figure (1.1) shows Palestenian map including the study area (Tubas district) [10]. Out of 23 villages within this district only 7 are connected to water network system and the rest still dependent on private systems (cisterns). It is worth note , that most areas with water network system are also have private water supply systems (cisterns).

1.2.3 Water Supply Systems in Tubas, Tammun, Tayasir, Aqqaba and El – Fara' Villages.

Tammun, Tayasir, Aqqaba and El-Fara' all are situated around Tubas city. They are about 3 – 7 km far from the city (Figure1.2) [10]. Tubas city is located in the nothern part of the West Bank, halfway between Nablus and Jenin cities.

Only Tubas, Aqqaba and El – Fara' are connected to water network. Tammun and Tayasir both are dependent on cistern water supply.

All of the five villages are not connected to sewage systems, and all are dependent on septic tanks to get rid of sewage.

Table 1.2 shows populations, buildings, cisterns, cesspits and other services in the five villages.

Table 1.2 Population, building, cisterns, cesspits, and other services in the five villages.*

Village	No. of population	No . of buildings connected with electricity net work	No . of buildings connected to water net work	No. of cisterns	No .of cesspits	No . of buildings connected to sewage system
Tubas	11771	1944	1903	1620	1912	-
Tammun	7640	1442	-	1210	1084	-
Aqqaba	4443	702	608	557	600	-
El-Fara'	4207	679	675	20	673	-
Tayasir	1754	313	-	261	247	-

* **After:** Palestinian Central Bureas of Statistics, 1999 **[10]**.

1.3 Water Storage Systems

Out of five villages studies only Tubas, El-Fara', and Aqqaba villages depend on water net work supply (piped water supply), and the rest (Tammun and Tayasir) still depend on householder rainwater, cisterns and communal sources as wells and springs (unpiped water supply).

The average rainfall in the area was 415 mm / year, which occurs predominantly between the month of October and April. Table **(1.3)** shows the average of precipitation in Tubas area (1969 – 2000) .

Table **1.3** Average of precipitation in Tubas area *.

Year	Mm / Year	Year	Mm / Year
1969/1970	323.2	1985/1986	365.5
1970/1971	351.4	1986/1987	558
1971/1972	509.3	1987/1988	522
1972/1973	282	1988/1989	247
1973/1974	583.1	1989/1990	293
1974/1975	373.3	1990/1991	363
1975/1976	364.5	1991/1992	921.5
1976/1977	383.6	1992/1993	462
1977/1978	284.5	1993/1994	462.5
1978/1979	222	1994/1995	444.5
1979/1980	644	1995/1996	378.5
1980/1981	345.5	1996/1997	508.5
1981/1982	226.9	1997/1998	514
1982/1983	624.5	1998/1999	184.5
1983/1984	374	1999/2000	385.7
1984/1985	364		

*After : Ministry of agriculture – Tubas .

1.3.1 Rainfed Cisterns

Rainfed cisterns are the main drinking–water source for households without a piped supply. In some areas they remain a preferred drinking water source, even when a piped supply has been installed. The taste of the cool cistern water may be preferred to the warm, chlorinated and some times slightly saline municipal supply. Cistern water also lathers better for washing **[6]**.

Cisterns are artificial reservoirs for collecting and storing rain water from impermeable area. For a long time, Palestinians have been constructing cisterns to collect and store the rainfall from roofs of their houses. These days, and inspite of the availability of water distribution systems in most cities and villages of the West Bank, people continue collecting rainwater in water cisterns, and this is due to the lack of contineous network water supply systems in addition to the limited winter season. Many cisterns, especially those that were constructed more than fifty years ago, have a pear shape that is usually pasted with a relatively thin layers of cement.

Currently, cisterns are designed and built with international standards and most municipalities encourage rain forced concrete cisterns

of a various shape and size. In general, construction of cisterns are socially acceptable, relatively cheap, environmental safe and don't require special permissions [8].

Water is channelled from the catchment to the cistern via pipes or open channels. In either case, it is normal practice not to collect the first flush of rain water after a dry spell, in order to avoid the washing of accumulated dirt from the catchment into the cistern. Various kinds of cloth and fibre filters are often used to strain solids from the collected water. Two main methods of water removal from cisterns are used :-

1- Water may be removed by a bucket.
2- Water may be removed by small electric pumps [6].

Possible sources that affect the bacterial quality of water in cisterns include: presence of cesspits, the distance between cesspits and cistern, the level of cesspit with respect to the cisterns, animal raising, water sources (catchment area), age of cistern, washing of cistern, styles of cisterns and treatment method [8].

Figure (1.3) Show main elements in the rainfed cistern collection system [6].

1.3.2 Roof Storage Tanks

A storage tank is commonly used to ensure water availability for the family needs [9]. Water is stored in a variety of ways in the West Bank households. The main kinds of containers used include the following: metal storage tanks, cement storage tanks, plastic storage tanks , ceramic jars, jery cans and plastic or glass bottles [6].

Most roof storage tanks are supplied with secure covers and usually filled on metal stands [8].

1.4 Persistence of Water Pathogens

After leaving the body of their host, pathogens and parasites gradually lose viability and the ability to infect. The rate of decay is usually exponential, and a pathogen will become undetectable after a certain period. Pathogens with low persistence must rapidly find a new host and more likely to be spread by person – to – person contact or faulty personal or food hygiene than by drinking-water. Because faecal contamination is usually dispersed rapidly in surface water, the most common waterborne pathogens and parasites are those that have high infectivity or possess high resistance to decay outside the body [3].

The persistence of most pathogens in water is affected by various factors, of which sunlight and temperature are among the most important [2]. Decay is usually accelerated by increasing temperature of water and may be mediated by the lethal effects of ultraviolet radiation in sunlight acting near the water surface.

Viruses and the resting stages of parasites (cysts, oocysts, ova) are unable to multiply in water, conversely relatively high amounts of biodegradable organic carbon, together with warm temperature and law residual concentrations of chlorine, can permit growth of *Legionella, Naegleria fowleri, Acanthamoeba,* the opportunistic pathogens *Pseudomonus aeruginosa and Aeromonus,* and nuisance organisms during water distribution [3]. Table **1-4** shows the usual persistence time of some excreted pathogens in fresh water at 20 – 30°C [8].

Table **1.4** The usual persistence time of excreted pathogens in fresh water at 20 – 30°C *

Type of pathogen	Survival time in days in fresh water
Viruses – *Enteroviruses*	< 50
Bacteria	
- Faecal coliforms	< 30
- *Salmonella spp*	< 30
- *Shigella spp*	< 10
- *Vibrio cholerae*	< 5
Protozoa – *Entamoeba histolytica cysts*	< 15
Helminthes – *Ascaris lumbricoides eggs*	Many months

***After**: Othman [8].

1.5 Infective Dose of Water Pathogens

Infectious dose (ID) is the minimum number of organisms required to cause infection and varies considerably by type of organism. In general , enteric viruses and protozoa have low infectious doses. Bacterial pathogens tend to require a larger dose to cause infection.

Median infectious dose (ID_{50}) is the number of organisms resulting in 50% infection rate **[11]**.

Ingestion of large numbers of pathogens on a single occasion of contaminated drinking-water is relatively small and this is mainly due to the fact that enteric pathogens can not normally multiply in water and they also have the tendency to disperse.

If polluted water is permitted to contaminate food, bacterial pathogens can multiply to produce very large doses and hence, infection through such contaminated food will be more serious**[3]**.

After ingestion of the pathogen, the development of an infection depend on the balance between host factors, such as age, sex, state of health, nutritional status, gastric acidity and intestinal immunity tending to remove it, and factors aiding the bacteria in their attempt to colonize the intestine, such as the possession of colonization and adhesions factors**[2, 3]**.

1.6 Water-Related Modes of Transmission of Infectious Agents

Water-related diseases include those caused by various pathogenic microorganisms associated with water such as: viruses, bacteria, protozoa, and helminthes, those related to chemical contaminates of water (flouride, heavy metals and nitrate), and those classified as non communicable diseases, due to lack of sufficient water for hygiene**[3]**.

The classification of water–related diseases by Bradley provides available framework for understanding the relationship between infectious disease transmission and water. This classification system facilitates planning effective prevention and control measures for a variety of water-related diseases, depending on the type of agent and type of transmission route involved.

Bradly described four main categories of water related infections: water-borne infections, water-washed infections, water-based infections and infections with water – related insect vectors [11].

1.6.1 Water – borne infections

Water-borne infections are caused by pathogens present in drinking- water, the source of these pathogens are excreta and sewage. Examples of water–borne diseases are: Typhoid, Cholera, Shigellosis, Giardaisis and Hepatitis [8].

Transmission of water-borne infection occurs by ingestion of contaminated water and depens on:

1- The concentrations of pathogen in water, which is determined by the number of infected persons in the community, the amount of faecal contamination in water, and the survival of the organism in water.
2- The infectious dose of the organism.
3- Individual ingestion (exposure) of the contaminated water.

The control of these infections is generally through improvement of microbiological water quality, by water treatment and/or source protection [11].

1.6.2 Water – washed infections

Water-washed infections are diseases due to poor personal and / or domestic hygiene. These diseases are not due to the presence of infectious agents in water, but rather are due to the lack of readily accessible water, which limits washing of hands and utensils and thus permits transmission of infectious agents, such as *shigella* species, by faecally contaminated hands and utensils [11].

Water-washed infections may be divided into the following three groups:-

1- Diseases transmitted by faecal–oral route such as hepatitis A, bacillary dysentry, and many diarrhoeal diseases.
2- Infections of the skin and eyes, such as trachoma, skin infections and fungal skin diseases.
3- Infections carried by lice or mites, such as scabies (mites), and louse–borne epidemic typhus (caused by *Rickettsia prowazeki* and transmitted largely by body lice) [5].

Control of these diseases is through provision of water for domestic purposes in adequate quantities and quality, closer and easier access to water, and education to improve personal and domestic hygiene [**5,11**].

1.6.3 Water – based infections

Water – based infections are worm infection in which the pathogen must spend a part of its life cycle in the aquatic environment.

Water–based infections are subdivided into those diseases acquired by ingestion of water and those diseases acquired by contact with water.

The prototype illness of water – based infections are:

1- Dracontiasis, which is due to the ingestion of water contaminated with guinea worm.
2- Schistosomiasis, which is transmitted by contact with water contaminated with species of the trematode genus *Schistosoma*.

Control of dracontiasis and schistosomiasis is through protection of the water source and the user by limiting skin contact with water and by eradication of intermediate hosts .

The types of water contact diseases most frequently encountered in developed countries are those associated with recreational water exposure to contaminated marine water, freshwater lakes, ponds, creeks, or rivers or treated water in swimming pools, wave pools, hot tubes and whirlpools. While many recreational water outbreaks are associated with ingestion of water, there are some diseases of the ear, eye and skin that are associated with actual water contact, as well as systemic illness associated with penetration of pathogen through an open wound or abrasion. Illness from recreational water contact can be due to enteric organisms in faecally contaminated water.

Outbreaks of recreational water have involved *Giardia* and *Cryptosporidium* species, *Shigella sonnei*, and *Escherichia coli* O157: H7 that presumably entered the gastrointestinal tract via ingestion. Other recreational water outbreaks of indigenous aquatic organisms such as *Naegleria, Pseudomonus, Legionella spp*, and several *Vibrio spp* and several *Mycobacterium spp*.

Epidemiological and microbiological studies indicate that *Staphylococcus aureus* skin and ear infections are often associated with recreational use of water[**11**].*Vibrio vulnificus* can cause serious wound infections when an injury to skin occurs in marine water.

Cynobacterial toxins have been associated with contact irritation after bathing in marine waters or fresh water.

Additional causes of recreational water infection are the *Leptospira spp* which are neither enteric nor aquatic organisms but enter water via the urine of infected domestic and wild animals [9].

1.6.4 Water – vectored infections

Water – vectored infections are those transmitted by insects which breed in water, such as mosquito vectors of malaria, or insects which bite near water, like the tsetse flies that transmit sleeping sickness .Control of these infections is through the applied of pesticides, destruction of breeding grounds, and construction of piped water supply[11].

1.7 Waterborne Pathogens

Infectious diseases caused by pathogenic bacteria, viruses, and protozoa or by parasites are the most common and widespread health risk associated with drinking-water.

Infectious diseases are transmitted primarily through human and animal excreta, particularly faeces. If there are active cases or carrieres in the community, then faecal contamination of water sources will result in the causative organisms being present in water. The use of such water for drinking or for preparing food, contact during washing or bathing and even inhalation of water vapor or aerosols may result in infection.

Pathogenic agents have several properties that distinguish them from chemical pollutants:

1- Pathogens are discrete and not in solution.
2- Pathogens are often clumped or adherent to suspended solids in water, so that the likelihood of acquiring an infective dose can't be predicted from their average concentration in water.
3- The likelihood of successful challenge by a pathogen, resulting in infection, depends upon the invasiveness and virulence of the pathogen, as will as upon the immunity of the individuals.

Because of these properties there *is no tolerance lower limit* for pathogens, and water intended for consumption, for preparing food and or for personal hygiene should thus contain no agents pathogenic for humans.

Pathogen – free water is attainable by selection of high – quality uncontaminated sources of water, by efficient treatment and disinfecting of water known to be contaminated with human and animal faeces, and

by ensuring that such water remains free from contamination during distribution to the user [3].

1.7.1 Agents of significance

Not all potentially waterborne human pathogenes are of equal public health significance [2]as shown in table 1.5.

Table 1.5 Waterborne pathogens and their significance in water supplies. *

Pathogen	Health significance	Main route of exposure[a]	Resistance to chlorine [b]	Relative infective dose [c]
Bacteria				
- *Camplylobacter jejuni*	High	O	Low	Moderate
- *Pathogenic E. coli.*	High	O	Low	High
- *S. typhi .*	High	O	Low	High
- *Other Salmonellae .*	High	O	Low	High
- *Shigella spp.*	High	O	Low	Moderate
- *V. cholerae .*	High	O	Low	High
- *P. aeruginosa.*	Moderate	C	Moderate	High (?)
- *Aeromonas spp.*	Moderate	O.C	Low	High (?)
Viruses				
- Adenoviruses	High	O.I.C	Moderate	Low
- Enteroviruses	High	O	Moderate	Low
- Hepatitis A	High	O	Moderate	Low
- Hepatitis E	High	O	?	Low
- Norewalk virus	High	O	?	Low
- Rotavirus	High	O	?	Moderate
- Small round viruses	Moderate	O	?	Low (?)
Protozoa				
- *Entamoeba histolytica*	High	O	High	Low
- *Giardia intestinalis*	High	O	High	Low
- *Cryptosporidium parvum*	High	O	High	Low
Helminths				
- *Dracunculus medinensis*	High	O	Moderate	Low
- *Schistosoma spp*	Moderate	C	Low	Low

[a] O: oral (ingestion); I: inhalation in aerosol; C: contact with skin.

[b] Resistance to chlorine:

1-low: agent completely destroyed.

2- Moderate: agent may not be completely destroyed.

3- ?: not known or uncertain.

[c] Dose required to cause infection in 50% of healthy adult.

*After: world Health organization, 1997[2].

Agents of significance are divided into:

1. Agents of high health significance:

Agents of high health hazard are those that cause serious risk of disease when ever present in drinking-water, these include:

a. Bacterial agents, such as: *Salmonella spp, Shigella spp,* Pathogenic *E.coli, V. cholera, Yersinia, Enterocolytica and Campylobacter spp.*
b. Viral agents, such: *Adenoviruses, Enterovirses, Hepatitis A, Hepatitis non A non B, Hepatitis E, Norewalk virus, Rotavirus, and small round viruses.*
c. Protozoal agents, such as: *E.histolytica, G.intestinalis, and C. parvum.*
d. Helminth agent, such as: *Drocunculus medianesis.*

Most of these pathogens are distributed world wide, and their elimination from water intended for drinking has high priority**[3,12]**.

2. Opportunistic pathogens:

Opportunistic pathogens are naturally present in the environment and not formally regarded as pathogens. They are able to cause disease in people with impaired local or general defense mechanisms, such as the elderly or the very young, patients with burns or extensive wounds, those undergoing immunosuppressive therapy, or those with acquired immune-deficiency syndrome (AIDS) **[3]**.

Water used by such patients for drinking or bathing, if it contains excessive numbers of these agents may produce a variety of infections involving the skin and mucous membranes of the eye, ear, nose and throat **[2]**.

Examples of such agents are *P.aeruginosa* and species of *flavobacterium, Acinetobacter, Klebsiella, Serratia, Aeromonas,* certain "slow growing" mycobacteria, *Legionella, Naegleria fowleri and Acanthameeba spp***[6, 3]**.

These organisms while clearly of medical importance, only require public health significance under certain conditions. Their removal from drinking- water may therefore be given moderate priority **[6]**.

3-Nuisance Organisms:

Nuisance organisms have no public health significance. However,

they produce problems of turbidity, taste and odour or appear as visible animal life in the water [13]. As well as being aesthetically objectionable, they indicate that water treatment and the state of maintenance repair of the system are defective.

The only positively identified health hazard from animal life in drinking-water arises with the intermediate stage of the guinea worm, *Dracunculus medinensis*, which parasites the water flea, *cyclops* [3].

4- Toxins from Cynobacteria

Blooms of *Cynobacteria* (commonly called blue-green algae) occur in lakes and reservoirs used for potable supply. Three types of toxin can be produced, depending upon species;

a. Hepatotoxins, produced by species of *Microcystis, Oscillatoria, Anabaena and Nodularia,* typified by microcystin LR: R, which induce death by circulatory shock and massive liver haemorrhage within 24 hours of ingestion.
b. Neurotoxins, produced by species of *Anabaena, Oscillatoria, Nostoc, Cylindrospermum* and *Aphanizmenon.*
c. Lipopolysaccharides [3].

1.7.2 Epidemic water borne disease:

1- Bacteria

A: Salmonella

Salmonella are members of the family Enterobacteriacea, they are gram negative bacilli growing aerobically and anaerobically at an optimum temperature of 37°C, readily killed by temperature above 55°C, they may be isolated from the intestine of man and animals and from food of animal origin [14].

The genus *Salmonella* was named in 1900 after the American veterinarian Dr Salmon who was the first to describe a member of the group *S. cholerasius* [15].

Contamination of drinking-water with *Salmonella* may cause outbreaks of gastroenteritis or typhoid. The source of the bacteria causing gastroenteritis may be either human or animal faeces. Severe gastroenteritis resulting in acute diarrhea and vomiting may also caused by *S.paratyphi*, which is transmitted only by humans or by other *Salmonella* carried by animals [9].

Salmonella are excreted in the faeces of infected humans and animals. Faecal contamination of groundwater, surfacewater, as well as insufficiently treated and inadequately disinfected drinking–water, are the main causes of epidemic waterborne outbreaks caused by *Salmonella ssp.*

Salmonella can be found in open wells as a result of the drainage or folding of contaminated surfacewater into unprotected well shafts. It is uncommon for *Salmonella* to be isolated from piped water supplies, whether treated or untreated.

Penetration of pathogens into water sources must be avoided by protection of groundwater and surfacewater catchment area **[2]**.

In the early development of taxonomic schemes, determinant biochemical reactions were used to separate *salmonella* into separate groups. The Kaufmann-White scheme stands predominetly as the first attempt to systemically classify *Salmonella* by using these specific scientific parameter**[12]**. At present, more than 2300 serovars are known to exist based on the 67 O – antigen groups and the numerous H-antigens **[16]**.

The virulence of *salmonella spp*, depends on serotype and strain specificity in host range and infective dose and on host status. *S.typi* is a specific human pathogen. In particular, *S.typhi, S.paratyphi A, and S. paratyphi B* are able to invade tissues and cause septicemia with high temperature rather than diarhoea. This is known as enteric fever **[10]**.

B:Shigella

Shigella are gram-negative, non-spore forming, non–motile rods, capable of growth under both aerobic and anaerobic conditions. Metabolism is both respiratory and fermentative; acid, but not usually gas, is produced from glucose. Catalase is usually produced, except by *Shigella dysenteria* type 1, but oxidase is produced by one sereotype only. Nitrates are reduced to nitrites **[2]**.

Shigella can be differentiated from *Salmonella* by the absence of motility and by inability of *Shigella* to produce hydrogen sulfide. *Shigella* is aerobic and grows well on nutrient media at 37°C. Neither *Salmonella* nor *Shigella* ferment lactose. This characteristics is used to differentiate them from *E.coli* **[9]**. *Shigella* are serotyped on the basis of their somatic O antigenes.

Shigella have no natural hosts other than higher primates, and humans are the only effective source of infection. Of the enteric bacterial pathogens, *Shigella* seem to be the best adapted to cause human disease. Direct transmission between susceptible individuals is the usual route of infection, and the infective dose is lower than for other bacteria[**2**].

The isolation of *Shigella* from drinking-water indicates recent human faecal contamination and is of crucial public health significance. Contamination of drinking-water or food with faeces containing *Shigella* causes epidemics of bacillary desentry. *Shigella* is execreted in faeces and urine during the active phase of the disease, *Shigella* does not grow in natural waters and rarely survives more than 10 days.

Shigella is able to resist gastric acidity, this allow very small inoculum to cause disease. *Shigella* invade the epithelial cells of the large bowel, causing a dysentery- like syndrome [**9**].Symptoms of shigellosis may vary from a mild transitory diarrhea to severe prostrating attacks accompanied by high temperatures, vomiting, and profuse bloody stool **[13]**.

C: V. cholerae

Vibrio species are motile, non spore forming, slightly curved gram-negative rods with a single polar flagellum: they are both aerobic and facultatively anaerobic. Their metabolism is both respiratory and fermentive without the production of gas. Both catalase and oxidase are formed, and nitrates are reduced to nitrites.

Among the vibrios, special attention has focused on the identity of those causing cholerae. *V.cholerae,* is divided into more than 80 serological types on the basis of the O or somatic antigens.

The isolation of *V. cholerae* O1 from water used for drinking is of major public health importance and is evidence of faecal contamination [**2**].

The diarrhea of cholerae may be very severe. The production of a powerful enterotoxin by the organism in the small intestine affects the mucosa in such a way that there is a profuse outpouring of fluids. The stool appears watery and gray and may contain flecks of mucin, giving the appearance of " rice water". The disease usually runs its course in 3 to 5 days in less severe infections; however, due to fluid and electrolyts loss, rapidly progressing dehydration, shock, and death may result **[17]**.

Studies with human volunteers show that the acidity of the stomach is responsible for the large inoculum needed to initiate cholerae. Although the ingestion of 10^8- 10^9 cholerae vibrios is generally required to cause cholerae, human volunteers given bicarbonate to neutralize gastric acidity developed the disease when only 10^4 cells were administrated [9].

Control of cholera depends primarily on satisfactory sanitation measures, particularly in the treatment of sewage and the purification of drinking water [18].

D: E. coli

E. coli is a gram–negative, non- spore forming, rod–shaped bacterium which can be either motile or nonmotile, growth is aerobic or facultatively anaerobic. Metabolism is both respiratory or fermentative; acid is produced by the fermentation of glucose and lactose. Catalase is produced but not oxidase, and nitrates are reduced to nitrites.

Serological typing are based on the somatic O antigens, the capsular K antigens, and the flagellar H antigens.

E.coli is found in large numbers in the faeces of humans and of nearly all warm – blooded animals; as such it serves as a reliable index of recent faecal contamination of water [2].

Most strains of *E. coli* are non pathogenic. However, subtypes able to cause gastroentestinal disease which is characterized by a profuse watery diarrhea with little mucous and no blood [2, 19].

Four classes of pathogenic *E.coli* responsible for diarrhea are recognized: enteropathogenic (EPEC), enteroinvasive (EIEC), enterotoxigenic(ETEC), and verocytoxin producing (VTEC).

Enterohemmorhagic *E.coli* O157: H7 is a pathogenic strain of *E.coli* that produces two potent toxins. This organism causes bloody diarrhea, and 2-7 % of infections result in hemolytic uremic syndrom, in which the erythrocytes are destroyed and the kidneys fail. Children and the elderly are the most susceptible to hemolytic uremic syndrome complications [11].

E.coli O157:H7 is an EHEC which was first isolated in 1975 in the USA from a woman with grossly bloody diarrhea [20].

In 1982 , *E.coli* O157: H7 has bee recognized as a causative agent of haemorrhagic colities in the USA, Canada, and the United Kingdom **[21, 22].**

Two waterborne outbreaks of *E.coli* O157: H7 have been reported in the United States. One out break was associated with drinking-water in a Missouri community in 1989, of the 243 people affected, one–third had bloody diarrhea, 32 were hospitalized, 2 had hemolytic uremic syndrome and 4 died. Unchlorinated well water and breaks in the water distribution system were considered to be contribution factors. The other waterborne outbreaks, 80 cases, was associated with recreational water exposure to a lake in Oregon in 1991. Faecal contamination from swimmers and poor water exchange in lake were believed to be contributing factors. The infectious dose is low, and incubation period is 12-60 hour **[11].**

E: Pseudomonas aeruginosa

Pseudomonas is a member of the family pseudomonadaceae and is a monotrichate, gram – negative rod. It can be recognized by its production of a blue – green fluorescent pigment (pyocyanin).

P. aeruginosa is commonly found in faeces, soil, water and sewage but can't be used as an indicator of faecal contamination, since it is not invariably present in faeces and sewage, and may also multiply in the enriched aquatic environment and on the surface of unsuitable organic materials in contact with water.

P. aeruginosa is an opportunistic pathogen. Most of the illness in humans for which it is responsible are caused, not by drinking-water, but by contact with it .Water containing these bacteria may also contaminate food, drinks, and pharmaceutical products, causing them to deteriorate and to act as secondary vehicle for transmission **[2].**

P. aeruginosa was found to be more resistance than acid-fast bacteria during ozonation process, which demonstrates its resistance to chemical disinfection and thus its usefullness in analysis of recreational waters which receive chemical disinfection **[11].**

Waterborne infections are usually associated with warm, moist environments; they include the skin rashes and pustules or outer ear chanal infections (otitis externa) reported in users of indoor swimming –

pools and whirlpools, where bacterial counts are high and disinfection is deficient.

The presence of this organism in water supplied to hospitals and for the manufacture of pharmaceutical preparations and dressings is a matter of concern because *P. aeruginosa* is a common pathogen in infections of wounds and burns and has caused serious eye infections after the use of contaminated eye drops.

The presence of this organism in potable water also indicates a serious deterioration in bacteriological quality, and is often associated with complains about taste, odour, and turbidity linked to low rates of flow in the distribution system and arise in water temperature **[2]**.

F :Cyanobacteria

Cyanobacteria (blue–green algae) occur naturally in fresh and brackish waters. Although these are not infectious agents, some species produce toxins during algal blooms, which are triggered by nutrient enrichment from natural water, agricultural fertilizer runoff, or domestic or industrial effluents.

Acute health effects in humans include gastroenteritis, liver damage, nervous system damage, pneumonia, sore throat, earache, and contact irritation of skin and eyes. It has been suggested that high rates of liver cancer in parts of China may be linked to cynobacterial hepatotoxins in drinking-water. A 1976 outbreak of intestinal illness in Pennsylvania was associated with cyanobacterial bloom in municipal water supply and affected 62% of populations. One outbreak of hepatoenteritis in the Palm Island Aboriginal Settlement on the Australian north east coast in 1979 affected 138 children and 10 adults, the majority of whom required hospitalization. The out break was linked to a dense algal bloom in the drinking water reservoir for the island that was treated with drinking copper sulfate, which caused lysis of *Cyanobacteria* and consequent release of toxins **[11]**.

Control of *Cyanobacteria* is problematic, because several studies indicate that the toxins can remain potent for days after the organisms have been destroyed by copper sulfate or chlorination. Only activated carbon and ozonation appear to remove or reduce toxicity **[11, 3]**.

2. Viruses

The viruses of greatest significance in the waterborne transmission infectious disease are essentially those that are multiply in the intestine of humans and are excreted in large numbers in the faeces of infected individuals. Discharges of sewage and human excreta constitute the main source of human enteric viruses in the aquatic environment **[2]**.

The enteric viruses are recently recognized waterborne pathogens. Depending on the virus, diagnosis of these infections may be by commercially available enzyme immunoassays for antigens and antibodies, electron microscopy, tissue culture, or molecular methods such as PCR and probe hybridization.

Unlike the case of enteric bacteria, concern of waterborne transmission of enteric viruses is generally limited to the strains that have humans as their natural reservoir. The infectious dose of these agents is low, typically in the range of 1-10 units, **[11]**.

Although viruses can't multiply outside the tissues of infected hosts, enteric viruses tend to be more persistent in the aquatic environment than most enteric bacteria, their survival depend on numerous physical, chemical , and microbial characteristic of the water as well as the virus type. Their prolonged survival times and small size enable viruses to move great distance in soil and water **[11, 2]**.

Enteric viruses can't multiply in the environment. Cultivable enteric viruses have been detected in surfacewaters, groundwater, and treating drinking-water. Viruses recovery from water samples is relatively poor, and currently many enteric viruses cannot be cultured in vitro **[11]**.

Inactivation and / or removal of enteric viruses by water treatment process varied by virus type and treatment conditions**[18]**.

From 1983 to 1992, there were 10 reported drinking-water outbreaks associated with viral agents in the United States. These outbreaks involved over 6,000 total cases, and all were attributed to the ingestion of untreated or inadequately treated groundwater **[11]**.

Viruses are the smallest obligate intracellular parasites. They range in size from 20-300 nm. There are more than 100 types of enteric viruses that are excreted in faeces and infect gastroentestinal tract and other parts of the human body **[9]**. Table **(1-6)** shows viruses pathogenic to humans which can occur in polluted water and diseases attributed to them **[2]**.

Table (1-6) Viruses pathogenic to humans which can occur in polluted water and diseases attributed to them*

Virus family	Members	No.of serotypes	Disease caused
Picornaviridae	Human polioviruses	3	Paralyses, meningitis, fever
	Human echoviruses	32	Meningitis,respiratory disease,rash,fever, gastroenteritis
	Human coxsackieviruses A1-22.24	23	Enteroviral vesicular pharyngitis,respiratory disease, meningitis,enteroviral vesicular stomatitis with exanthem (hand, foot and mouth disease)
	Human coxsackieviruses B1-6	6	Myocarditis,congenital heart anomalies,rash,fever,meningitis , respiratory diseases, epidemic myalgia(pleurodynia)
	Human enteroviruses 68-71	4	Meningitis,encephalitis, respiratory disease, rash, acute enteroviral haemorrhagic conjunctivitis, fever
	Hepatitis A virus	1	Hepatitis A
Reoviridae	Human reoviridae	3	Unknown
	Human rotaviruses	5	Gastroenteritis, diarrhoea
Adenoviridae	Human adenoviruses	41	Respiratory disease, conjunctivitis, gastroenteritis
Parvoviridae	Adeno-associated viruses	4	Latent infection following integration of DNA in to the cellular genome
Caliciviridae	Human caliciviriuses	5	Gastroenteritis in infants and young children
	Small round structured viruses(including Norewalk virus)	14	Gastroenteritis, acute viral gastroenteropathy(winter vomiting disease)
Caliciviridae(?)	Hepatitis E virus	?	Hepatitis E
Unknown	Astroviruses	1	Gastroenteritis,neonatal necrotizing enterocolitis
Papovaviridae	Papillomaviruses	2	Plantar warts

*After : World Health Organization [2].

A: Group B Rota virus

Group B rota virus was first reported in connection with a waterborne outbreak in China in 1984 and differs from group A rota viruses strains that commonly cause pediatric diarrhea.

Group B rota virus infections occur more frequently in adults than in children, are associated with severe, cholera –like illness, and have reported mainly in China **[11].**

Diagnosis is by electron microscopy and genome electrophoresis or by enzyme immunoassay. Many outbreaks involves tens to thousands of cases were attributed to faecally contaminated water. Rotaviruses have been detected in sewage, rivers, and lakes and in treated drinking-water in some countries **[11, 2].**

Transmission occurs via the faecal-to-oral route. The infection is usually associated with sporadic cases, but several large waterborne outbreaks have been well documented.

The Rotaviruses are of considerable public health importance as a common cause of acute diarrhoea, particularly in young children. They infect and multiply in mature or diffrentiated enterocytes located on the villi of the duodemun and small intestine, and are excreted in large numbers, as many as 1000 virus particles may be present per gram of faeces for approximately 8 days after the onset of symptoms **[2].**

B: *Hepatitis E virus*

HEV is the only known agent of enterically transmitted non-A, non-B infectious hepatitis. HEV is a single serotype of virus that has single –stranded, positive – sense RNA. HEV is believed to be either new RNA virus or a member of *caliciviridae* family.

In contrast to Hepatitis A virus, the majority of cases in epidemics occur among young adults, and there is a high case fatality rate among pregnant women.

Large outbreaks involving thousands of cases have been reported in developing areas in Africa, Asia, and Mexico and have been linked to faecally contaminated water and inadequate chlorination. In 1991, the largest documented waterborne HEV outbreak affected an estimated 79,000 persons in Kanpur, India, and was associated with contaminated surfacewater **[11].**

HEV cause infections of the liver typically accompanied by lassitude, anorexia, weakness, nausea, vomitting, headache, abdominal discomfort, fever, dark urine, and jaundice [2].

HEV has been detected in polluted rivers and in drinking-water. Control of infectious hepatitis is dependent on adequate treatment of sewage and water. Viruses are more resistant to chlorination than bacteria[23, 9].

C: *Coronaviruses*

Coronaviruses are pleomorphic, enveloped RNA viruses that are established causes of diarrhea in animals. They were first observed in faeces of persons with gastroenteritis, by electron microscopy in 1975.

Epidemiological evidence suggests fecal–oral transmission and personal hygiene may be key factors in transmission, since several studies noted that the highest prevalence rates were among population with low socioeconomic status and poor personal hygiene [11].

D: *Norewalk like viruses*

NV and related small round structured viruses are the leading cause of epidemic viral gastroenteritis in older children and adults. Numerous NV and small round structured virus outbreaks linked to drinking-water, recreational water, ice, shellfish, various food items and environmental contamination have been documented.

NV has been proposed as a member of *caliciviridae* family which includes Snow Mountain agent, Hawaii agent, and Taunton agent.

In USA 50-70% of adults have NV antibodies by the fifth decade of life, these high rates of antibody prevalence indicate that much of population is frequently exposed to NV viruses. NV and related viruses may be responsible for 23% of waterborne outbreaks of active gastroenteritis [11].

Norewalk agent infects the villi of the jejunum. Virus shedding in stool occurs during the first 72 hrs after the onset of illness. The virus is transmitted by the faecal-to-oral route. Of all Norewalk–related outbreaks, water seems to be responsible for about 40%, the type of water involved including drinking -water supplies, recreational bathing water, and shellfish –harvesting water [2].

E: *Adenoviruses*

Adenoviruses generally infect conjunctival, respiratory, intestinal, epithelial and lymphoid tissue. Several species have been isolated from sewage, rivers, lakes, groundwater and water used for drinking and swimming. Waterborne transmission occurs by the faecal –to-oral route ,by inhalation of adenovirus aerosols into the lower respiratory tract, and by eye contact when the cojunctival surface is mildly irritated **[2]**.

Adenoviruses are found in small numbers in faeces of some patients with diarrhea, but occasionally in great numbers.

Of the various species of adenoviruses, two (type 40 and 41) can't replicate in cell culture and are called fastidious variants**[9]**.

3:Protozoa

Intestinal parasitic infections are important public health problems through out many parts of the world especially in underdeveloped countries. It was estimated that 60% of the world population in 1998 were infected with intestinal parasites. It is known that the infections of intestinal parasites flourish wherever poverty, inadequate sanitation, insufficient health care, over crowding, and lack of clean water to maintain personal hygiene **[24]**.

Drinking-water plays a major role in the spread of three of the intestinal protozoa pathogenic for humans, namely *G. intestinalis, C. parvum,* and *E. histolytica. Balantidium coli* infection is uncommon, although the parasite has a world wide distribution. These pathogenic intestinal protozoa can be transmitted to humans by any mechanism where by material contaminated with faeces containing viable organisms from infected individuals can reach the mouth. However, infections with pathogenic *Naegleria fowler*, and *Acanthamoeba spp* are associated primarily with recreation and the inhalation of warm soil contaminated water **[2]**.

A: *Giardia*

Giardia lambia are flagellated protozoa that parasitize the intestine of humans **[2]**. *Giardia* organisms are widely distributed in nature and have been reported as occurring in more than 40 species of animals including amphibians, birds, and mammals **[25]**.

Giardia can be transmitted by any mechanism where by material contaminated with faeces containing viable organisms from infected

individuals can reach the mouth. Route of exposure include drinking-water, recreational water, food, and person-to-person contact [2]. Most of the outbreaks in USA have been attributed to contaminated surfacewater treated only by disinfection [26].

The protozoal cells, called trophozites produce a resting stage called a cyst, and this is the primary form, transmitted by water. Cysts germinate in the gastroentestinal tract and bring about the symptoms of giardiasis: An explosive, watery diarrhea and intestinal cramps, nausea, and malaise [9].

The time between the ingestion of the organism and the appearance of the parasite in the stool is about 9-14 days, while the incubation period may range from 1 to 75 day. *Giardia* isolated from humans and animals have been found to be associated with bacteria, virus – like particles and mycoplasma-like organisms.

B: *Cryptosporidium spp*

Cryptosporidium spp are intracellular parasites of the gastroentestinal and respiratory tracts of many animals, including mammals, birds, and fish, and have a worldwide distribution. Six species are known, namely *C.parvum* and *C. muris*, which infected mammals, *C.baileyi* and *C.meleagridis* which infect birds, and *C. serpentis* and *C.nasorum*, which infected reptiles and fish [2].

C.parvum is the major species responsible for clinical disease in humans and domestic animals. Human cryptosporidiosis was first described in 1976, and the first reported outbreak in 1984 [2, 9].

Cryptosporidium complete its life cycle within a single host. The infection is more common in children than in adults. The infective dose is thought to be small.

Cryptosporidium can be transmitted by any mechanism where by material contaminated with faeces containing viable organism from infected humans or animals can reach the mouth.

Humans and other animals are reservoirs for infection, and the communication of water supplies with either human or animal sewage can lead to the transmission of *cryptosporidium* through drinking-water. Oocysts can survive several months in water at 4°C and are among the most chlorine –resistent pathogens known.

While infection may be asymptomatic, it is usually associated with diarrhoea. Gastrointestinal symptoms include vomiting, anorexia and flatulence, symptoms typically last 7-14 days, and prolonged excretion of oocysts is unusual [2].

C: *Entamoeba histolytica*

E. histolytica is distributed world wide and exist in trophozoite and cyst stages. Infection occurs by ingestion of cysts. Humans are the primary resevoir for infection with *E. histolytica*, so the contamination of water supplies with domestic sewage can lead to the transmission of this organism through drinking-water.

Dysentric individuals pass only trophozoites, while most or all of the parasites in the active amoeboid stage are destroyed by gastric juice. The cysts can survive for several months in water at 0°C, 3days at 30°C, 30 minutes at 45°C and 5 minutes at 50°C, and are extremely resistent to chlorination.

Person-to-person spread and contamination of food spread by infected food handler appears to be the most significant means of transmission[2].

Because most infections with *E. histolytica* are asymptomatic, death can occur. The usual clinical manifestations are gastroenteritis with symptoms ranging from mild diarrhoea to bloody dysentry. Pathogenicity appears to depend both on strain virulence and on host factors [27].

There are only two absolute criteria by which we can identify *E. histolytica* from other amoeba species:

* By the demonstration of unidirectional, purposeful motility by the trophozoite.
* By the presence of ingested erythrocytes within the trophozoite cytoplasm [17].

1.8 Microbial Indicators of Water Quality

Indicators organisms are used globally as a warning of possible contamination and as an index of water quality deterioration. The recognition that faecally polluted water is responsible for spreading enteric diseases led to the development of sensitive methods of verifying that drinking-water is free from faecal contamination. Even though many waterborne pathogenes can be detected, the methods are often difficult, relatively expensive, and time–consuming. Furthermore, pathogens are

shed into water only from infected people and animals, and it is not possible to examine water for every possible pathogen that might be present [2].

Faecal indicator bacteria should fulfill certain criteria to give meaningful results: they should be universally present in high number in the faeces of humans and warm- blooded animals, readily detectable by simple methods, they should not grow in natural water, their persistence in water and their degree of removal in treatment of water are simillar to those of waterborne pathogens [3].

The criteria described above, for an ideal faecal indicator, are not expected to be met by any single organism, however, many of them are fulfilled by the thermotolerent coliform *E.coli,* thus thermotolerent coliform *E.coli* is considered as the first choice indicator [2].

The major indicator organisms of faecal pollution are *E.coli* the thermotolerent, total coliform bacteria, faecal *Streptococci,* sulfite-reducing *Clostridia* and coliphages.

1.8.1 *Total coliform*

Total coliform organisms have long been recognized as suitable indicator of drinking-water quality, as they are easy to detect and enumerate in water. The term coliform organisms refer to a group of gram-negative, rod shaped, non spore former bacteria, capable of aerobic and facultative anaerobic growth in the presence of bile salts. They are also able to ferment lactose and posses β- *galactosidase* gene [3, 2].

Traditionally coliform bacteria were regarded to belong to the genera: *Escherchia, Citrobacter, Klebsialla* and *Enterobacter*. However, modern taxonomical methods consider this group as a heterogeneous group that include lactose fermenting bacteria, such as *Enterobacter cloacae* and *Citrobacter freundii.* Organisms belonging to this group can be found in faeces, soil, decaying plant material, and in drinking-water rich in nutrients. Thus, its presence in water, as coliform bacteria could be due to other sources than faecal contamination. A good example for this is the finding of *Serratia fonticola* species in water [8].

The existance both of non-faecal coliform bacteria that fit the definitions of coliform bacteria, and of lactose negative coliform bacteria (*Salmonella, Shigella*) limits the applicability of this group as indicators of faecal pollution. Coliform bacteria should not be detectable in treatd water supplies and, if found, suggest inadequate treatment, posttreatment

contamination, or excessive nutrients. The coliform test can therefore be used as an indicator of treatment efficiency and of the integrity of the distribution system. Thus, this test is useful for monitoring microbial quality of water supply, especially when coliform organisms are found in the absence of thermotolerant coliform organisms [3].

Based on total coliform count the WHO classified contaminated drinking-water into four degrees, and accordingly treatment of such water depends on its degree of contamination[8]. Table 1.7 shows WHO classification for contamination degree and treatment methods.

Table 1.7 WHO classification for contamination degree and treatment methods.

Total coliform count	Contanimation degree	Treatment method
0-3	0	-
Greater than 3 to 50	1	Disinfiction
Greater than 50 to 50.000	2	Agglutination, filtration, disinfection
Greater than 50.000	3	Special treatment

1.8.2 *Thermotolerant coliforms bacteria*

Thermotolerant coliforms are defined as the group of coliform organisms that are able to ferment lactose at 44-45°C. They comprise the geneus *Escherichia, klebsiella, Enterobacter*, and *citrobacter*. Of these organisms, only *E. coli* is specially of faecal origin, being always present in the faeces of humans, and other mammals, and birds in large numbers , and rarely found in water or soil that has been not subjected to faecal pollution. Thermotolerant coliforms other than *E. coli* may also originate from originally enriched water such as industrial effluents or from decaying plant materials and soils [3, 2].

Regrowth of thermotolerant coliform organisms in the distribution system is unlikely unless sufficient bacterial nutrients are present or unsuitable material are in contact with the treated water, the water temperature is above 15°C, and there is no free chlorine residual [3].

Thermotolerant coliforms are less reliable indicators of faecal contamination than *E.coli*, although under most circumstances their concentration are directly related to *E.coli* concentration in water. Their use for water-quality examination is therefore considered acceptable.

The detection and identification of these organisms as faecal organisms or presumptive *E.coli* provide strong evidence of recent faecal contamination **[2]**.

E.coli is abundant in human and animal faeces, where number may attain 10^9 per gram of fresh faeces. It is found in sewage, treated effluents and all natural waters and soils that are subjected to recent faecal contamination.

E.coli is a member of the family Enterobacteriacea, and is characterized by the possession of enzymes β-galactosidase and β-glucuronidase. It grows at 44-45°C on complex media, ferment lactose and mannitol with the production of acid and gas, and produces indole from tryptophan **[3]**.

WHO classified contamination of drinking water based on thermotolerant coliform *E.coli* count into five risk levels according to the count of thermotolerant coliform *E. coli* CFU /100 ml **[24,8]**.WHO classification is shown in table **(1.8)**.

Table **1.8** WHO classification for *E. coli* counts & risk *

Count/100ml	Risk
0	Not risk
Greater than 0 to 10	Low risk
Greater than10 to100	Intermediate risk
Greater than100 to 1000	High risk
Greater than1000	Very High risk

* **After** : Othman **[8]**.

1.8.3 *Faecal Streptococci*

Faecal *Streptococci* which are gram – positive bacteria, are useful as indicators of microbiological water quality since they are common inhabitants of the intestinal tracts of the humans and lower animals **[28]**.

Faecal *Streptococci* refer to those *Streptococci* generally present in the faeces of humans and animals, all possess the lancefield group D antigen. They belong to the genera *Enterococcus and Streptococcus* **[2]**.

The genus *Enterococcus* has recently been defined to include all *Streptococci* sharing certain biochemical properties and having a wide tolerance of adverse growth conditions. It includes the species *E. avium, E. casseliflavus, E. cecorum, E. durans, E. faecalis, E. facium,*

E. gallinarum, E. hirae, E. malodoratus, E.munditii, and E. soliarius. Most of these species are of faecal origin and can be regarded as specific indicators of human faecal pollution under many practical circumstances **[3].**

In the genus *Streptococcus,* only *S. bovis and S. eqinus* possess the group D antigen and are members of the faecal *Streptococcus* group. They occur mainly in animal faeces **[2].**

Faecal *Streptococci* rarely multiply in polluted water and are more persistent than *E.coli* and coliform bacteria. Their main value in assessing water quality is therefore an additional indicator of treatment efficiency **[2].**

Streptococci are highly resistent to drying and may be valuable for the purposes of routine control after new mains have been laid or distribution system repaired, or for detecting pollution by surface run-off to ground water or surface waters **[3].**

1.8.4 *Sulfite –reducing clostridia*

These are anaerobic, spore-forming organisms, of which the most characteristic *Clostridium perfringens* is normally present in faeces, however, they are not exclusively of faecal origin and can be derived from other environmental sources **[2].**

Clostridium spores can survive in water much longer than organisms of coliform group and will resist disinfection. Their presence in disinfected waters may thus indicate deficiencies in treatment **[3].**

1.8.5. *Bacteriophages*

Bacteriophages are viruses that infect bacterial host cells. They usually consists of a nucleic acid molecule (genome) surrounded by protein coat (capsid).

Bacteriophages may contain either DNA or RNA as the genome and may have a very simple, cubic structure or a more complex one with heads, tail fiber, or other attachments. They are in the size range 25-100 nm **[3].**

Bacteriophages have been proposed as indicators of water quality, particularly with respect to human enteric viruses, both because of simillar nature and because they are easy to be detected in water samples **[2].**

1.8.6 : Miscellaneous indicators

The *Befido* bacteria and the *Bacteroides fragilis* group are very numerous in faeces but have not been considered as suitable indicators of faecal pollution because they decay more rapidly in water than coliform bacteria and because method of examination are not very reliable and have not been stranderized **[3]**.

1.9 Current Status of Water Supply and Quality in West Bank

Over the past 15 years several attempts were made in order to determine quality of drinking-water in various locations in the West Bank. Most of these studies based on total coliform count and faecal coliform count.

A study by smith C. (1985) on bacterial quality of drinking-water in seven villages in the Hebron region **[29]**, shows that the piped chlorinated water was free from dangerous bacterial contamination. The majority of cistern revealed faecal contamination, which, by WHO standards, rendered them unfit as drinking -water source. The central spring in Sa'ir showed high levels of contamination. The Higra well was considerably contaminated.

A study by Smith C. (1985) at cistern of Abu Shkeidem village **[30]**, indicates that the average quality of 75 cistern in the village was 5.01 CFU of FC /100 ml, the average faecal coliform concentration in cisterns where electric pump was used was 2.61 CFU of FC /100 ml, while in cisterns where bucket were used for removal of water was 7.61 CFU/100ml.

A study by Smith C. (1984) on bacterial quality of drinking water in eight villages in the Jordan Valley **[31]**, indicates that water of unacceptable quality was being consumed in all eight villages.

A study by Smith C .(1985) on seasonal aspects of faecal coliform concentrations in nine springs in Ramallah area which are used for drinking-water **[32]**, indicates that a seasonal trend of higher faecal concentration during the rainy season.

A study by Birzait University Community Health unit. (1990) on water quality in the West Bank **[8]**, presents a brief definition of clean drinking-water, the concentration of faecal coliform used as indicator for the level of water pollution in cistern, the result shows that cisterns which

were fed by water originated from streets had more than 100 FC /100 ml, while cisterns which were fed by home yards has less level of faecal coliform pollution. Cisterns which are located at distance of more than 50 meters from sewage soakage pits had 50% less pollution than cisterns which are located shorter distance from soakage pits.

A study by Othman (2000) at cisterns and roof storage tanks in two villages (Beit-leed and safarine) in district of Tulkarm **[8]**, indicates that the total coliform count were 16.1 and 12 CFU/100 ml water for cisterns and roof storage tanks respectively, while average count of thermotolerant *E.coil* were 7.0 and 4.5 CFU/100 ml water for cisterns and roof storage tanks respectively.

A study by AL- Kahah (2000) of water borne pathogenes with relation to gastroenteritis in three villages (Burqin, Farkha, and Kafir ad-Dik) in Salfeet district**[9]**, indicates that the average counts of total coliform (CFU/100ml) in spring water were 15.4, in rainwater 19.4, and zero in water network. The average of faecal coliform (CFU/100ml) in spring water were 9.4, in rainwater 11.4, and zero in water net work **[9]**.

In the West Bank it is unusual for a household not to have a latrine and drinking–water source. Existing data on water quality suggests that piped water supply are providing good quality water, and that most rainfed cistern provide good quality water.

Some of the diseases which are associated with severe environmental contamination–for example cholerae and typhoid–are rare in the West Bank. Intestinal protozoa are common infections, and gastrointestinal infections are a major source of child morbidity **[6]**.

1.10 Aims of the Study

The present work aimed at :

1-Carry out sanitary survey in five villages in Tubas district, with the aim of evaluating the extent of faecal pollution of drinking-water.

2-Assessing drinking –water quality by measuring total coliform bacteria, and faecal coliform bacteria. With the aim of determining the degree of water contamination and the associated health risk level according to WHO standards.

3-Measure the bacterial quality of drinking – water in cisterns and household vessels in order to evaluate whether the different collections and storage practices had an influence on the quality of water consumed.

4-Study relationship between the presence of total and faecal coliform

and the presence / absence of *Salmonella* and *E.coil* 0157: H7 in drinking – water.

5-Search for possible sources of water contamination in the studied areas.

6-Study possible relationship between contamination of water and infection of people using this water by pathogens isolated from stool samples – an epidemiological study– .

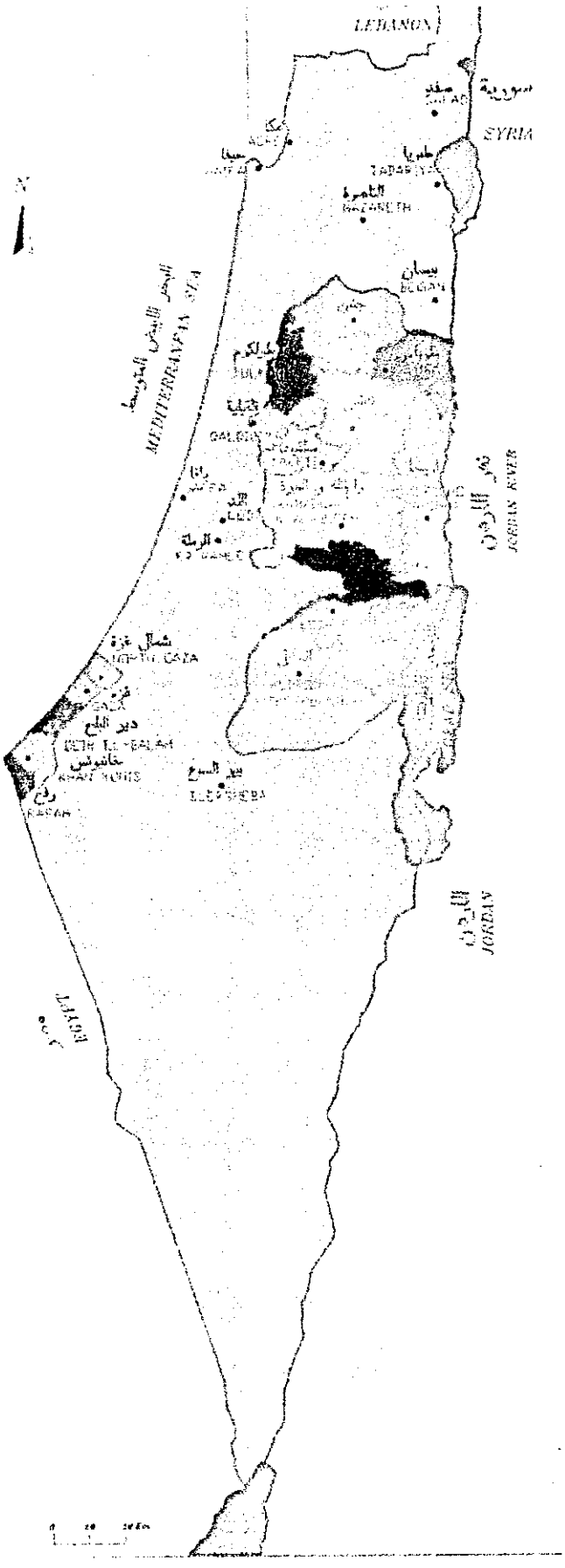


Fig. **1.1** Palestine map including the study area (Tubas district)**[10].**

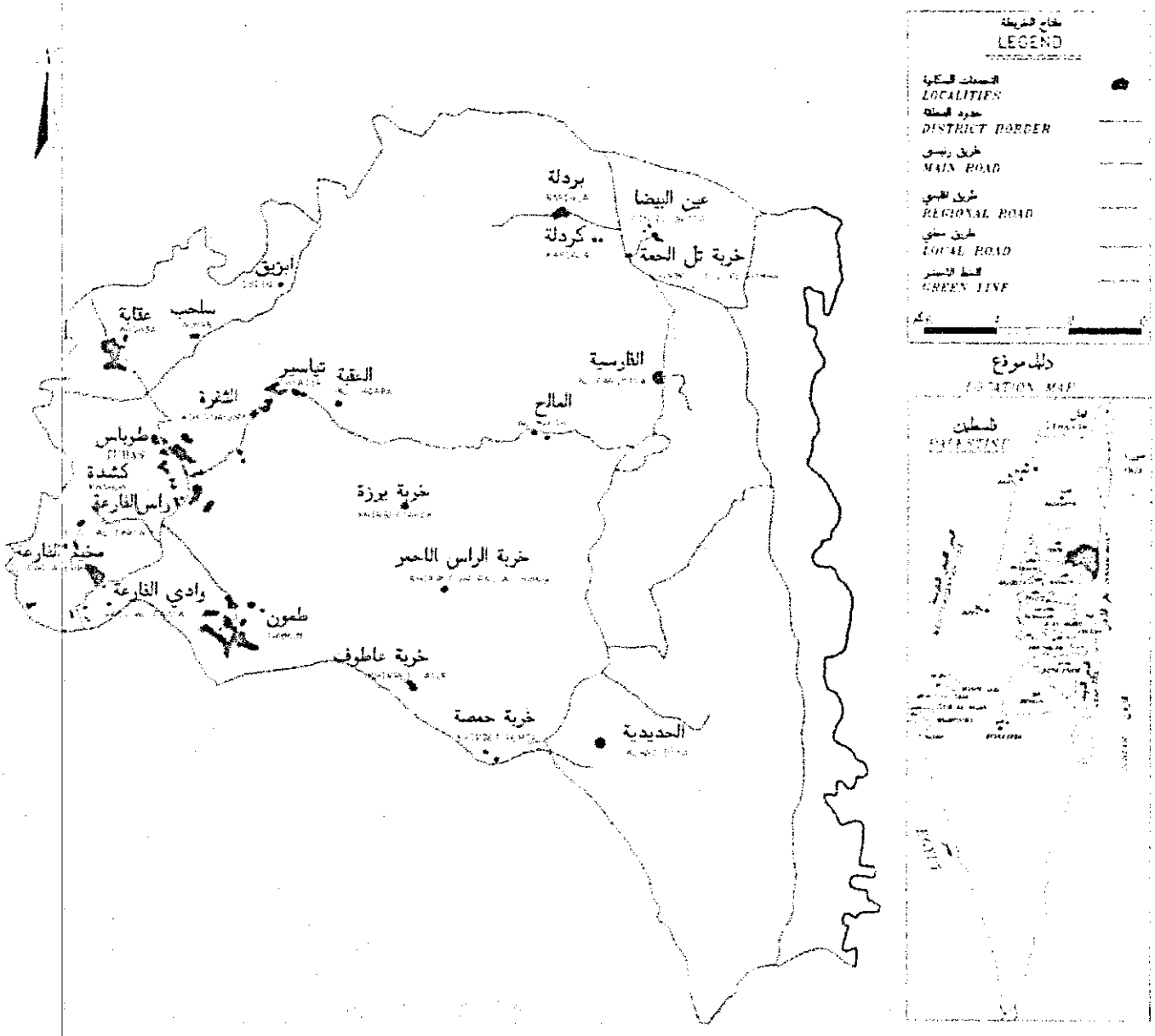


Fig. **1.2** Tubas district map including the study villages**[10]**.

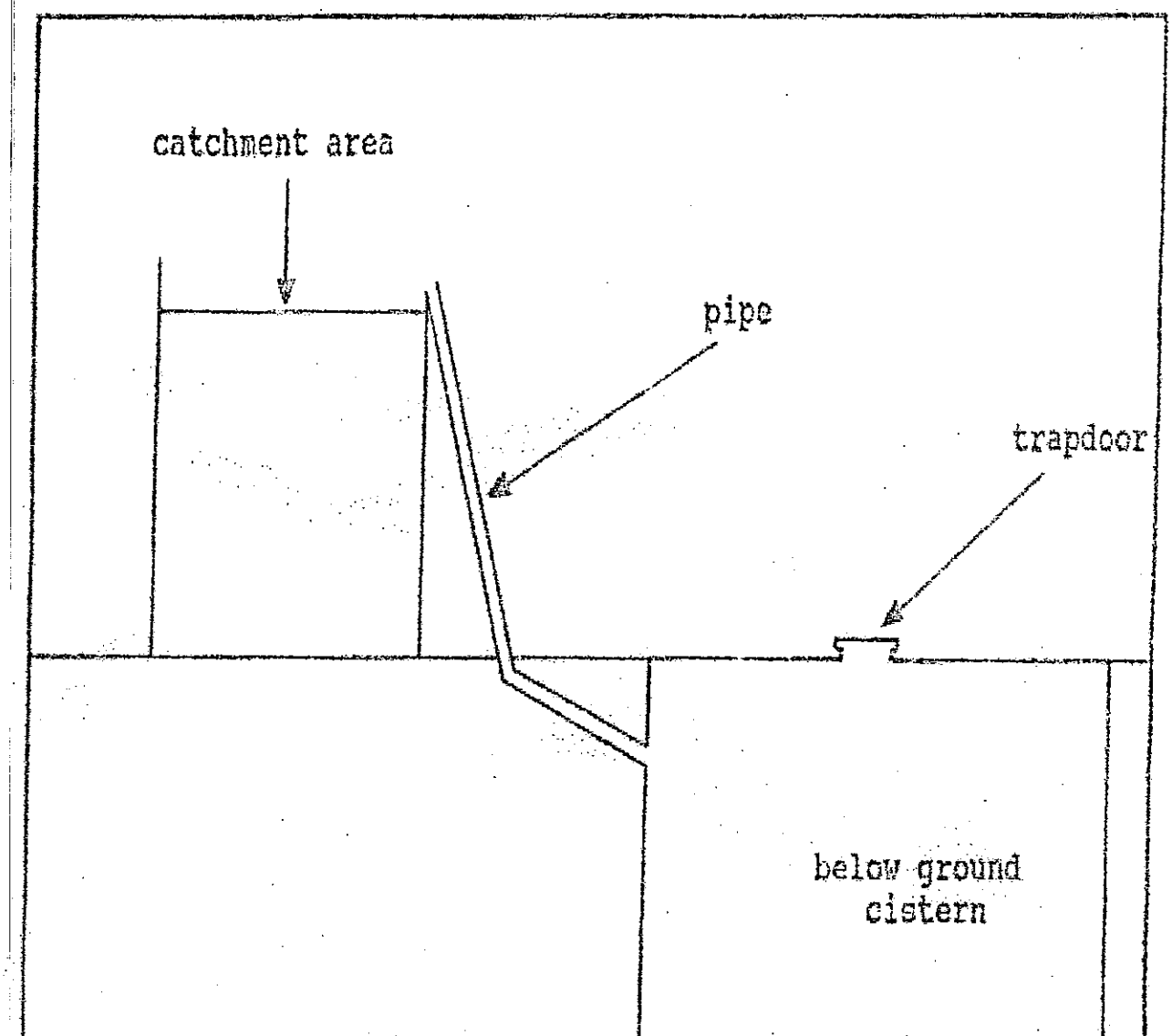


Fig. **1.3** Main element in the rainfed cistern collection system[6].

Chapter 2

Materials & Methods

CHAPTER II
MATERIALS AND METHODS

2.1 Water Sample Collection

A total of 200 samples of water including 75 rainwater, 19 spring water, and 106 municipality water, were collected randomly from houses of five villages(Tubas, Tammun, 'Aqqaba, El-Fara', and Tayasir) in Tubas district, in the period between March 15,& June 20, 2000.

Water samples were collected aseptically in autoclaved 330 ml glass bottle, following WHO instructions. Collecting water samples from tap water was carrid as follows :

- The tap was cleaned by clean cloth, to remove any dirt.
- The tap was opened at the maximum flow for 1-2 minutes.
- The tap was flamed for a minute using a gas burner.
- The tap was opened before sampling for 1-2 minutes at a medium flow rate.
- The bottle was opened and filled with water and stoppered .

Figure **2.1** shows checklist for collecting water samples from tap water .

Collecting water samples from dug wells was carried as follows :

- A clean weight to the sampling bottle was attached with a piece of string.
- A 20-m length of string rolled around a stick was taken and tied to the bottle, then the bottle was opened.
- The bottle was lowered down into the well .
- The bottle was immersed completely in water.
- The bottle was raised.

Figure **2-2** shows checklist for collecting water samples from dug wells.

2.2 Water Samples Transport

The samples were immediately sent refrigerated in a light proof insulated box containing melting ice or ice – packs with water to ensure rapid cooling to the microbiology laboratory at An-Najah National University within 2 hours of collection **[5].**

Samples where either analysed immediately or stored at – 4°C until testing.

The following information was obtained from householder :

1- The source of water in the cistern (rainwater, spring water or municipality water).

2- If chlorine or any disinfectant is used ?
3- The last year the cistern was cleaned.
4- Does the cistern have a trap door?
5- How far is the nearest latrine from the cistern?
6- Are animals kept in the household?

2.3 Water Samples Filtration and Culture Media.

A total of 1.5 – 2 ml of memberane laurly sulfate lactose broth (Oxoid MM 615) was poured into the pad of Millipore Specialized 47 mm perti dish (PD 100470) [9].

For each water sample, two petri dishes were used, one for the total coliform count, and the other for the faecal coliform count.

1- The Erlenmeyer flask was connected to the vacuum source and the porous support was placed in its position.
2- The sterile petri dish was opened and absorbed pad was placed in it.
3- Broth medium was added to saturate the pad.
4- The filtration unit was assembled by placing a sterile membrane filter on the porus support, using forceps sterilized by flaming.
5- The upper container was placed in position and secured by clamp.
6- About 100 ml of sterile buffered water was poured into the funnel before adding the sample.
7- The water sample bottle was shaked vigorously, from which a measured volume of the sample (100ml) was filtered through the membrane using pressure vaccum pump.
8- The membrane was then placed aseptically on an absorbent pad saturated with the culture broth using sterile forceps.
9- Two volumes were filtered for each plate: one for total coliform count and the other for faecal coliform count.
10-After which, the funnel was rinsed with a volume of sterile buffered water that equals the total a mount of the liquid filtered followed by running the second sample.
11-The total coliform count plates were incubated at 37°C, and faecal coliform count plates at 44.5 °C both for 24 hours.
12-After 24 hours incubation, the plates were examined for the presence of yellow colonies[5,9].

Figure 2.3 shows membrane filter technique for determining coliform content of water [5].

2.4 Identification of Bacteria

2.4.1 Identification of coliform bacteria (Lactose fermenter).

Four well – separated yellow colonies, which were suspected to be coliform from uncrowded part of the plate were randomly choosen for biochemical identification.

The tests for identification were gram stain, oxidase, catalase, triple - sugar iron, urea, and IMVIC tests, which were described below.

Gram strain

1- Using an inoculating loop, part of an isolated colony was emulsified with a drop of sterile saline on a glass slide to prepare a thin smear. The smear was fixed by gentle heating by passing the slide several times on flame.
2- The smear was flooded with crystal violet stain for 10 seconds then poured of and washed with gram iodine solution.
3- Then the smear was decolorized with absolute ethanol acetone solution (1:1) until no further color flew from the slide.
4- The smear was then countered stained by safranin for 30 seconds, followed by washing with water, and air dried.
5- After that the smear was examined under the microscope using the oil immersion lens.

Gram – positive organisms appeared blue in color, while Gram – negative organisms appeared red in color.

Oxidase test:

The test was carried out as follows **[33].**

1. Several drops of oxidase reagent (tetramethyl paraphenyline diamine dihydrochloride) were used to moisten piece of filter paper.
2. A small portion of the colony to be tested was removed and smeared with sterile wooden stick on the moistened filter paper.
3. A positive reaction (oxidase positive) was recognized by dark purple color, developed in 5 to 10 seconds followed by black color due to death of bacteria.

Coliforms are oxidase negative, colonies of *E.coli* (oxidase negative) and *Pseudomonus aeruginoza* (oxidase positive) were used as control.

Catalase test (3% hydrogen peroxide (H_2O_2) solution

The test was carried out as follows **[33].**

1. With a sterile wooden stick, part of an isolated colony from the agar media was tranferred aseptically to the surface of a clean, dry glass slide.
2. Immediately a drop of 3% hydrogen peroxide (H_2O_2) was placed on to a portion of the colony on the slide.

A positive reaction was recognaized by elaboration of oxygen, which developed immedately in the form of bubbles .

Coliform is castalase positive. Colonies of *Staphylococcus aureus* (catalase positive) and *Staphylococcus pyogenes* (catalase negative) were used as control.

Triple Suger Iron (TSI) test

The test was carried out as follows **[33].**

1. The very center of the colony to be tested was lightly touched with a sterile inoculaing needle.
2. The TSI agar (oxide, CM 277) slant, was aseptically inoculated by streaking the slant and stabbing the butt.
3. The tube was incubated at 37 °C for 24 hr.
   Coliform organisms are classified according to their several different possible reaction on TSI (Table **2.2**).

Table 2.2 Basic reactions of Enterobacteriaceae on TSI agar.

[1]K/A gas	K/A No gas	[2]A/A gas	K/A H_2S	A / A H_2s	Possible species
+	+	+	-	-	*Escherichia coli*
+	+	-	-	-	*Hafina alvei*
+	-	+	+	+	*Morganella morganii* *Providencia* *Alcalifaciens* *P.rettgri* *P. stuartii* *Serratia spp*
+	-	+	-	-	*Citrobacter spp*
+	+	-	+	-	*Enterobacter aerogenes* *E. cloacae*
-	+	+	-	-	*Salmonella spp* *Shigella*
-	-	+	-	-	*Yersinia spp*
-	-	-	+	+	*Klebiella spp*
-	-	-	+	-	*P. mirabilis* *p. vulgaris*
-	+	+	-	-	*Edwardsiella trada*

"1" k/A : Alkaline slant (red) and acid butt (yellow).
"2" A/A : Acid slant (yellow) and acid butt (yellow).

Urease test:

The test was carried as follows **[33].**

1. With a sterile inoculating needle, the colony was lightly touched and inoculated aseptically into urea agar (Oxoid, CM 33) slant by streaking the slant only.
2. The tube was incubated at 35 °C for 24 hour.
   Positive test is indicated by development of pink color, where as in negative test no change in the color of the media can be detected.

Colonies of *Proteus* (usease positive) and *E.coli* (urease negative) were used as control.

Indole – Methyl Red – Voges Proskauer – Citrate (IMVIC) test:

A: Sulfide, Indole and Motility (S.I.M) test:

The test was carried as follows **[33].**

1. A sterile tube of S.I.M media (oxoid, CM 435) was aseptically inoculated with a portion of bacterial colony by stabbing the media once to a depth of 1 / 2 – 1 / 4 in. with a sterile inoculating needle.
2. The tube was incubated overnight at 35 °C.

Reaction on S.I.M

S: blackening of tube – sulfide production present (sulfied positive), no color change in tube (sulfide negative).

M: cloudiness throughout medium or brush like growth around line of inoculation – positive motility.

I : Means that this medium was used for detection of indole production, this was achieved by:

1. Adding 1 ml of xylene to the S.I.M medium.
2. The tube was shaken well and allowed to stand for few minutes untill the solvent rised to the surface.
3. About 0.5 ml of Ehrlich reagent was gently added down the sides of the tube.

A distinct brilliant red ring, which developed just below the solvent layer, represented a positive test while absence of such red ring represented a negative test . Colonies of *E . coli* (indole positive) and *P. aeruginosa (* indole negative) were used as control .

B : Methyl Red – Voges Proskauer (MR – VP) test :

The test was carried as follows **[34]** :-

1- Portion of the bacterial colony is inoculated into a sterile tube of 2 ml MR – VP broth (Oxoid , CM43) using a sterite loop.

2- Incubation for 18 hours at 35 °C.

3- For methyl red test, 0.5 ml of MR- VP culture was transferred aseptically to another sterile tube, and the remainder was incubated for another 30 hours at 35 °C .

4- One drop of methyl red reagent was added aseptically to the tube (0.1gm methyl red dissolved in 300 ml ethyl alcohol and then completed to 500 ml by distilled water). A positive reaction was indicated by distinct red color, while negative reaction was indicated by yellow color.

6- About 0.6 ml of 5% 1 – naphthol solution (5 gm of 1 – naphthol in 100ml of ethyl alcohol) and 0.2 ml of 40% potassium hydroxide with creatine (40 gm KOH in 100ml distilled water) were added to the remainder incubated culture of MR - VP tube and mixed .
6- The tube was shaken and left to stand for 10 – 20 minutes.

A positive reaction was indicated by a bright orange – red color in the medium. Negative reaction was indicated by colorless or yellow color.

C : Citrate Utilization :-

The test was carried as follows **[33]:**

1- From a portion of bacterial colony, a very light inoculum was picked with a sterile inoculating needle (to prevent false positive reactions because of carry over of substrates from previous media).
2- Under aseptic conditions, bacteria were inoculated to sterile simmon citrate agar (Oxoid , CM 155) slant by streaking the slant .
3- The tube was incubated for 24 hours at 35 °C with a loose cap.

A positive reaction was indicated by growth of the organism on the slant, with change of color indicator from green to blue, where as, no growth or very little growth and no color change indicated a negative reaction.

Colonies of *K. pneumonia* (citrate positive) and *E . coli* (citrate negative) were used as control .

2.4.2 Isolation and Identification of *E.coli* O157 : H7 .

E. coli strains isolated from water were further identifed for *E. coli* O157 : H7 by biochemical and serological tests .

A: Biochemical confirmation :-

For each suspected colony, biochemical tests including TSI, urease and IMVIC tests were done according to standard biochemical reactions **[33].**

E. coli O157 : H7 shows the same biochemical reactions like other *E. coli* strains. So all cultures which produce acid (yellow) slant and acid (yellow) butt in TSI, urease negative and + + - - IMVIC tests were retained as potential *E . coli* O157 : H7 isolates and were submitted for serological test, while cultures which gave negative results were discared.

1. With sterile loop, small amount of growth was aseptically streaked on to eosin methylene blue agar (EMB).
2. The plates were incubated overnight at 37 °C.
   A positive reaction was indicated by green metalic sheen on EMB was suspected to be *E.coli* : O157:H7.
3. With a sterile inoculating loop, a portion of bacterial colony was steaked on to the MacConkey sorbitol agar.
4. The plates were incubated overnight at 37 °C.
   A positive reaction for *E. coli* O157:7 was indicated by the appearance of colorless colonies.

B: Serological test:

E.coli O157:H7 latex test (oxoid, DR 620 M) had been used for identification of *E .coli* serogroup 0157: H7.

1. After the reagent had been brought at room temperature, vigorous shaking mixed the latex suspensions.
2. One drop of the test latex was dispended onto a circle on the reaction card close to the edge of the circle.
3. A Pasteur pipette drop of saline was added to the circle, but the latex and saline were not mixed at this stage.
4. A partial of the bacterial growth was picked off aseptically, by using a loop, from fresh nutrient agar slant culture and then emulsified carefully in the saline drop.
5. By using a sterile loop, the test latex and suspension were mixed together and spreaded to cover the reaction area.
6. The loop was flamed. The card was rocked in a circular motion for only one minute.

Negative reaction was indicated by agglutination with the test reagent within one minute. In this case a further portion of the colony was tested with the control latex reagent to ensure that the isolated colony was not an auto – agglutinating stain.

2.4.3 Isolation of *Salmonella* .

Isolation of *Salmonella* was carried out as follows [35] :-

1- A sterile membrane filter was placed into a sterile holding apparatus .
2- The water sample was allowed to pass through the filter under vaccum pressure, and the bacteria were traped on this filter .
3- The filter membrane was removed with sterile forceps and was placed onto 15–20 ml of tetrathionate broth (Oxoid, CM 29) enriched media.
4- The broth was incubated for 24 hr at 37 °C

A: Selective growth :

1- After incubation of tetrathionate broth, the cups were mixed by using a vortex .
2- Using a sterile loop, a loopfull was taken from the tetrathionate broth and streaked on Xylose deoxychaolate agar (X . L . D) (Oxoid, CM469) and Salmonella – Shigella agar (S. S) (Oxoid, CM99) **[34]**.
3- The plates were incubated at 37 °C for 24 hr .
4- In the following day, the plates were examined for the presence of *Salmonella* suspected colonies .

On X. L . D agar, *Salmonella* appeared as pink – red colonies with or without black centers, while on S . S . agar *Salmonella* appeared as transparent (colorless) colonies usually with black centers .

B: Screening :

1- The colonies typical or suspected to be *Salmonella* were selected from each selective agar for further identification .
2- The very center of the colony to be picked was lightly touched with sterile inoculating needle and TSI agar slant was aseptically inoculated by streaking the slant and stabbing the butt .
3- The TSI agar slant was incubated for 24 at hours 35 C° with a loose cap to maintain aerobic conditions while incubating slant and to prevent excessive H_2S production .

Cultures, which produced alkaline (red) slant and acid (yellow) butt with or without production of H_2S (Blackening) in TSI agar, were retained as potential *salmonella* and were submitted for biochemical and serological tests.

2.4.4 Identification of *Salmonella* .

A : Urease test [36] :-

The steps of urease test were mentioned previously. All cultures that give negative urease (no change in color of medium) were retained for further identification, whereas all cultures giving positive urease test (pink color) were discarded .

B : Lysine decarboxylase broth test [36] .

1- With needle, small amount of growth from TSI agar slant suspicious for *Salmonella* was asepticically inoculated into lysine decorboxylase broth (Oxoid, CM 308) .
2- The cap was replaced tightly and the tube was incubated for 24 hr at 35°C.

Cultures that cause alkaline reaction indicated by purple color through the medium were considered as suspected *Salmonella* and were retained for further identification whereas cultures caused development of yellow color throughout the medium were discarded .

C : S . I . M test [33] .

S.I.M test was carried out as mentioned previously.All *Slamonella* are Indole negative as indicated by absence of a brilliant red ring below the solvent layer after the addition of Ehrlic reagent.

Most *Salmonella* are motile organisms as indicated by producing cloudiness in the medium or by growing in brush – like patterns around the line on inoculation.

Some *Salmonella* species produce hydrogen sulphide as indicated by blackening of the line of inoculation whereas others can not .

Under aseptic conditions, growth on the TSI agar slant, biochemically identified as *Salmonella* was inoculated into sterile nutrient agar (Oxoid,CM 3) slant by streaking the slant with a sterile inoculating needle. The slant was incubated for 24 hr at 35 °C.

From fresh nutrient agar slant culture, gram stain ,oxidase, catalase and serological tests were done .

D : Serological test :

Serological test was carried out by *Salmonella* polyvalent somatic (O) antiserum (Murex ZCO 2).

1- On a clean slide, a portion of the fresh pure suspected colony, which had been cultured on nutrient agar, was emulsified in sterile saline with sterile loop to obtain smooth, fairly dense suspensions .
2- One drop of undiluted antiserum was added to the suspension followed by mixing .
3- The slide was then rocked gently for one minute and observed for agglutination using indirect light over a dark background .

As control, one drop of saline was added to another bacterial suspension .

2.5 Stool Sample Collection .

During the period from May, 26/2000 to July 21/ 2000, 100 stool specimens were collected from patients presenting with diarrhea at four Public Health Unit located on Tubas city, Tammun village and El-Fara' camp in Tubas district .

The specimens were collected as follows :

2.5.1. Collection and Transport of Stool Specimens

1- In every morning of saturday of every week stool specimens were collected in sterile plastic containers, and rectal swabs were used when the stool specimins were not obtainable .
2- A list of names, ages and places of residence was compiled of all patients .
3- All Samples were transported refrigerated in an icebox to the Microbiology Laboratory at An – Najah National Univesity, and examined there immediately .

2.5.2. Microscopic Examination .

1- A small portion of faecal material was emulsified in a drop of physiologic saline on a microscope slide using wooden stick to make a thin film .
2- A cover slip was applied onto the smear and the preparation was examined under the microscope under low power objective {10X} and low light intensity , then examined under high – dry power { 40X}, to identify the possible parasites, trophozoites, cysts or ova of parasite .

2.5.3 Isolation of *Salmonella* and *Shigella* .

Isolation of *Salmonella* and *Shigella* were carried aut as follows :

A . Selective enrichment :

1. About one gram of stool sample was transferred to 10 ml of tetrathionate broth .
2- The broth was incubated at 37 °C for 18 hours .

B . Selective growth :

Selective growth for *salmonella* and *Shigella* was carried out as mentioned previously.

On S . S ager, *Salmonella* and *Shigella* appeared as transparent colonies usally with or without black centers, while on X . L . D agar, *Salmonella* and *Shigella* appeared as pink – red colonies with or without black centers.

2.5.4. I dentification of *Salmonella* and *Shigella*

Identification of *Salmonella* and *Shigella* was caried out as mentioned previously.

2.5.5 Isolation and Identification of *E. coli* O157 : H7

Isolation and identification of *E. coli* O157 : H7 was carried out as mentioned previously.

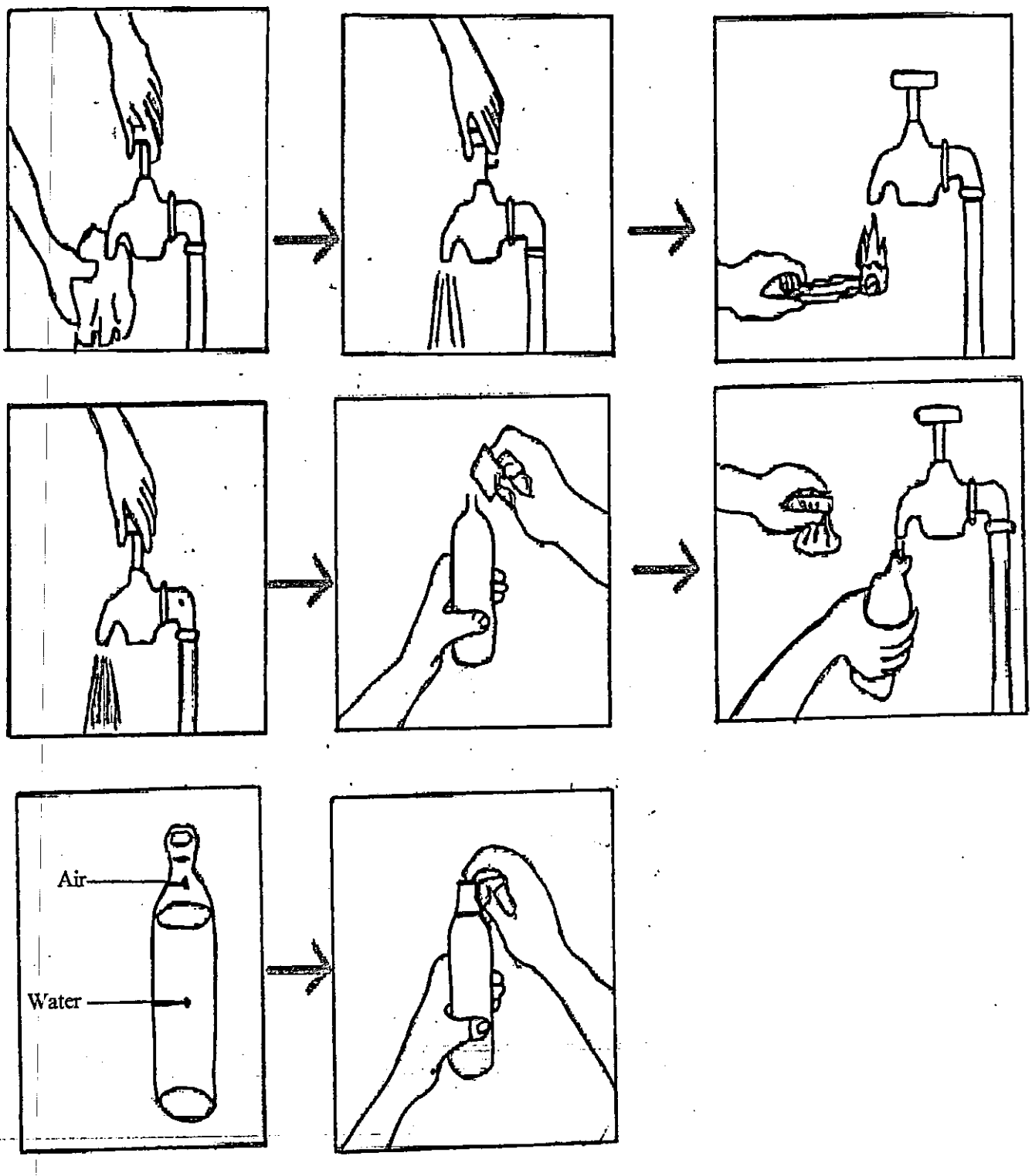
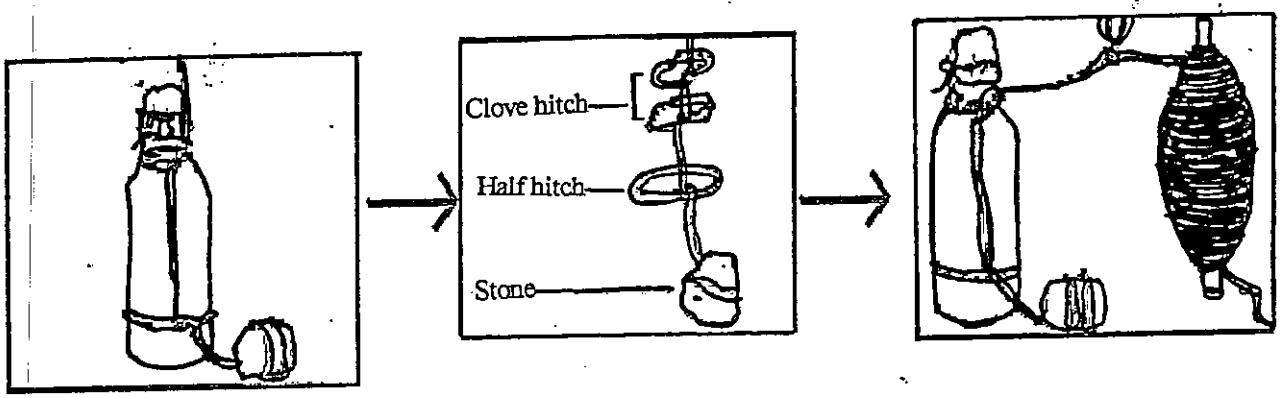


Fig. **2.1**Checklist for collecting water sample from tap**[5]**.



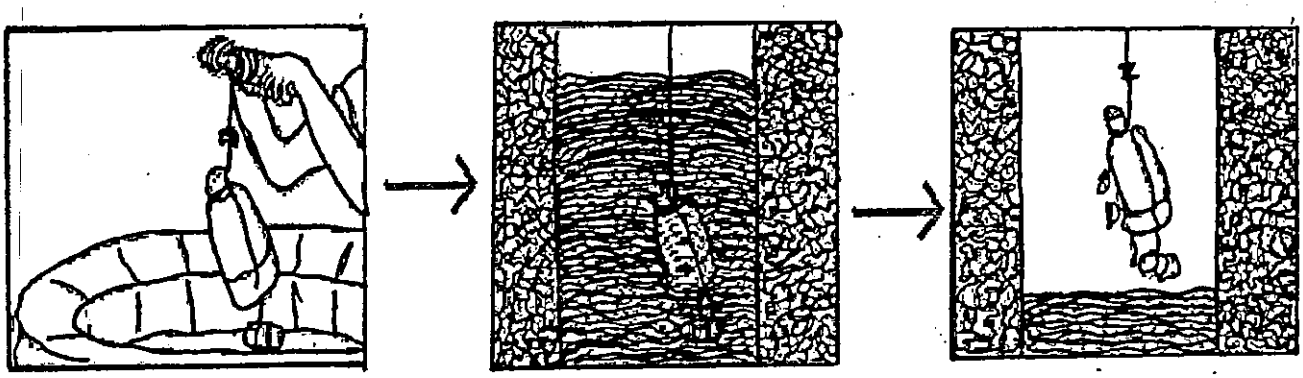


Fig. **2.2** Checklist for collecting water sample from dug wells **[5]**.

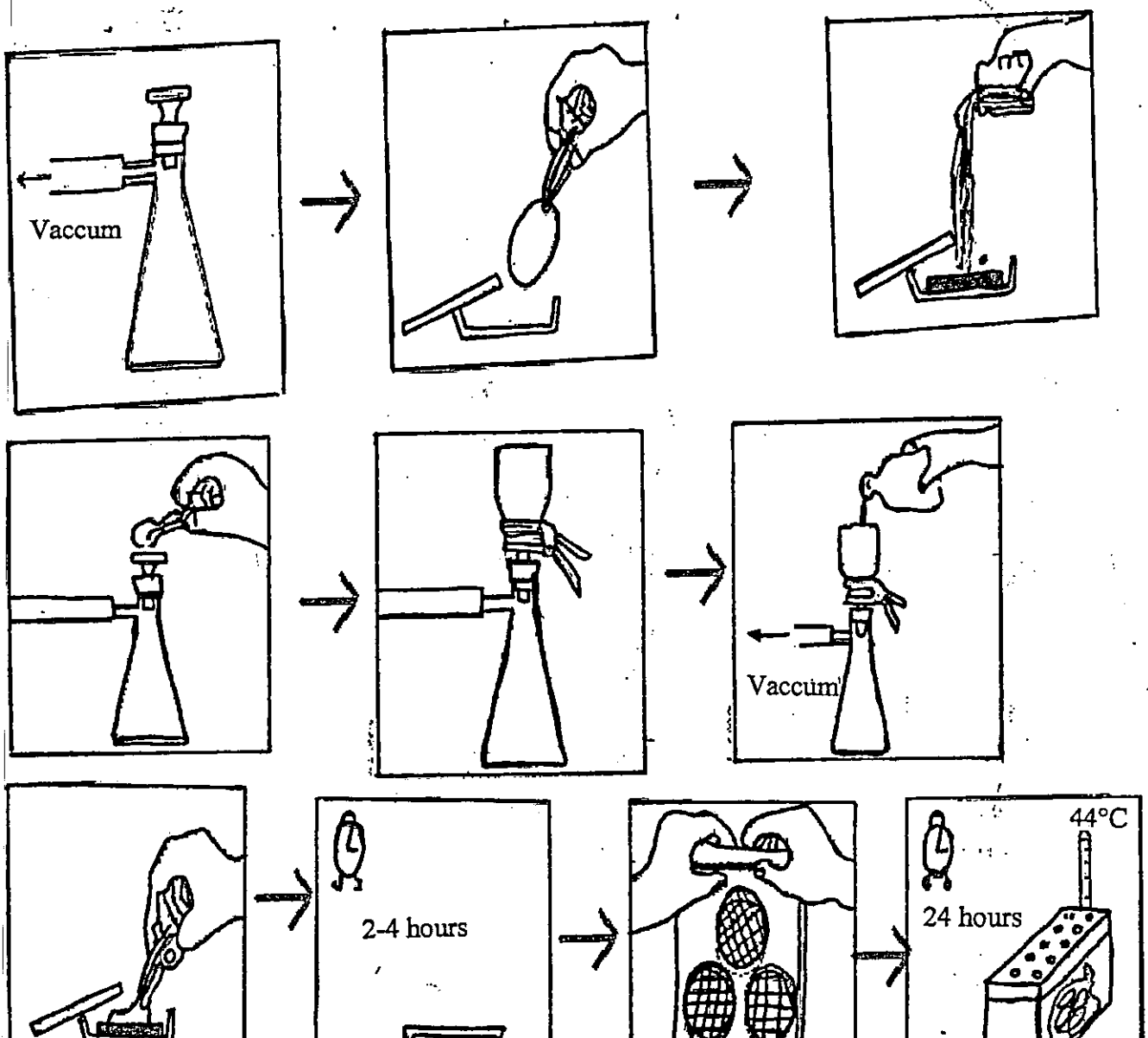


Fig. **2.3** Member filter technique for determining coliform content of water **[5]**.

Chapter 3

Results

Chapter III
RESULTS
3.1 Bacterial quality of drinking water according to water sources.

Data presented in table **3.1** shows the average total coliform and faecal coliform counts in municipality water, rain water and spring water.

Table **3.1** Association between average counts of bacterial indicators and water sources using analysis of variance (ANOVA) test.

Water sources	No.of samples	Percentage	TCC			FCC		
			Average count	F	Sig	Average count	F	Sig
Municipality	106	54.0	0.056	25.171	0.000*	0.0	25.041	0.000*
Rain	75	37.5	20.73			9.97		
Spring	19	9.5	16.79			9.26		
Total	200	100	-	-	-	-	-	-

* significant at ($\propto = 0.05$)

The average total coliform count in municipality water was 0.056 CFU/100 ml, rain water 20.73 CFU/100, and spring water 16.79 CFU/100 ml water.

The average faecal coliform count in municipality water was 0.0 CFU/100 ml, rain water 9.97 CFU/100ml, and spring water 9.26 CFU/100 water. Figure **1.3** shows graphical presentation of these data.

From the table above we notice that, the computed F-value of total coliform and faecal coliform counts according to water sources are 25.171 and 25.041 respectively. This means that, there is no significant difference between rain water sources and spring water sources, but there is significant differences between spring water and rain water on one side and the municipality water on the other side at ($\propto = 0.05$).

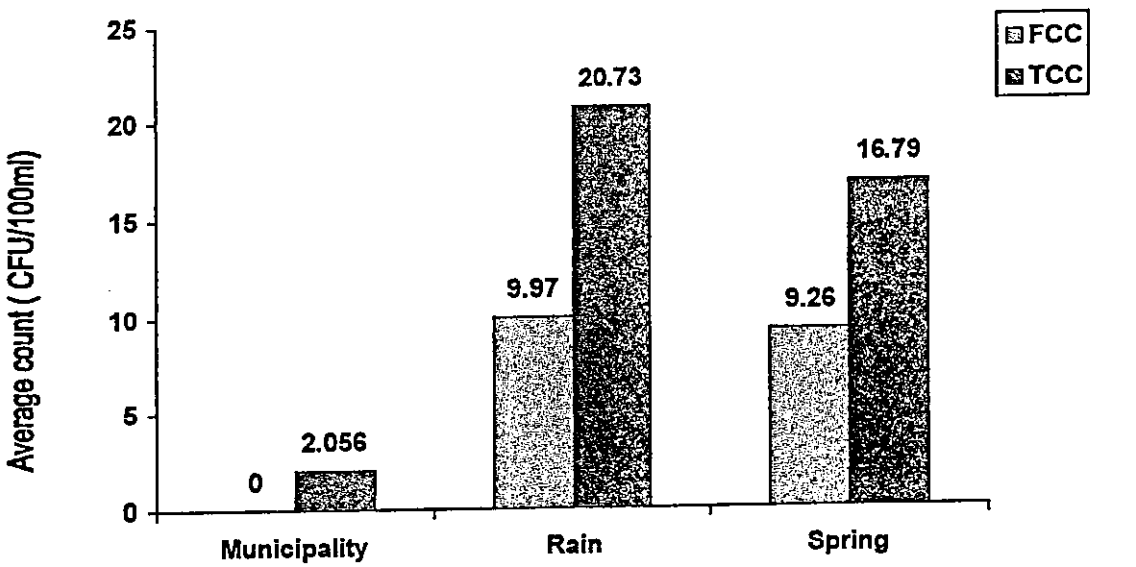


Figure **3.1**.Average counts of bacterial indicators in municipality water, rain water, and spring water.

3.2 Bacterial quality of drinking water in Tubas, Tammun, El-Fara', Tayasir, and Aqqaba villages.

Data presented in table **3.2** shows the average total coliform and faecal coliform counts of the studied villages.

Table **3.2** Average counts of bacterial indicators in storage systems of the studied villages using analysis of variance (ANOVA) test

Village	No.of samples	Percentage	TCC			FCC		
			Average count	F	Sig	Average count	F	Sig
Tubas	101	50.5	3.87	10.466	0.000*	0.82	12.399	0.000*
Tammun	41	20.5	16.63			7.43		
El – Fara'	18	9	5.44			1.44		
Tayasir	15	7.5	30.60			15.60		
Aqqaba	25	12.5	18.48			11.04		
Total	200	100	-	-	-	-	-	-

- significant at ($\propto = 0.05$)

The average total coliform count in storage systems of Tubas, Tammun, El-Fara', Tayasir, and Aqqaba was 3.87, 16.63, 5.44, 30.60, and 18.48 CFU/100ml water, respectively. The average faecal coliform count in storage systems of Tubas, Tammun, El-Fara', Tayasir, and Aqqapa was 0.82, 7.43, 1.44, 15.60, and 11.04 CFU/100 ml water, respectively. Figure **3.2** shows graphical presentation of these data.

The observed differences of the average total coliform count in storage systems in both Tubas and EL-Farra' were of no significance, also there is no significant differences between Tammun and Aqqapa. But there is significant differences between Tubas and El-Fara' on one side and Tammun, Tayasir, and Aqqaba on the other side, also there is significant differences between Tammun and Tayasir, and between Aqqaba and Tayasir, with F-value 10.466 at ($\alpha = 0.05$).

The difference of the average faecal coliform count in storage system of both Tubas and EL – Fara', Tammun and Aqqaba, and Tayasir and Aqqaba villages were of no significance. But there is significant difference between Tubas and El-Fara'on one side, and Tammun, Tayasir and Aqqaba on the other side, also there is significant difference between Tammun and Tayasir, with F-value 12.399 at (α=0.05).

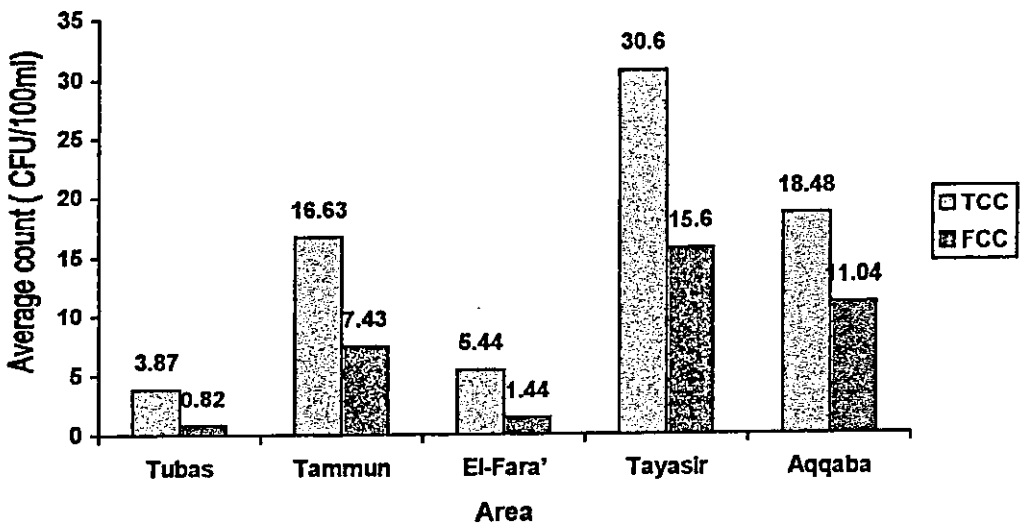


Fig. **3.2** Average counts of bacterial indicators in storage system for Tubas, Tammun, El- Fara', Tayasir, and Aqqaba.

3.3 Degree of contamination of drinking water with respect to water sources.

Table **3.3** shows the result of municipality water, rain water, and spring water, according to the WHO classification for the degree of contamination, with respect to total coliform count.

Table **3.3** Distribution of municipality water, rain water, and spring water according to contamination degree.

Total colifom count (CFU/100ml)	Contamination	No. of municipality samples	(%)	No. of rain samples	(%)	No. of spring samples	(%)
0-3	0	92	86.8	12	16.0	2	10.5
3-50	1	12	11.3	54	72.0	16	84.2
50-50.000	2	2	1.9	9	12.0	1	5.3
> 50.000	3	-	-	-	-	-	-
Total	-	106	100	75	100	19	100

Data presented in table **3.3** shows that 92 samples (86.6%) of municipality water, 12 samples (16.0%) of rain water, and 2 samples (10.5%) of spring water were free of contamination (degree 0.0). While 12 samples (11.3%) of municipality water, 54 samples (72.0%) from rain water, and 16 samples (84.2%) of spring water were in the first degree of contamination. Two samples (1.9%) of municipality water, 9 samples (12.0%) of rain water, and one sample (5.3%) of spring water were with the second degree of contamination. None of the studied water sources were with the third degree of contamination. Figure **3.3** shows graphical presentation of these data.

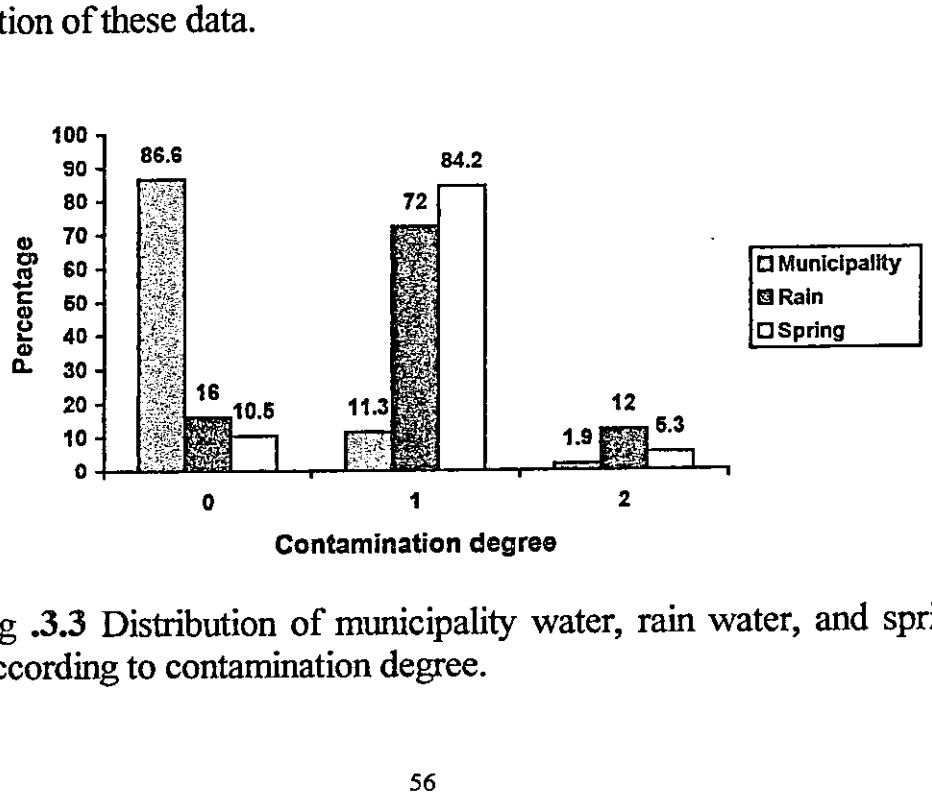


Fig **.3.3** Distribution of municipality water, rain water, and spring water, according to contamination degree.

3.4 Contamination of water sources as potential risk factors for human health

Table 3.4 shows the finding in both rain water and spring water according to WHO classification for risk levels of contaminated water, with respect to faecal coliform *E. coli* count.

Table 3.4. Distribution of rain water and spring water with respect to *E. coli* count and risk levels.

FCC/count (CFU/100ml)	Risk*	Rain water	(%)	Spring water	(%)
0	No risk	28	37.3	8	42.1
0-10	Low risk	25	33.3	5	26.3
10-100	Intermediate risk	22	29.3	6	31.6
100-1000	High risk	-	-	-	-
>1000	Very high risk	-	-	-	-
Total	-	75	100	19	100

* **Risk as defined by WHO.**

Data presented in table 3.4 shows that 28 samples (37.3%) of rain water and 8 samples (42.1%) of spring water and all samples of municipality water were free of contamination (no risk). While 25 samples (33.3%) of rain water and 5 samples (26.3%) of spring water were with low risk level. 22 Samples (29.3%) of rain water and 6 samples (31.6%) of spring water were with an intermediate risk level, and non of these water sources show high or very high risk level. Figure 3.4 shows graphical presentation of these data.

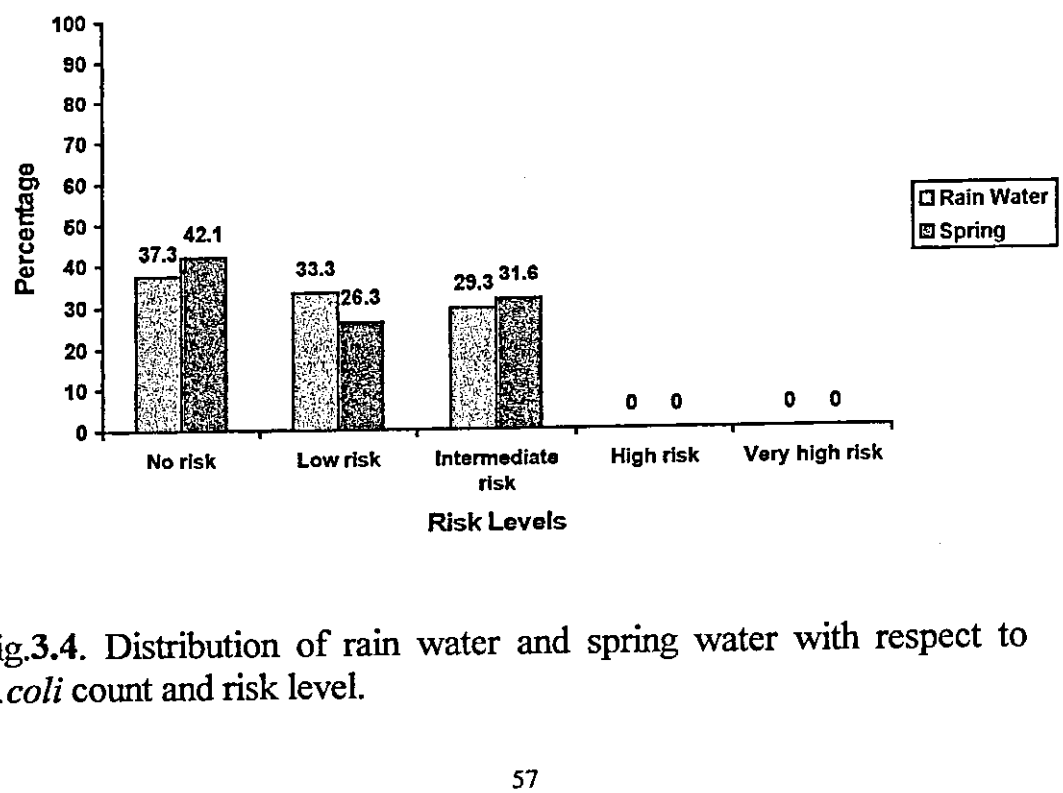


Fig.3.4. Distribution of rain water and spring water with respect to *E.coli* count and risk level.

All samples that are positive for coliforms were cultured for *Salmonella, Shigella, and E.coli O157:H7* in municipality water, rain water, and spring water.

Table **3.5** shows that the total 87 water samples positive for coliforms were cultured for *K. pneumoniae, Proteus spp, P. aeruginosa, E. aerogenes, E.coli, Salmonella, Shigella,* and *E.coli O157:H7*

Table **3.5**. Number (N) and percentage occurrence (%) of bacteria in water sources.

Water sources	No. of samples	*K. pneumoniae*		*Proteus spp,*		*P. aeruginosa*		*E. aerogenes*		*E.coli*		*Shigella*		*Salmonella,*		*E.coli O157:H7*	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Rain water	58	8	13.8	1	1.7	11	19	14	24.1	24	41.4	0	0	0	0	0	0
Spring water	17	5	29.4	1	5.9	2	11.8	3	17.6	6	35.3	0	0	0	0	0	0
Municipality water	12	2	16.6	0	0	2	16.6	6	0.5	2	16.6	0	0	0	0	0	0
Total	87	15	17.2	2	2.3	15	17.2	23	26.4	32	36.8	0	0	0	0	0	0

Data presented in table **3.5** shows that 8 samples (13.8%) of rain water, 5 samples (29.4%) of spring water, and 2 samples (16.6%) of municipality water were *K.pneumoniae* positive. One sample (1.7%) of rain water, and one sample (5.9%) of spring water were *Proteus spp* positive, but municipality water was free from *Proteus spp.* Eleven samples (19.0%) of rain water, 2 samples (11.8%) of spring water, and 2 samples (16.6%) of municipality water were *P.aeruginosa* positive. Fourteen samples (24.1%) of rain water, 3 samples (17.6%) of spring water, and 6 samples (0.5%) of municipality water were *E.aerogenes* positive. Twenty four samples (41.4 %) of rain water, 6 samples (35.3%) of spring water, and 2 samples (16.6%) of municipality water were *E.coli* positive. None of these water sources were positive for *Salmonella, Shigella,* and *E.coli O157H : 7.* Fig **3.5** shows graphical presentation of these data.

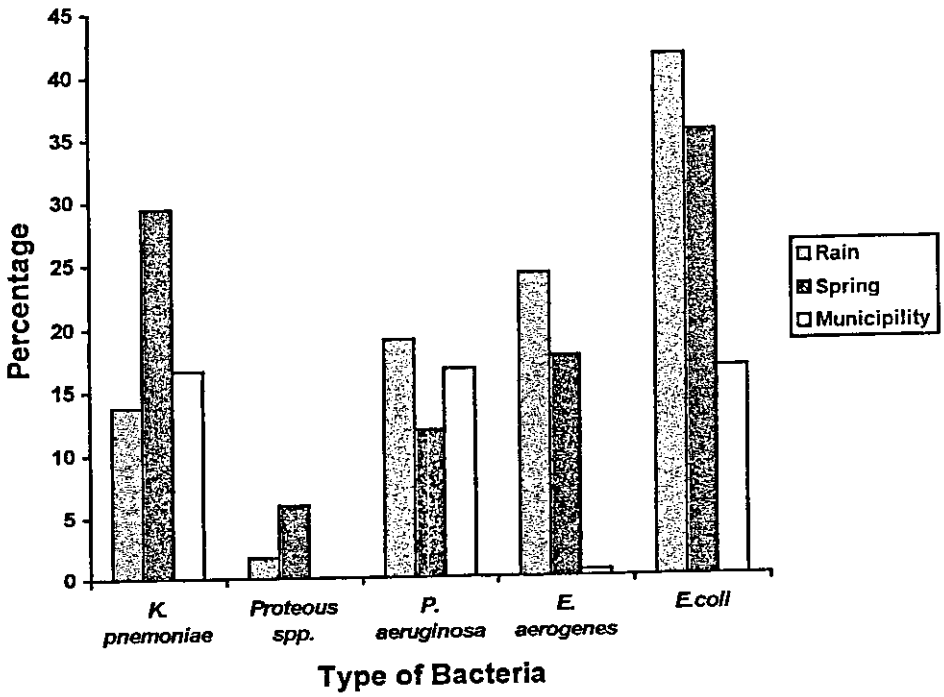


Fig.3.5 Distribution of bacteria in rain water, spring water, and municipality water (examined samples = 87).

The findings indicate that no significant difference in the degree of contamination with bacteria between the different water sources studied.

For Identification of bacteria in water, different biochemical reactions were used. Table **3.6** shows the different biochemical reactions for identification of bacteria in different studied water sources.

Table **3.6.** Biochemical reactions for identification of bacteria in water.

Colony number	Frequency	Gram stain	Oxidase	Catalase	MR	VP	Simmon	SIM			Urea	TSI				Identification
								Motility	Indole	Sulfide		Gas	H 2 S	Butt	Slant	
1	23	-	-	+	-	+	+	+	+	-	d	d	-	A	Alk/A	*Enterobacter*
2	14	-	+	+	-	-	+	+	-	-	d	-	-	Alk	Alk	*P.aeruginosa*
3	13	-	-	+	-	+	+	-	d	-	d	+	-	A	Alk/A	*Klebsiella*
4	2	-	-	+	+	-	-	+	-	+	+	+	+	A	A	*Proteus*
5	31	-	-	+	+	-	-	+	-	-	-	d	-	A	Alk/A	*E.coli*
6	0	-	+	-	-	+	-	+	-	-	-	d	d	A	Alk	*Salmonella*
7	0	-	-	+	-	+	-	-	-	-	-	-	-	A	Alk	*Shigella*
8	4	-	-	+	-	+	-	+	+	+	-	-	-	Alk	Alk	*N.I.*

TSI : Triple sugar iron agar.
MR : Methyl red .
VP : Vogas Proskaeur.
Alk: Alkaline reaction , which gives red color.
Alk / A : some strains give alkaline results, others give acidic results.
A : Acid reaction, which gives yellow color.
+ : Positive result.
- : Negative result.
d : Different strains give different results.
N . I : Not identified, need other biochemical reactions.

3.5 Prevalence and distribution of intestinal parasites according to water sources

Data presented in table **3.7** shows among the one hundred stool samples tested, to identify the prevalence and distribution of intestinal parasites in persons depending on rain water, spring water and municipal water for drinking.

Table 3.7. Number (N) and percentage (%) of parasites in stool samples.

Water sources	No . of stool samples	*G. lamblia*		*E.histolytica*		*H. nana*	
		N	%	N	%	N	%
Rain water	39	7	17.9	9	23.1	2	5.1
Spring water	46	4	8.7	6	13.0	2	4.4
Municipality water	15	1	6.7	1	6.7	0	0
Total	100	12	12	16	16.0	4	4.0

Data presented in table **3.7** shows that 7 stool samples (17.9%) taken from persons depending on rain water, 4 samples (8.7%) taken from persons depending on spring water, and 1 sample (1.2%) taken from persons depending on municipality water, were *G. lambia* positive. Nine samples (23.1%) taken from persons depending on rain water, 6 samples (13%) taken from persons depending on spring water, and 1 sample (6.7%) taken from persons depending on municipality water were *E. histolytica* positive. Two samples (5.1%) taken from persons depending on rain water, and 2 Samples (4.4%) taken from persons depending on spring water, were *H. nana* positive, but samples that were taken from persons depending on municipality water were free from *H. nana* . Figure **3.6** . shows graphical presentation of these data .

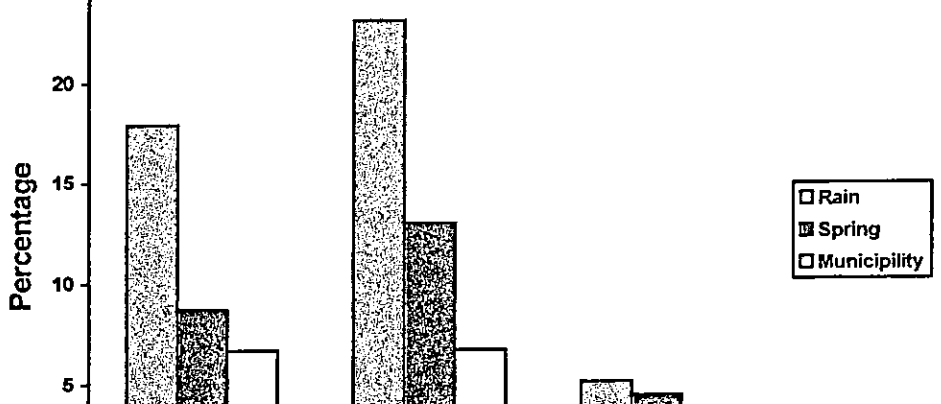


Fig . **3.6**. Distribution of intestinal parasites in persons depending on rain water, spring water, and municipality water.

Association between the sources of drinking- water and numbers of intestinal parasitic infections encountered in this study was very weak and not clear.

Table **3.8** Biochemical reactions for identification of bacteria in different stool samples .

Colony number	Frequency	Gram stain	Oxidase	Catalase	MR	VP	Simmon	SIM			Urea	TSI				Identification
								Motility	Indole	Sulfide		Gas	H 2 S	Butt	Slant	
1	15	-	-	+	-	+	+	+	+	-	d	d	-	A	Alk/A	*Enterobacter*
2	2	-	-	+	+	-	+	+		d	-	d	-	A	Alk	*Providancia*
3	15	-	-	+	-	+	-	-	d	-	d	+	-	A	Alk/A	*Klebsiella*
4	23	-	-	+	+	-	-	+	-	+	+	+	+	A	A	*Proteus*
5	32	-	-	+	+	-	-	+	-	-	-	d	-	A	Alk/A	*E.coli*
6	0	-	-	+	-	+	-	+	-	-	-	d	-	A	Alk/A	*Hafina alvei*
7	0	-	-	+	+	-	-	+	+	-	-	-	+	A	Alk/A	*Edwardsiella tarda*
8	4	-	-	+	-	+	-	+	+	+	-	-	-	Alk	Alk	N . I .

TSI : Triple sugar iron agar.
MR : Methyl red.
VP : Vogas Proskaeur.
Alk : Alkaline reaction, which gives red color.
AlL / A : Some strains give alkaline results, others give acidic results.
A : Acid reaction, which gives yellow color.
+ : Positive result.
- : Negative result.
d : Different strains give different results.
N . I : Not identified, need other biochemical reactions.

3.6 Factors that affect water quality in storage systems (cisterns and roof storage tanks).

3.6.1 Distance between the cesspits and cisterns.

Table **3.9** shows association between average total coliform and faecal colifrom counts and distances between cesspits and cisterns.

Table **3.9** Association between average counts for bacterial indicators and distances between cesspits and cisterns, using analysis of variance (ANOVA) test.

Sewage position (meter)	No . of samples	Percentage	TCC			FCC		
			Average count	F	Sig	Average count	F	Sig
< 10	35	37.2	24.77	2.344	0.102	12.31	1.323	0.271
10 – 20	45	47.9	19.51			8.97		
> 20	14	14.9	8.00			5.28		
Total	94	100	-			-	-	-

Sig = significant

According to collected data, 35 cisterns (37.2%) were less than 10 m from cesspits. The average total coliform and faecal coliform counts were 24.77 CFU / 100 ml and 12.31 CFU / 100 ml water, respectively . While 45 cisterns (47.9%) were between 10 – 20 m from cesspits. The average total colifrom and faecal clifrom counts were 9.51 CFU / 100 ml and 8.97 CFU / 100 ml water, respectively. Fourteen cisterns (14.9%) were more than 20 m from cesspits. The average total coliform and faecal coliform counts were 8.00 CFU / 100 ml and 5.28 CFU / 100 ml water. Figure **3.7** shows graphical presentation of these data.

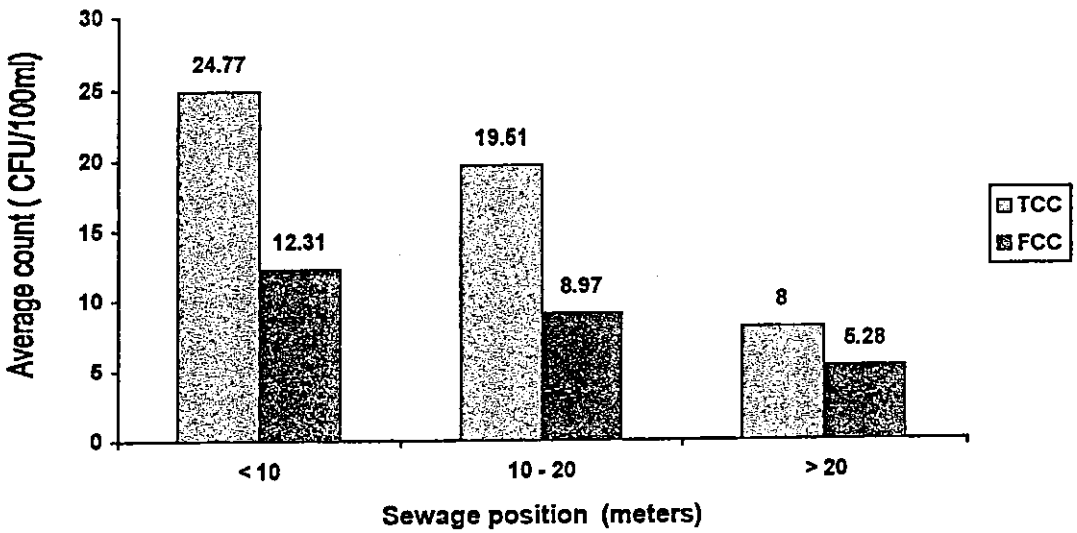


Fig. **3.7.** Average count of bacterial indicators according to cesspits distance

A noticeable decrease in both indicators is clear up to a distance of 20 meters and above.

Using analysis of variance (ANOVA) test, the computed F values of total coliform and faecal coliform counts according to distance between the cesspits and cisterns in every household were 2.344 and 0.271, respectively, this means that, there are significant difference, between cisterns located less than 20m from cesspits, and cisterns located more than 20 m from cesspits, at ($\propto = 0.05$).

3.6.2 Animals raising

Data presented in table **3.10** shows the association between average total coliform and faecal coliform counts according to animals raising in every household, using analysis of variance (ANOVA) test.

Table **3.10.** Number, percentages of cisterns according to animals raising in households, using analysis of variance (ANOVA) test.

Animal raising	No . of samples	Percentage	TCC			FCC		
			Average count	F	Sig	Average count	F	Sig
YES	40	42.6	31.85	19.79	0.000*	19.52	51.075	0.000*
NO	54	57.4	10.79			2.37		
Total	94	100	-	-	-	-	-	-

Sig = Significant

According to collected data, 40 cisterns (42.6%) were found near animal keeping areas. The average total coliform and faecal coliform counts were 31.85 CFU / 100 ml and 19.52 CFU / 100 ml water, respectively. On the other hand 54 cisterns (57.4%) were with no close animal keeping areas. The average total coliform and faecal coliform counts were 10.79 CFU / 100 ml and 2.37 CFU / 100 ml water, respectively. Figure **3.8** shows graphical presentation of these data.

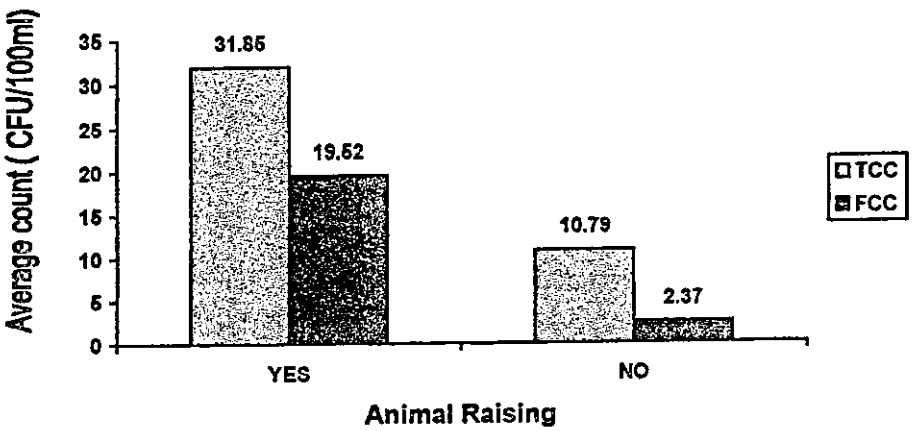


Fig. **3.8**. Average counts of bacterial indicators according to animal raising in house hold .

Using analysis of variance (ANOVA) test, the computed F-values of total ciliform and faecal coliform counts according to animal raising in household are 19.79 and 51.075, respectively, this means that, there is significant difference between animal raising in household and no animals raising in households at ($\propto$ = 0.05).

3.6.3 Cistern location .

Data presented in table **3.11** shows average total coliform and faecal coliform counts according to cistern location in every household.

Table **3.11** Numbers Perentages of cisterns according to cisterns location in every household, using analysis of variance (ANOVA) test.

Cistern location	No . of samples	Percentage	TCC			FCC		
			Average count	F	Sig	Average count	F	Sig
External	45	47.9	20.80	0.151	0.699	10.33	0.185	0.668
Internal	49	52.1	18.79			9.06		
Total	94	100	-	-	-	-	-	-

Sig = Significant

According to collected data, 45 cisterns (47.9%) were located external the house. The average total coliform and faecal coilform counts were 20.80 CFU / 100 ml and 10.33 CFU / 100 ml water, respectively. While 49 cisterns (52.1%) were located internal the house. The average total colifrom and faecal coliform counts were 18.79 CFU/ 100 ml and 9.06 CFU / 100 ml water. Figure **3.9** shows graphical presentation of these data.

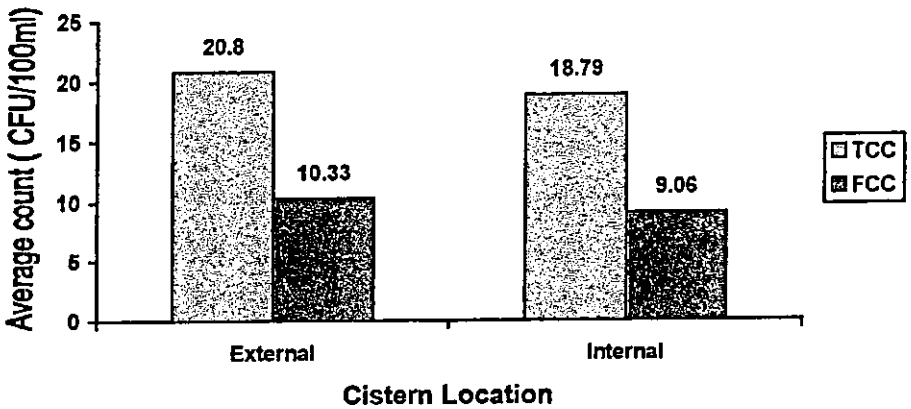


Fig. **3.9.** Average counts of bacterial indicators according to cistern location in every house.

Using analysis of variance (ANOVA) test, the computed F- value of total coliform and faecal coliform counts according to cistern location in every house are 0.151 and 0.185, respectively, this means that, there is no significant diffrence between cisterns located external the house and cisterns located internal the house at ($\propto = 0.05$).

3.6.4 Type of Storage Systems (cisterns and roof storage tanks)

Data presented in table **3.12** shows average total coliform and faecal coliform counts according to type of storage systems in very household .

Table **3.12** Number, percentage of samples according to the type of storage systems, using analysis of variance (ANOVA) test.

Tank type	No . of samples	Percentage	TCC			FCC		
			Average count	F	Sig	Average count	F	Sig
PVC	75	37.5	1.90	15.887	0.000*	0.00	15.915	0.000*
Cistern	94	47	19.75			9.67		
C. I	20	10	3.6			0.00		
Direct	11	5.5	0.00			0.00		
Total	200	100	-	-	-	-	-	-

Sig = Significant

According to collected data, 75 samples (37.5%) were taken from PVC roof strong tanks. The average total coliform and faecal coliform counts were 1.9 CFU / 100 ml and zero CFU / 100 ml water, respectively. While 20 samples (10%) were taken from C.I roof storage tanks. The average total coliform and faecal coliform counts were 3.6 CFU/100 ml and zero CFU/100 ml water, receptively. Ninety four of samples (47%) were taken from cisterns. The average total coliform and faecal coliform were 19.75 CFU/100 ml and 9.67 CFU / 100 ml water, respectively. 11 samples (5.5%) were taken directly from municipality water networks. The average total coliform and faecal coliform counts were zero. Figure **3.10** shows graphical presentation of these data.

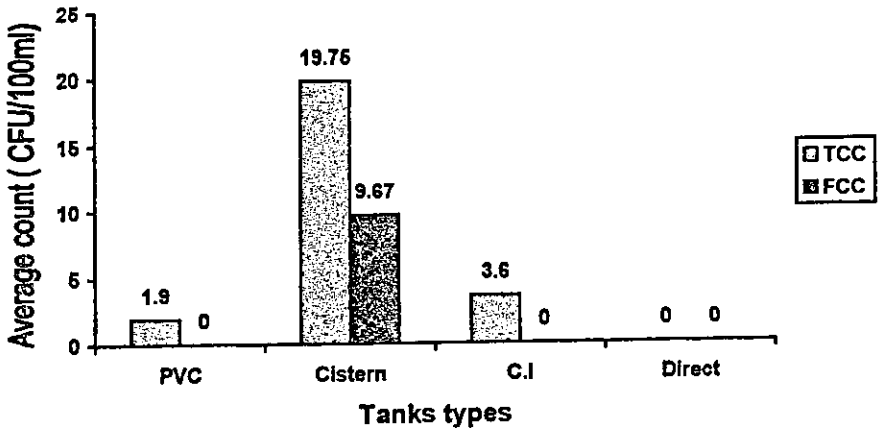


Fig **3.10**. Average counts of bacterial indicators according to type of storage systems.

Using analysis of variance (ANOVA) test, the computed F- value of total coliform and faecal coliform counts according to type of storage system in every house are 15.603 and 15.497, respectively, this means that, there are significant difference between cisterns on one side and PVC roof storage tank, C.I roof strong tanks, and those that are taken

directly from municipality water network on the other side. But there are no significant difference between PVC roof storage tanks on one side, and C.I roof storage tanks and those that are taken directly from municipality water network on the other side. Also there is no significant difference between C.I roof storage tanks and those that are taken directly from municipality water network at ($\propto = 0.05$).

3.6.5 Usage of disinfectant (chlorine)

Data presented in table **3.13** shows average of total coliform and faecal coliform counts according to usage of chlorine as disinfectant in every house hold .

Table **3.13** Number, percentages of samples according to usage of chlorine as disinfectant, using analysis of variance (ANOVA) test.

Chlorine factor	No. of samples	Percentage	TCC			FCC		
			Average count	F	Sig.	Average count	F	Sig
YES	107	53.5	2.177	48.871	0.000	0.14	47.945	0.000
NO	93	46.5	19.98			9.77		
Total	200	100	-	-	-	-	-	-

Sig = significant

According to collected data, 107 samples (53.5%) used chlorine as disinfectant . The average total coliform and faecal coliform counts were 2.177 CFU/100ml and 0.14 CFU/100ml water, respectively . While 93 samples (46.5%) do not use any disinfectant. The average total coliform and faecal coliform counts were 19.98 CFU/100ml and 9.77 CFU/100ml water, respectively.Figure **3.11** shows graphical presentation of these data.

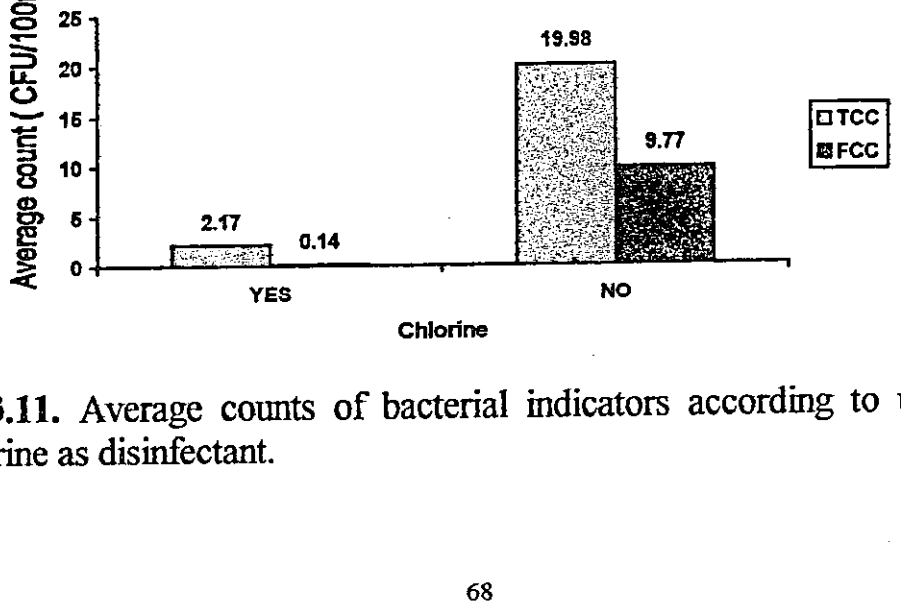


Fig.**3.11.** Average counts of bacterial indicators according to usage of chlorine as disinfectant.

Using analysis of variance (ANOVA) test, the computed F-value of total coliform and faecal coliform counts according to usage of chlorine as disinfectant in every house hold are 48.871 and 47.945, respectively, this means that, there is significant difference between the water in which chlorine is used as disinfectant and the water in which no disinfectant is used at ($\alpha = 0.05$)

3.6.6 Storage time .

A: Cisterns

Data presented in table **3.14** shows average total coliform and faecal coliform counts according to storage time in cisterns.

Table **3.14** . Number, percentages of samples according to storage time in cisterns, using analysis of variance (ANOVA) test.

Storage time	No. of samples	Percentage	TCC			FCC		
			Average count	F	Sig.	Average count	F	Sig.
<1year	10	10.5	7.3	11.864	0.000*	3.10	5.822	0.001*
1-5	75	79.7	16.1			8.6		
5-10	9	9.6	45.0			15.7		
>10	3	3.2	71.66			37.0		
Total	94							

Sig = significant

According to collected data, 10 samples (10.5%) were collected from cisterns in which storage time is less than one year, The average total coliform and faecal coliform counts were `7.3 CFU/100ml and 3.1 CFU /100ml water, respectively. While 75 samples (76.7%) were collected from cisterns in which storage time is between one to five years. The average total coliform and faecal coliform were 16.1 CFU/100ml and 8.6 CFU/100ml water, respectively. Nine samples (9.6%) were collected from cisterns in which storage time is between five to ten years. The average total coliform and faecal coliform counts were45.0 CFU/100 ml and 15.7 CFU/100ml water, respectively. Three samples (3.2%) were collected from cisterns in which storage time is more than ten years. The average total coliform and faecal coliform counts were 71.66 CFU/100ml and 37.0 CFU/100ml water, respectively. Figure **3.12** shows graphical presentation of these data.

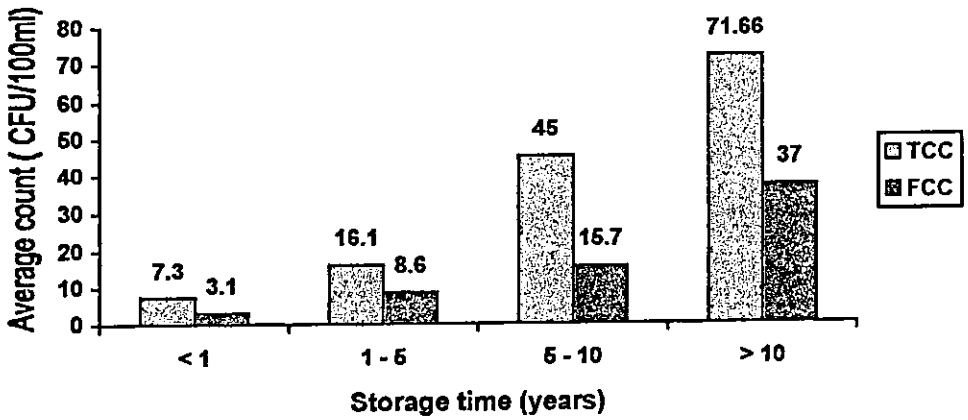


Fig.**3.12.** Average counts of bacterial indicators according to storage time in cisterns

Using analysis of variance (ANOVA) test, the computed F-value of total coliform and faecal coliform counts according to storage time in cisterns are 11.864 and 5.822, respectively, this means that, there is no significant difference between cisterns in which storage time is less than one year and cisterns in which storage time is between one to five years. But there is significant difference between cisterns in which storage time is less than one year on one side and cisterns in which storage time is between ten to five years, and cisterns in which storage time is more than ten years, on the other side.

Also there is significant difference between cisterns in which storage time is between one to five years on one side, and cisterns in which storage time is between five to ten years, and cisterns in which storage time is more than ten years on the other side. There is significant difference between cisterns in which storage time is between five to ten years and those in which storage time is more than ten years at ($\alpha = 0.05$).

B : Roof Storage tanks (PVC and C.I)

Data presented in table **3.15** shows average total coliform and faecal coliform counts according to storage time in roof storage tanks.

Table **3.15** Number, percentages of samples according to storage time in roof storage tanks, using analysis of variance (ANOVA) test.

Storage time	No .of samples	Percentage	TCC			FCC		
			Average count	F	Sig.	Average count	F	Sig.
< 1 year	43	45.3	0.907	1.371	0.319	0	-	-
1-5	51	53.7	3.25			0		
5-10	1	1	10.0			0		
Total	95		-					

Sig = Significant

According to collected data, 43 samples (45.3 %) were collected from roof storage tanks in which storage time is less than one year. The average total coliform was 0.907 CFU/100 ml water. Fifty one samples (53.7%) were collected from roof storage tanks in which storage time is between one to five years. The average total coliform count was 3.25 CFU/100 ml water. Only one sample (10 %) was collected from roof storage tank in which storage time is more than five years. The average total coliform count was 10.0 CFU/100 ml water. All samples that were collected from roof storage tanks have zero faecal coliform counts. Figure **3.13** shows graphical presentation of these data .

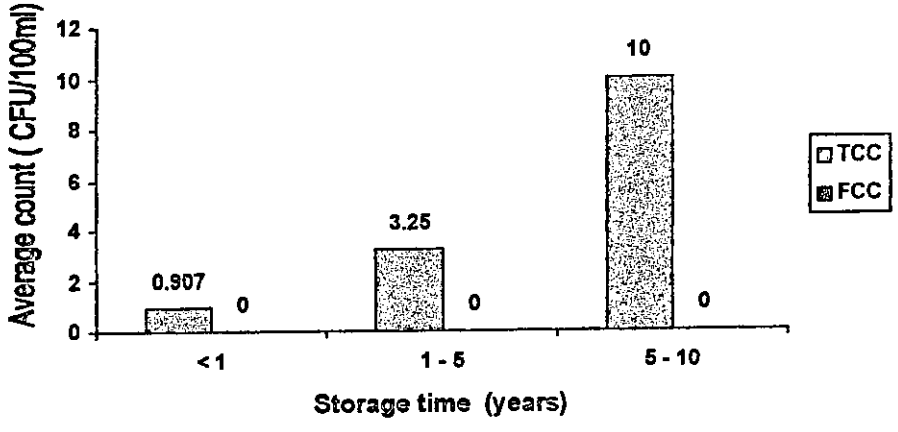


Fig. **3.13** Average counts of bacterial indicators according to storage time in roof storage tanks .

Using analysis of variance (AVOVA) test, the computed F-value for total coliform count according to storage time in roof storage tanks is 1.371, this means that, there is no significant difference between roof storage tanks with respect to storage time at ($\alpha = 0.05$).

Chapter 4

Discussion

Chapter IV
Discussion

4.1 Average counts of total coliform and faecal coliform in water sources (municipality water, rain water, and spring water)

Average counts of total coliform in municipality water, rainwater and spring water was 2.056, 20.73, and 16.79 CFU/100ml water respectively (Table 3.1).

According to international standards set by WHO, only municipality water values were within the safe limit, which is 0-3 CFU/100 ml water, but rain water and spring water values were higher than the safe limit. Such finding could be due to the fact that, only municipality water was treated by disinfectant (chlorine), and the rest (rain water, and spring water) were not treated by any of the used disinfectants for this purpose .

Average counts of faecal coliform in municipality water, rain water and spring water were 0, 9.97, and 9.26 CFU/100ml water respectively (Table 3.1)

According to the international standards set by WHO, only municipality water values were within the safe limit, which is zero, but rain water and spring water values were higher than the safe limit. The finding of higher average counts of faecal coliform *E.coli* in the studied water sources (spring water and rainwater) strongly indicates that such sources were exposed to faecal contamination .

Behind such findings in the studied villages is the improper sewage disposal (cesspits). All included households don't have sewage networks for sewage water disposal and they rely on cesspits that are designed to serve single household, these cesspits required digging into ground which increases the risk of water contamination. The risk of contamination depends on the soil permeability and drainage capacity, in impermeable and poor drainage capacity soil, frequent hypersaturation and flooding will occur, while in permeable and high drainage capacity soil rapid filtration through the soil will occur, especially during the rainy

seasons. Both hypersaturation of cesspits and rapid filtration of waste water result in contamination of these cisterns [8].

Differences for both indicators were significant between municipality water on one side, and rain water and spring water on the other side, but differences for both indicators were not significant between rainwater and spring water in storage systems. Both indicators were with very much lower values in municipality water compared to rain water and spring water (Table 1.3) . This is an expected observation, because rain water collected from roof top of the house, and storing in the house cistern , and spring water collected from a near by spring by tanker trucks and storing in the cistern, then using electric pumps, to transfer water from the cistern to roof storage tanks, which is connected to a tap in the house. From this we notice that both source of the water located under the same condition. But municipality water network connected to the roof storage tanks directly from water network. On the other hand municipality water network supply is chlorinated, from this, municipality water is safe for drinking.

4.2 Bacterial quality of water storage systems in Tubas, Tammun, El-Fara', 'Aqqaba, and Tayasir Villages.

Table (3.2) represents a comparison between different storage systems in the studied villages. Differences for both indicators were similar. Differences between both indicators were with no significant values between Tubas and El-Fara' and between 'Aqqaba on one side and Tammun and Tayasir on the other side . But differences in average counts were with significant values between Tubas and El-Fara' on one side, and Tammun,'Aqqaba, and Tayasir on the other side, also there was significant difference between Tammun and Tayasir. Both indicators were with very much lower values in Tubas and El-Fara' compared to Tammun, 'Aqqaba, and Tayasir. This is an expected observation, because most people in these two villages depend on municipality water network to supply them with drinking –water, which is chlorinated. Although 'Aqqaba village was connected to municipality water network, most people preferred to drink from their cisterns rather than municipality chlorinated water supply, because in their opinion, the taste of the cool cistern water was preferred to the warm, chlorinated municipal supply, and also because cistern water lathers better for washing. Both indicators were with very much higher values in Tammun, 'Aqqaba, and Tayasir,

so, these villages are suffering from contamination of water sources. Economical, cultural, life styles, land uses, sewage systems and geographical nature similarities are behind the finding of very similar average count values for both indicators.

4.3 Degree of contamination in storage systems.

Estimation of the degree of contamination was based on the WHO classification (Table3.3), which indicates that (86.8%) of municipality water, (16%) of rainwater, and (10.5%) of spring water were free of contamination .

Only (11.3%) of municipality water, (72%) of rainwater and (84.2%) of spring water were with first degree of contamination . While (1.9%) of municipality water, (12%) of rainwater, and (5.3%) of spring water were with second degree of contamination . According to WHO recommendation, such contamination degree is hazardous to human health, and therefore, water sources (municipality water, rainwater, spring water) required treatment that involve the regular use of disinfectant (chlorine). Whether chlorine in our storage systems is going to be effective or not, will depend on water turbidity levels and pH [8].

Water sources with second degree of contamination can be treated with agglutination, filtration, and disinfection.

4.4 Contaminated water sources as potential risk factor for human health

Estimation of risk level was based on WHO classification, which indicates that all municipality water, (37.3%) of rain water and (42.1%) of spring water were with no risk level. While (33.3%) of rain water and (26.3%) of spring water were within low risk. Only (29.3%) of rainwater and (31.6%) of spring water were within intermediate risk levels (table3.4). An increase in the risk level is an indicator of increase levels of faecal contamination, thus our finding indicates that the majority of our storage systems (cisterns) were exposed to faecal contamination from cesspits, animal raising, and latrines.

Annual reports by the Palestinian Ministry of Health (see annex II) regarding waterborne diseases show an alarming number of cases indicative of bad water quality [8].

4.5 Bacterial quality of water sources

Table 3.5 represents a comparison between the bacterial quality of the three water sources (municipality water, rain water, and spring water). Our findings indicate that all water sources examined were free from *Salmonella, Shigella, and E.coli 0157:H7.*
Municipality water was with lower percentage values for different types of bacteria. Both sources (rainwater and spring water) were similar and differences in percentage occurrence of bacteria were with no significant values. Our finding strongly indicates that both sources are suffering from contamination. Economical, social, cultural, life style, land uses, sewage systems and geographical nature similarities in most houses studied were behind the finding of very similar percentage values for different types of bacteria .

4.6 Association between intestinal parasites and water sources.

Table (3.7) represents the relationship between drinking - water source and intestinal parasitic infections. Our finding indicates that the percentage occurrence of intestinal parasite was with lower values in persons depending on municipality water (6.7%, 6.7%, and 0)for *G.lambia, E.histolytica*, and *H.nana*, respectively. While for persons depending on rain water sources, the percentage occurrence of *G.lambia, E.histolytica,* and *H.nana* was 17.9%, 23.1%, and 5.1%, respectively. However for persons depending on spring water sources, the percentage occurrence of *G.lambia, E.histolytica,* and *H.nana* was 8.7 %, 13%, and 4.4%, respectively.

This indicates that the three parasites infected persons depending on municipality water, rain water, or spring water source, which means that there were no association between drinking-water source and intestinal parasite in this study. These parasites were found in the small intestine of humans and other mammals, and this cause prolonged diarrheal disease.

4.7 Factors that affect water quality in cisterns and roof storage tanks .

4.7.1 Distance between the cesspits and cisterns.

Cesspits (improper sewage systems) are usually accused in water contamination. Thus, cisterns should be located a safe distance from all surrounding cesspits and other sources of pollution. The safe distance should be determined from the time taken by contaminates to travel from their source to the source of drinking-water, this will depend on local

condition of the area and influenced by geographical conditions, hydrological conditions, quantity of faecal matter likely to be discharged and land use and ownership. Our findings with respect to distance and level of contamination based on average counts for both indicators were associated with distance up to 20 meters away from cesspits.
Both indicators showed noticeable decrease on there average counts in cisterns far away from cesspits 20 meter and above (table 3.9).

Such finding are in agreement with the study by Birzeit University Community Health unit (1990) on water quality in the West Bank [8], with results in a study by Othman (2000) at cisterns roof storage tanks in Beit-leed and Safarine villages [8], and with results in a study by AL-Kahah (2001) at waterborne pathogenes with relation to gastroenteritis in Salfeet district [9], As we mentioned earlier it seems to be, that, it is very difficult to specify minimum distance, as this will depend on the slope of the land, as will as the soil texture.

4.7.2 Animal Raising

Animal raising is one from other possible sources of water contamination, especially during the rainy season as flooding is some times seepaging to cisterns. Association between animal raising and water contamination is clear as both indicators showed higher average counts in household with history of animal raising in the living vacinity compared to those with no history of animal raising (Table 3.10).

Our findings is in agreement with the findings of a study by Smith C. (1985) at cisterns of Abuskheidem village [30], a study by Othman (2000) at cisterns and roof storage tanks in Beit – lead and Saffarine villages [8], and a study by Al – Kahah (2001) at water borne pathogens with relation to gastroenteritis in Salfeet district [9].

4.7.3 cistern location

Trap doors location of the cisterns, are another possible source of water contamination. During the rainy season, factors which could enhance water contamination in cisterns with external trap doors such as flooded floors of houses and animals residuals. This seepaging to cisterns developed many thicker sedimentation layers that in true will increase food sources [9].

Both indicators showed similar average counts in households with cisterns having external trap doors and those with internal trap doors

(Table 3.11).This can be explained by the fact that there are many possible sources of water contamination in cisterns (distance between the cesspits and cisterns, animal raising, and cisterns age).

4.7.4 Storage time

Cisterns with increased age are most likely to develop many thicker sedimentation layers that in true will increase food sources and turbidity. These factors are most likely to have a positive effect on bacterial growth, thus influencing average counts of bacterial indicators. On the other hand, with increased age one expect to have more cracks especially in the upper part of the cisterns allowing more seepage of contamination from adjacent sources [8].

Our finding is in agreement with the above mentioned factors, as both indicators showed higher average counts with increased storage time (Table 3.14).

4.7.5 Disinfection of water

Microbiological quality of drinking – water can be enhanced by treating the raw water with chlorine which has been widely applied in treating water supplies.

A comparison between water sources that were treated by chlorine and those that were not treated for both indicators was in favor of water sources that are treated by chorine (Table 3.13).

Conclusions and Recommendations

As a result of limitation in current supplies of water in the West Bank, cisterns are used as alternative sources of water.Cisterns have a large risk of bacteriological and chemical contamination, from the water flowing on the ground surface during the rainy seasons, due to the existence of large numbers of contaminant sources in the rural area such as cesspits, animal farms, solid wastes and agrochemical. Contaminated cisterns are not recommended for use as water supply when a more satisfactory source is available, but if the satisfactory source is not available cisterns should be protected and treated from all sources of contamination [8].

The current study recommended that:

1- Roof storage tanks should be covered with a secure cover to prevent the entry of debris, contaminants and to restrict access by children and animals.

2- Roof storage tanks should be provided with a tap for withdrawl of water.

3- Roof storage tanks should be inspected, cleaned and disinfected at least once a year if the water comes from protected source, but if the source of water is not protected, the tank will require more frequent cleaning, depending on the water quality [5].

4- When a household storage tanks and pipes for drinking-water are installed, they should ideally filled with water containing 50 mg / liter of chlorine and left to stand overnight, so that the system is disinfected before use [5].

5- Effective physical protection for cisterns should be done to prevent the entrance of a surface drainage or contaminats such as [8].

a- Secure cover

b- Concrete apron around the cistern head, at least 2 meters in diameter should be above the soil level and sloped towards the drainage channel.

c- Surface drainage ditch located uphill from the cistern to intercept surface water run off and carry it away from the cistern.

6. Rainwater should be collected from clean catchment area such as house roofs. House roofs should be cleaned completely from the contaminants and debris which have been accumulated on the roof and the gutters. It is therefore, recommended that the water running of the roof after the first storm of the season and preferably for the first five to ten minutes afterwards or until it runs cleaned [5].

7- Mesh should be placed between guttering and the down pipe to prevent the entery of coarse debris[5].

8- Cistern should be emptied and cleaned completely from the water of the previous year to remove the sediments and to keep water turbidity in acceptable range.

9- Water should be pumped from cisterns to houses by electric pumps and not by buckets or other manual containers.

10- Monitoring the quality of the cisterns water by means of periodic checks for bacterial contamination by standard methods.

11- Municipalities should encourage the construction of inforced concrete cisterns.

12- The cesspits and other pollutants sources should be located downhill of cisterns wherever possible and this based on the topography, subsurface geology, land ownership and land use [8].

13- The sewage disposal systems (cesspits) should be replaced by sewage net systems.

14- A minimum safe distance for all potentially pollutant activity should be fixed during the planning stage for the water source.

15- It is essential to improve the disinfecting practices and the chlorination efficiency in the distribution networks by:

a- Rehabilitation of the non –functional dosing unit.

b- Implementation of training program on the operation and maintenance.

16- Suitable treatment process should be done to protect the consumer from pathogens and impurities that may be offensive or injurious to human health.

17- The method and intensity of treatment must depend on the degree of contamination and risk level of the water source.

18- It is necessary to clean and disinfect the main pipes when any repair has to be done and to introduce a regular flushing and disinfection program of pipes.

19- Implementation of research studies to determine the relationship between microbial contamination of drinking water and epidemic diseases.

20- Environment health education programs on the hazards of intestinal parasites and hygienic habits should be carried out as soon as possible to create awareness of the problem among children.

21- Comprehensive hygiene educational programs should be developed and implement, to insure that the community [9].

   a- Is aware of the importance of water quality and its relation to health and of the need for safe water supplies.

   b- Is aware of the hazards of intestinal parasites and its relation to the contlamination of water.

22- Municipalities should monitor the quality of water transported to homes by tankers and the sources of this water.

References

1- Bates . A . J, (2000) . Water as Consumed and Impact on the Consumer – do we Understand the variables ?
Food and Chemical Toxicology **38** (2000) 529 – 5 36. United Kigdom.

2- World Health Organization, (1997). Guidelines for Drinking Water Quality, 2nd edition,Vol .2 . Geneva.

3- World Health Organization, (1993). Guidelines for Drinking Water Quality, 2nd edition, Vol . 1 . Geneva .

4- Abdul–Jaber, Q ., Rabbo, A . A., Scarpa, D ., Qannam, Z ., and Younger, P. (1999). Wells in the West Bank, Water Quality and Chemistry. Bethlehem University, Palestinian Hydrology Group, and University of Newcastle .

5- World Health Organization , (1997). Guidelines for Drinking Water Quality , 2nd edition , Vol .3 . Geneva .

6- Smith, C., (1987) . Water Supply, Sanitation and Health in the West Bank. Birzeit University. Community Health Unit .

7- JMCC, (1994). Water the Red Line. Jerusalem : Jerusalem Media and Communication Center .

8- Othman, S . M . H, (2000). Bacterial Quality of Drinking - Water in Rain–Fed Cisterns and Roof Storage Tanks in Beit–Leed and Safarine Villages. MSc. Thesis, An – Najah National University, Nablus, Palestine.

9- Al–Kahah, A . B . I, (2001). Water – Borne Pathogens with Relation to Gastroenteritis in Salfeet District: An Epidemiological Study . MSc . Thesis, An – Najah National University, Nablus, Palestine.

10- Palestinian Central Bureau of statistics, (1999). Population, Housing , and Establishemt Census–1997. Final Results – Housing Report -Tubas District, First part. Ramallah–Palestine .

11- Moe, C . L., (1997).Water borne Transmission of infectious agent. In : C .J. Hurst, G. R . knudesn, M . J . Mclnerney, L . D . Stetzenbach and M . V . Walter (eds) . Manual of Environmental Microbiology. United State of America . PP . 136 – 147 .

12- Doyle, M . P ., Beuchat, L . R., and Montville, T . J., (1997) Food Microbiology Fundamentals and Frontiers . Washington D . C, USA : ASM Press .

13- Mitchell , R., (1972) . Water Pollution Microbiology. New York : John Wiley and Sons, Inc .

14- Hobbs, B .C, and Roberts, D . (1987) Food Poisoning and Food Hygiene . 5 th edition, London : Edward Arnold.

15- Frazier, W .C., and Westhaff, D. C. (1978) Food Microbiology, 3rd edition . London : Mc Grow .

16- WHO Expert Committee (1988) Salmonellosis Control : the rule of animal and product hygiene. Geneva : World Health Organization .

17- Koneman E . W. ; Allen S. T. ; Dowell , Jr . V. R . and Sommers, H . M. (1983) . Color Atlas and Textbook of Diagnostic Microbiology. 2nd ed. , J . B. Lippencott Company, PP. 56-70 .

18- Madigan , M . T. , Martinko, J . M . , and Parker, J . , (2000) . Brock Biology of Microorganisms , 9th edition .United States of America : Prentice-Hall, Inc .

19- Gaddad, S . M., and Rodgi, S . S., (1987). The Effect of Temperature on the Growth and Biochemical Activities of *Escherichia coli* in Sewage . Environmental Pollution **43** : 313 – 321 .

20- Rilry , L . W. , Remis , R. S., Helgerson, S . D. , Mc Gee , H . B . , Wells , J . G ., Davis , RJ et al . (1983) Haemorrhagic Colitis Associated with a rare *Escherichia coli* Serotype . *N . Engl. J . Med* , **308** , 681 – 685 .

21- Todd, E . C . D ., Szabo, R . A ., Peterkin, P ., Sharpe , A . N ., Parrington, L ., Bundle , D ., Gidney, M . A . J. and Perry, M . B . (1988) Rapid hydrophobic grid membrane filter enzyme- labelled antibody procedure for identification and enumeration of *Escherichia coli* O157 : H7 in Foods. Applied and Environmental Microbiology, **54** , 2536-2540.

22- Weeranta, R . D ., and Doyle, M . P . (1991) Detection and Production of verotoxin 1 of *Escherichia coli* O157 : H7 in food . Applied and Environmental Microbiology , **57** , 2951 – 2955 .

23- Flewett, T . H. , and Woode , G . N . (1978) . The Rotaviruses . Archives of virology , **57** : 1-23 .

24- Orabi, M . A ., (2000) . Epidemiological Study of Intestinal Parasites in Pre – School Children in AL – Jiftlic Area – Jordan Vally. MSc Thesis , An – Najah University , Nablus , Palestine .

25- US Environmental Protection Agency . (1989) . National Primary Drinking Water Regulations : filtration, disinfection, turbidity, *Giardia lambia* , viruses, *Legionella* and heterotrophic bacteria; final rul . *Fedral register*, **54** (124) .

26- Craun, G . F . (1990 . Waterborne Giardiasis . In : Meyer EA, ed Human parasitic diseases , volume 3 , Giardiasis . Amsterdam , Elsevier .

27- Hoff, G . C (1979) . Disinfection resistance of *Giardia* cysts : origin of current conccpts and research in progress .In Jakubowski, W. , Hoff . J . C . eds . Waterborne transmission of giardiasis . Cincinnati , O . H, US Environmental Protection Agency, 1979 : 231 – 239 .

28- Mcfeters, G . A, and Toranzos, G . A . (1997). Detection of Indicator Microorganisms in Environmental Fresh water and Drinking- water . In : C . J . Hurst , G . R . Knudsen, M . J . Mclnerney , L . D . Stetzenbach and M . V . Walter (eds) . Manual of Environmental Microbiology . PP . 184 – 192 .

29- Smith, C ., (1985) . Bacterial Quality of Drinking–Water in Seven Villages in the Hebron Region. Birzeit University . Community Health Unit .

30- Smith , C ., (1985), Faecal Colifrom Concentrations of Cistern and Stored Household Water in the Palestinian Village of Abu Shkheidem . Birzet University . Community Health Unit .

31- Smith , C, (1984) . Bacterial Quality of Drinking – Water in Eight Villages in the Jordan Valley . Birzeit University . Community Health Unit.

32- Smith, C ., (1985) . Seasonal Aspects of Faecal Coliform in Nine Springs in the Ramallah Area . Birzeit University. Community Health Unit .

33- Baron , E. J ., Peterson , L . R ., and Finegold, S . M . (1994) Bailey and Scotts Diagnostic Microbiology, 9th edition united States of America : Mosby .

34- Abu – Seir, S . Gh . Kh ., (2000) . Prevalence and Senovar Distribution of Some Species of Enterobacteriacea in Fresh Meat with Special Emphasis on *Salmonella* and *E . coli O157 : H7* . MSc . Thesis , An – Najah Nationa University Nablus , Palestine .

35- Bridson , E , Y . , (1995) . The OXOID Manual, 7th edition United Kingdom : Alphaorint , Alton , Hants .

36- Andrews, W . (1992) Manual of Food Quality Control 4 . Rev . 1 . Microbiological Analysis . Rome : FAO.

Appendix I
The Questionnaire

This questionnaire was used to search for possible sources of drinking -water contamination in storage systems.

Part I: General information

Name of household..... Village name:.....

Part I I : Questions

1.What are the sources of water that supply the cisterns (catchment area)?
.....

2. Is there animal keeping area in the household ? ☐ Yes ☐ No

3. Is there a cesspit in the household ? ☐ Yes ☐ No

4.How far is the cesspit from the cistern ?.....meters .

5. Is the water of the previous seasons removed out from the cisterns ?
☐ Yes ☐ No

6. How many times is the well cleaned after its construction?.....Times.

7. Is the cistern having a trap door ? Yes ☐ No ☐

8. Where the trap door of the cistern located ?

☐ External the house ☐ Internal the house ☐ Others,.....

9. Are the disinfectant materials used ? Yes ☐ No ☐

10. Was the child diagnosed with following parasites :

☐ *E.histolytica* ☐ *G. lamblia*

☐ Others, ... ☐ *H. nana*

Appendix II
*Water related diseases in Palestine in 1998**

Diseases	West Bank	Gaza Strip	Total
Hepatitis A	1558	712	2270
Malaria	1	4	5
Typhoid, Paratyphoid	33	66	99
Amoebiasis Trophozite	414	5176	5590
Giardiasis Trophozite	0	3303	3303
Shigellosis	965	15	980

*** Ministry of health-Palestine**

الملخص

الممرضات المحمولة في الماء وعلاقتها بالأمراض المعوية في منطقة طوباس – دراسة وبائية

أجريت هذه الدراسة في قرى طوباس ، طمون ، الفارعة ، عقابا ، تياسير في منطقة طوباس خلال فصل الصيف لعام ٢٠٠٠ حيث جمعت ٢٠٠ عينة ماء شرب عشوائياً من آبار الجمع ، الخزانات السطحية ، أو مباشرة من شبكة المياه التابعة للمجالس البلدية .

اشتملت الدراسة على ١٠٦ عينات مصدرها شبكة المياه و ٧٥ عينة مصدرها مياه المطر و ١٩ عينة مصدرها ماء النبع حيث تم تحليل هذه العينات باستخدام طريقة الترشيح الغشائي (Membrane filtratio method) وذلك عن طريق فحص مؤشرات التلوث المستخدمة في فحص المياه وهي بكتيريا (Total coliform) وبكتيريا Faecal coliform، والعينات التي أظهرت نتيجة إيجابية للمؤشرين السابقين تم زراعتها مرة أخرى لعزل كل من *E.coli O157:H7 ، Shigella ، Salmonella*.

دلت نتائج كل من مؤشرات الفحص البكتيري أن معدل التلوث في كل من آبار الجمع والخزانات السطحية ذات قيم أعلى مما هو موصى به من قبل منظمة الصحة العالمية للمياه الآمنة الشرب حيث كانت معدلات القراءة لبكتيريا Total coliform في المياه التي مصدرها شبكة مياه المجالس البلدية ، والمياه التي مصدرها المطر والمياه التي مصدرها النبع ٣,٠٥٦ و ٣٠,٧٣ و ١٦,٧٩ مستعمرة بكتيرية لكل ١٠٠مل ماء على التوالي .في حين كانت معدلات القراءة لبكتيريا Faecal coliform في المياه التي مصدرها شبكة مياه المجالس البلدية والمياه التي مصدرها المطر والمياه التي مصدرها النبع صفر و ٩,٩٧ و ٩,٣٦ مستعمرة بكتيرية كل ١٠٠ مل ماء على التوالي. نلاحظ من هذه النتائج ان المياه التي مصدرها شبكة مياه المجالس البلدية في الأكثر أمانا حيث يقع معظمها ضمن القيم الموصى بها من قبل منظمة الصحة العالمية للمياه الآمنة للشرب بينما التي مصدرها المطر والنبع فهي عالية التلوث حيث إنها أعلى من القيم الموصى بها من قبل منظمة الصحة العالمية للمياه الآمنة للشرب .

عند مقارنة درجات التلوث بين الخمس قرى الموجودة في الدراسة باستخدام المؤشرات السابقة تبين أن لافرق بين مستويات التلوث بين كل من طوباس والفارعة ، طمون وعقابا ، تياسير وعقابا حيث أنها متشابهة وغير دالة إحصائيا ، كما تبين انه يوجد فرق كبير بين مستويات التلوث بين كل من طوباس والفارعة من جهة وطمون ،تياسير ، عقابا من جهة أخرى وهي ذات دلالة إحصائية كما يوجد فرق بين مستويات التلوث بين كل من طمون وتياسير وهي ذات دلالة إحصائية .

فيما يتعلق بدرجة التلوث المقدرة بالاعتماد على Total coliform في كل من الماء الذي مصدره شبكة مياه البلديات أو المطر أو النبع فكانت النتائج متشابهة للعينات التي تم أخذها من مياه مصدرها المطر أو النبع حيث كانت أغلبية الخزانات السطحية ضمن الدرجة الأولى من التلوث حسب معايير منظمة الصحة العالمية ، بينما كانت معظم العينات المأخوذة من مياه مصدرها شبكة مياه البلديات خالية من التلوث (درجة التلوث صفر) حسب معايير منظمة الصحة العالمية ، أما فيما يتعلق بعامل الخطورة المقاس بالاعتماد على Faecal coliform فكانت جميع العينات المأخوذة من مياه مصدرها شبكة مياه البلديات خالية من التلوث (لاخطر) أما العينات التي تم أخذها من ماء المطر وماء النبع فقد صنفت على إنها ضمن درجات الخطورة المتدنية والمتوسطة وذلك حسب معايير منظمة الصحة العالمية .

بينت الدراسة فيما يتعلق بدرجة التلوث والبعد ما بين آبار الجمع وحفر الامتصاص ضمن الوحدات السكنية انه لا يوجد أي فروقات في معدلات قراءة كلا من المؤشرين ولغاية ٣٠متر في حين لوحظ انخفاض واضح في معدلات كلا من المؤشرين ما بعد ٣٠ متر علماً بان هذه الفروقات ذات قيم إحصائية غير دالة .

بينت الدراسة فيما يتعلق بدرجة التلوث وتربية الحيوانات في الوحدات السكنية انه يوجد فروقات في معدلات قراءة كلا المؤشرين وهذه الفروقات ذات قيم إحصائية دالة ، أما فيما يتعلق بدرجة التلوث وموقع البئر (داخل الوحدات السكنية أو خارج الوحدات السكنية) بينت الدراسة انه لا يوجد فروقات في معدلات قراءة كلا المؤشرين ، أما فيما يتعلق بدرجة التلوث ومدّة تخزين المياه في آبار الجمع والخزانات السطحية فقد بينت الدراسة انه يوجد فروقات كبيرة في معدلات قراءة كلا المؤشرين بين الآبار والخزانات السطحية التي يتم تنظيفها سنوياً وبين تلك التي لا تنظف لفترات طويلة وهذه الفروقات ذات قيم إحصائية دالة .

بينت الدراسة فيما يتعلق بدرجة التلوث واستعمال معقمات المياه (الكلور) انه يوجد فروقات كبيرة في معدلات قراءة كلا المؤشرين بين المياه التي يتم تعقيمها والتي لا يتم تعقيمها وهذه الفروقات ذات قيم إحصائية دالة ، أما فيما يتعلق بدرجة التلوث ونظام التخزين (آبار الجمع ، خزانات سطحية) فقد بينت الدراسة انه يوجد فروقات كبيرة في معدلات كلا المؤشرين بين المياه التي يتم تخزينها في الخزانات السطحية وتلك التي تم تخزينها في آبار الجمع وهذه الفروقات ذات قيم إحصائية دالة .

خلال هذه الدراسة تم جمع ١٠٠ عينة براز من أشخاص مصابين بالإسهال من قرى طوباس، طمون و الفارعة ، بمساعدة جمعية الهلال الأحمر الفلسطيني ، لجان اتحاد العمل الصحي ، وكالة الغوث الدولية، لدراسة الطفيليات المعوية السائدة وعلاقتها بمصدر المياه ، وحيث اظهرت الدراسة ان اكثر الطفيليات شيوعاً هي:

Giardia lambia 12%, Entamoeba histolytica 16%, and Hymenolepis nana 4% .

أظهرت الدراسة ان هناك علاقة بسيطة وضعيفة بين الطفيليات المعوية ومصدر الماء الذي يستعمل للشرب .