An-Najah National University
Faculty of Graduate Studies

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

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TO

MY DEAR MOTHER, FATHER,

AND MY WIFE FOR THEIR

ENCOURAGEMENT

WITH LOVE AND RESPECT

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WHO:	World Health Organization.
CFU:	Colony Forming Unit.
TC:	Total coliform
FC:	Faecal coliform
X^2:	Chi-sequre
d.f:	Degree of freedom
P-value:	Probability (Significant Level)

ABSTRACT

A total of two hundred drinking water samples were collected randomly from roof storage tanks, during the summer season from June to August 1999. The area of study includes three villages (Bruqin, Farkha, and Kafr ad-Dik) in the district of Salfeet. Both the average total coliform counts and faecal coliform counts were used as indicators for water quality using the membrane filtration technique. Also water sample positive for coliform were further cultured for the isolation of *Salmonella*, *Shigella,* and *E.coli 0157:H7.*

Both indicators gave average counts higher than that recommended as safe drinking water by the WHO. 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 counts of faecal coliform (CFU/100ml) in spring water were 9.4, in rainwater 11.4, and zero in the water network. These samples of the water network were free of contamination, while spring water and rainwater samples showed high contamination and *Salmonella* was isolated from spring water.

Based on the average counts for both indicators, no significant variations were observed on the quality of drinking water in the studied villages.

The degree of contamination based on total coliform counts in both spring and rain water was similar and the majority were with first degree

of contamination according to the WHO classification, while all water network samples were free of contamination, (degree 0).

Based on faecal coliform *E.coli* with respect to the risk levels, both spring and rain water were within low and intermediate risk levels according to WHO classification.

The households with history of animal raising, external cisterns door, distance of septic tanks from cisterns less than 20m, and not cleaned cisterns seem to have further effect on the contamination and risk levels compared to findings on households with no history of animal raising, internal cistern door, distance of septic tank from cistern more than 20m, and cistern cleaning every year.

During the period from 14 August to 19 October 1999 about 102 of stool specimens were collected from patients with diarrhea at two Public Medical Units located on Bruqin & Kafr ad-Dik villages. Prevalence rates of encountered parasites were as follows: *G. lamblia* 10.8%, *E. histolytica* 17.3%, and *H. nana* 5%. The findings indicated very weak association between drinking water source and intestinal parasites in this study.

CHAPTER I

1.1 Introduction

Water is essential to sustain life, and a satisfactory supply must be made available to consumer's [25]. A supply of drinking - water should be sufficient in quantity, whole some, and not injurious to health. The history of water – supply engineering has repeatedly shown that the provision of safe drinking – water is the most important step, which can be taken to improve the health of a community by preventing the spread of water-borne diseases [26]. Protection of water supplies from contamination is the first line of defense [25]. It was recognized that faecally polluted water is responsible for spreading enteric infectious diseases [26].

Waterborne infectious diseases caused by pathogenic bacteria, viruses, and protozoa or parasites are the most common and widespread health risk associated with drinking water [25]. Many enteric pathogens transmitted via ingestion of faecally contaminated water can also be transmitted from person to person by contact with faecally contaminated food [19].

A serious risk of disease whenever present in drinking - water include a number of pathogens such as *Salmonellas spp.*, *Shigella spp.*, pathogenic *Escherichia coli*, *Vibrio cholera*, *Yersinia enterocolitica*. *Camylobacter jejuni*, and *Campylobacter coli*, and the enteric viruses *Adenoviruses*, *Enteroviruses*, *HepatitisA*, *HepatitisE*, *Norwalk virus*, *Rotavirus*, and the

parasites *Dracunculus medinesis*, *Giardia spp.*, *Cryptosporidium spp.*, and *Entamoeba histolytica* [25].

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 [25]. Frequent examinations for faecal indicator organisms remain the most sensitive and specific way of assessing the hygienic quality of water. Faecal indicator bacteria should fulfil certain criteria to give meaningful result [25]. The bacteria selected as indicators of faecal pollution should be universally present in the faeces of humans and warm - blooded animals in large numbers. Other desirable properties of fecal indicators are that they should be readily detected by simple methods and that they do not grow in natural water [26].

The use of normal intestinal organisms as indicators of faecal pollution rather than the pathogens themselves is universally accepted for monitoring and assessing the microbial safety of water supplies [26].

1.2 Water resources in the West Bank and Gaza Strip

Since Israeli occupation of the West Bank and Gaza Strip in the 1967, Israel has been exploiting the 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 ground water wells [13,21].

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 annual Israeli consumption (with a population of nearly 5 million) is 2 milliard m^3, which gives an average of 400 m^3/capita/year, which is four times as much as the Palestinian individual consumers [21].

1.2.1 Water supply systems In the West Bank

After the Israel occupation in 1967, the West Bank has administratively divided into eight districts, namely: Jenin, Tulkarm, Ramallah, Nablus, Jerusalem, Bethlehem, Hebron and Jerico. These districts depend on water - net work supply systems (piped water) and house hold rainwater; cisterns and communal sources (unpiped water)[21]. Table 1.1 shows water 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: Othman [21].

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 [21].

Most of these villages rely on the following systems for their water supply: Household rainwater (cistern), and communal sources as wells, springs and canals. Where communal sources are used, water is transported to homes by tankers and animals.

At present most of out - water net work supply depends on, private wells (35.8%), Mekorote systems (27.6%) and West Bank department wells or springs (36.6%) [21].

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 about 30-31 mcm/year, 22 mcm from wells, 4 - 5 mcm from mekorote [21].

The average daily consumption of water for domestic and industrial sector in the West Bank was estimated as follows: 100 - 120 litters/capita / day in cities, 70 - 90 litters /capita/day in towns, and 40 - 60 litters/capita/ day in villages [21].

1.2.2 Water Supply systems In Salfeet district

In 1997, the Palestinian National Authority divided the West Bank area into 11 districts by considering Qalqillia, Salfeet and Tubas as new districts. According to this, the number of villages around Salfeet town was 23 villages with a total population number of 46688 (figure 1.1) [22]. Out of 23 villages with in this district only 14 are connected to water network system and the result still dependent on private systems (cisterns). It is worth noting that most areas with water network systems are also having private water supply systems (cisterns).

1.2.3 Water Supply systems in Bruqin, Farkha and Kafr ad Dik Villages

Bruqin and Kafr ad Dik villages are situated in the western part of Salfeet city. They are about 17 - 19 km far from the city, while Farkha situated in the southern part of salfeet and far from it about 3 km (figure1.2). The three villages are dependent on cisterns water supply. Table 1.2 Shows Population, buildings, cisterns, septic tanks and other services, in the three villages studied.

Table 1.2. Population, buildings, cisterns, septic tanks, and other services in the three villages*.

Village	No. of building	No. of Population	No. of Cisterns	No. of Septic tanks	No. of Buildings connected to water network
Bruqin	392	2659	392	381	-
Farkha	150	1115	140	146	10
Kafr ad-Dik	541	3747	541	535	-

*After: Palestinian Central Bureau of Statistical. 1999.[22].

1.3 Water Storage Systems

The three villages studied are without a piped water supply, the majority of households drinking from rain fed underground cement cisterns supplied by roof tap catchments. The average rainfall in the area is 660 mm/year, which occurs predominantly between the months of October and April table 1.3. [11].

Table 1.3. Average of precipitation in Salfeet area*.

Year	Mm/year	Year	Mm/year
1972/1973	986.7	1986/1987	Not count
1973/1974	749.55	1987/1988	811.5
1974/1975	580.8	1988/1989	418.5
1975/1976	640.5	1989/1990	Not count
1976/1977	646.2	1990/1991	433.2
1977/1978	433.2	1991/1992	411.25
1978/1979	952.6	1992/1993	1223
1979/1980	815.9	1993/1994	836
1980/1981	621	1994/1995	643
1981/1982	1063.5	1995/1996	807
1982/1983	566.8	1996/1997	648
1983/1984	536.4	1997/1998	745
1984/1985	536.04	1998/1999	762
1985/1986	157.1	1999/2000	555

*After: Ministry of agriculture - Salfeet.

1.3.1 Cisterns

Cisterns are artificial reservoirs for collecting and storing rainwater from impermeable area. For a long time, Palestinians have been constructing cisterns to collect and store the rainfall from the roofs of their houses.

Harvested rainwater have been used for domestic and irrigation purposes, and it constitutes favorable alternative source of water. Due to the cost consideration and the limitation in current water supplies, with respect to

quantity and quality, cistern of rainwater have started to attention as available and important source of water [21,23].

Many cisterns, especially those that were constructed more than 50 years ago, have a pear shape that is usually pasted with a relatively thin layer of cement. Currently, cisterns are designed and built with international standards and most municipalities encourage rain forced concrete cisterns of various shape and size. Cisterns are usually equipped with overflow system and their openings are securely covered in order to prevent the entry of pollutants that may contribute to water contamination. Electrical pumps are usually used to pump water from a cistern to small-galvanized metal reservoirs (roof storage tank) [21,3].

Possible sources that affect the bacterial quality of water in cisterns include, presence of septic tanks, the distance between septic tanks and cisterns, animal razing, water catchment area (water sources), washing of cisterns and treatment methods. To search for these possible sources a questionnaire was used (see appendix I).

1.3.2 Roof storage tanks

A storage tank is commonly used to ensure water availability for the family needs. A storage tank is made of metal, plastic or asbestos with volume range (1-2) m^3. Most roof storage tanks are supplied with secure covers and usually fitted on metal stand [21].

1.4 Persistence and Infective Dose of Water Pathogens

After leaving their host, pathogens gradually lose viability and their ability to infect. The numbers of a pathogen introduced on a given occasion will tend to decline exponentially with time, reaching in significant and undetectable levels after a certain period [26]. Pathogens with low persistence must rapidly find a new host and they are more likely to be spread by person contact rather than by drinking contaminated water. Because faecal contamination is usually dispersed rapidly in water the most common waterborne pathogens and parasites are those that have high ineffectivety or possess high resistance to decay out side the host body [21].

The persistence of most pathogens in water is affected by various factors, of which sunlight and temperature is among the most important. Lifetimes are shorter, at warmer temperature [26]. Table 1.4 Shows the persistence time of some excreted pathogens in fresh water at 20 - 30°C [21].

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 cholera*	<5
Protozoa -*Entamoeba Histolytica cysts*	<15
Helminthes- *Ascaris Lumbricoides eggs*	Many months

*After: Othman [21].

Infective dose (ID) determines the number of organisms needed to produce either a clinically apparent infection or intestinal colonization in human subjects, ID_{50} (dose required to cause infection in 50% of healthy adults) [26]. Experimental studies of infectivity provide relative information, but it is doubtful whether the infective doses obtained are relevant to natural infections. 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 cannot normally multiply in water and they also have the tendency to disperse [21].

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 [21].

After ingestion of the pathogen, the development of an infection depends on the balance between host factors, such as gastric acidity and intestinal immunity, tending to remove it, and factors aiding the bacteria in their attempt to colonize the intestine [26].

1.5 Microbial indicators of water quality

The coliform organisms are well established as faecal indicators for water contamination. The finding of large numbers of these organisms in food and water is taken to indicate faecal pollution or contamination since

the water – borne diseases are generally intestinal diseases, the existence of pollution is taken to indicate the possibility that the etiologic agents of these diseases may be present. Whether or not intestinal pathogens are present, the presence of faecal matter in water is undesirable [1].

Indicator organisms are used globally as a warning of possible contamination and as an index of water quality deterioration [16].

Gastroenteritis is the most common affliction associated with water borne pathogens. Al though for most of the population in developed countries minor gastroenteritis may simply mean several hours of discomfort, in developing countries up to 10 million people die every year as a direct result of the consumption of contaminated water [16].

The presence of enteric pathogens in drinking and recreational waters is of great concern. As a result of the danger to public health due to the presence of pathogens [16].

The following groups of microorganisms are used to determine the biological safety of the waters:

1.5.1 Total Coliforms

They are aerobic and facultatively anaerobic, gram – negative, nonspore forming, rod-shaped bacteria that ferment lactose with gas and acid production in 24 to 48h at 35°C [16].

Coliform bacteria belong to the family *Enterobacteriaceae* and usually include *Escherichia coli* as well as various members of the genera *Enterobacter* , *Klebsiella* , and *Citrabacter* [16].

These bacteria are classically used as indicators of faecal contamination or water pollution from sewage and thus are of sanitary significance [16].

The persistence of these bacteria in aquatic systems is comparable to that of some of the water borne bacterial pathogen [16].

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 [10,21]. Table 1.5. Shows this classification.

Table 1.5. WHO classification for contamination degree and treatment methods.*

Total coliform count	Contamination Degree	Treatment method
0 - 3	0	-
Greater than 3 to 50	1	Disinfection
Greater than 50 to 50,000	2	Agglutination, filtration, disinfection
> 50,000	3	Special treatment

*After: Othman [21].

1.5.2 Faecal coliforms

These are defined as the group of coliform organisms that are able to ferment lactose at 44-45°C. They comprise the genus *Escherichia* and to a lesser extent, species of *Klebsilla*, *Enterobacter*, and *Citrobacter*. Of these organisms, only *E. coli* is specifically of faecal origin, being always present in the faeces of humans, other mammals, and birds in large numbers, and

rarely found in water or soil that has not been subject to faecal pollution [26].

E. coli is abundant in human and animal faeces, where numbers may attain 10^9 per gram of fresh faeces table 1.6. The presence of *E.coli* in water always indicates potentially dangerous contamination requiring immediate attention [26]. *E.coli* is a member of the family *Enterobacteriaceae*, it grows at 44 - 45°C on complex media, ferments lactose and mannitol with the production of acid and gas, and produce indole from tryptophan [25]. Some strains can grow at 37°C, but not at 44 - 45°C, and some don't produce gas. *E.coli* dose not produces oxidase or hydrolyse urea [25].

Table 1.6. Number of indicator bacteria commonly found in human faeces*.

Indicator	**Cells per gram of faeces (wet weight)**
Bacteroides spp.	10^7 - 10^{11}
Bifidobacterium spp.	10^7 - 10^{11}
Clostridium perfringens	10^3 - 10^{10}
Coliforms Faecal	10^6 - 10^9
Nonfaecal	10^7 - 10^9
Faecal streptococci	10^5 - 10^8

*After: Moe, C. L. [19].

Ingestion, contact, or inhalation of water has transmitted numerous infectious agents. Disease out comes associated with water borne infections include mild to life – threatening gastroenteritis, hepatitis, skin infections, wound infections, conjunctivitis, respiratory infections, and generalized infections [19]. Most microbial waterborne pathogens of concern originate in the enteric tracts of humans or animals and enter the aquatic environment via faecal contamination. The concentration of these pathogens in a

community water supply will depend in part on the number of infected persons and / or animals in the community and the opportunities for faeces from these individuals to enter the water supply [19,20].

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 [25,21]. WHO classification is shown in table 1.7.

Table 1.7. WHO classification for *E.coli* counts and risk*

Count / 100 ml	Risk
0	No risk
Greater than 0 to 10	Low risk
Greater than 10 to 100	Intermediate risk
Greater than 100 to 1000	High risk
Greater than 1000	Very high risk

*After: Othman [21].

1.5.3 Faecal streptococci

The term "faecal streptococci" refers to those *Streptococci* generally present in the faeces of humans and animals. All possess the lancefield group D antigen. Taxonomically, they belong to the genera *Enterococcus*, and *Streptococcus* [25]. The genus *Enterococcus* has recently been defined to include all *Streptococci* sharing certain biochemical properties and having a wide tolerance of adverse growth condition. It includes the species *E.avium, E.casseliflavus, E.cecorum, E.durans, E.faecalis, E.faecium, E.gallinarum, E.hirae, E.malodoratus, E.mundtii,* and *E.solitarius.*

Most of these species are of faecal origin and can generally be regarded as specific indicators of human fecal pollution under many practical circumstances [26].

In the genus *Streptococcus*, only *S.bovis* and *S.equinus* possess the group D antigen and are members of the faecal streptococcus group. Their sources are mainly animal faeces. Faecal streptococci rarely multiply in polluted water, and they are more persistent than *E.coli* and coliform bacteria [25].

1.5.4 Sulfite - reducing clostridia

These are anaerobic, spore-forming organisms, of which the most characteristic, *Clostridium perfringens*, is normally present in feces, though in much smaller numbers than *E.coli* [26]. They are not exclusively of faecal origin and can be derived from other environmental sources. Their presence in disinfected waters may thus indicate deficiencies in treatment.

1.5.5 Bacteriophages

Bacteriphages are viruses that infect bacterial host cells. They usually consist of a nucleic acid molecule (genome) surrounded by a protein coat (capsid). Bacteriophages may contain either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) as the genome and may have a very simple, cubic structure or a more complex one with heads, tails, tail fibers, or other

and skin, such as trachoma, conjunctivitis, and scabies, that are related to lack of water for bathing.

Control of these diseases is through provision of greater quantities of water, closer, easier access to water, and education to improve personal and domestic hygiene [19].

1.6.3 Water- based infections

Water-based infections are worm infections in which the pathogen must spend apart of its life cycle in the aquatic environment, and these are divided into diseases acquired by ingestion of water and diseases acquired by contact with water as species of the trematode genus *Schistosoma.*

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, or rivers or to possibly treated water in swimming pools. Illness from recreational water contact can be due to enteric organisms in faecal contaminated water. Outbreaks of recreational water have involved *Giardia* and *Cryptosporidium* species, *Shigella sonnei*, and *Escherichia coli 0157:H7* that presumably entered the gastrointestinal tract via ingestion. Other recreational water out breaks of indigenous aquatic organisms such as *Naegleria, Pseudomonas, Legionella spp.*, several *Vibrio spp*, and several *Mycobacterium spp.* [19].

Epidemiological and microbiological studies indicate that *Staphylococcus aureus* skin and ear infections are often associated with recreational use of water.

Vibrio vulnificus can cause serious wound infections when an injury to skin occurs in marine water [19].

Cyanobacterial toxins have been associated with contact irritation after bathing in marine waters or freshwaters.

An additional cause of recreational water infections are the *Leptospira spp.*, which are neither enteric organisms nor aquatic organisms but enter water via the urine of infected domestic and wild animals [19].

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. These at times may be found inside houses for instance, water tanks or storage jars [19,24]. Control of these infections is through the application of pesticides, destruction of breeding grounds, and construction of piped water supplies [19].

1.7 Waterborne Pathogens

Microorganisms transmitted in water generally grow in the intestines and leave the body in faeces.

Faecal pollution of water supplies may then occur, and if the contamination is not identified or eliminated by disinfecting, then a new host may consume the water and the pathogen may colonize the intestine and cause disease [15].

The majority of human diseases associated with microbial contaminated water are infectious in nature and the pathogens are including numerous bacteria, viruses, and protozoa [2] table 1.8.

Table 1.8. Waterborne pathogens and their significance in water supplies.*

Pathogen	Health significance	Main rout of exposure[a]	Resistance to chlorine[b]	Relative infective dose[c]
Bacteria				
- *Campylobacter jejuni*	High	O	Low	Moderate
- Pathogenic *E.coli*	High	O	Low	High
- *Salmonella typhi*	High	O	Low	High
- Other salmonella	High	O	Low	High
- *Shigella spp.*	High	O	Low	Moderate
-*Vibrio cholerae*	High	O	Low	High
- *Pseudomonas 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
- Norwalk virus	High	O	?	Low
- Rotavirus	High	O	?	Moderate
- Small round virouses	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. [26].**

1.7.1 Bacteria

1. *Salmonella*

The genus *Salmonella* is a member of the family Enterobacteriaceae [26]. It is motile Gram - negative rod that dose not produce spores. It is aerobic and grows well on most nutrient media. The temperature optimum for growth is 37°C.

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 be caused by *S. paratyphi,* which is transmitted only by humans or by other *Salmonellae* carried by animal's [5].

Salmonella spp. are able to invade the intestinal mucosa. Some strains produce an enterotoxin. *S. typhi*, *S.paratyphi* Λ, and *S. cholera – suis* are more likely to invade systemically, entering the blood stream and causing serious febrile disease. Because *Salmonella spp.* are susceptible to gastric acid, it was thought that a large inoculum (approximately 10 organisms) must be ingested. One third of all cases of Salmonellosis occur in children less than 5 years old [2].

Salmonellae are excreted in the faeces of infected humans or animals. Faecal contamination of ground water or surface water is the main causes of epidemic water borne outbreaks caused by *Salmonella spp.*

Salmonella can be found in open wells as a result of the drainage or flooding of contaminated surface water in to unprotected well shafts. Penetration of pathogens into water sources must be avoided by the protection of ground water and surface water catchment areas [26].

2. *Shigella*

Shigella is a non- motile Gram- negative rod that does not form spores. It can be differentiated from *Salmonella* by the absence of motility and by the 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 characteristic is used to differentiate them from *E.coli* [5].

Contamination of drinking water or food with faeces containing *Shigella* causes epidemics of bacillary dysentery. *Shigella* is excreted in faeces and urine during the active phase of the disease. The organism does not grow in natural waters and rarely survives more than 10 days.

Shigella spp. are carried primarily by humans and are not disseminated in nature. The organism is able to resist gastric acidity, allowing a very small inoculum (as few as 10 organisms) to cause disease. The organisms invade the epithelial cells of the large bowel, causing a dysentery-like syndrome [2]. Symptoms of shigellosis may vary form a mild transitory diarrhea to

severe prostrating attacks accompanied by high temperatures, vomiting, and profuse bloody stool [18].

3. *Vibrio cholerae*

Cholera is caused by a short curved Gram- negative rod, aerobic, motile, and non-spore forming. It causes the disease cholera in human, which results from eating food or drinking water contaminated by faeces containing the Vibrios [5]. *Cholera* is characterized by sudden diarrhea with profuse, watery stools, vomiting, rapid dehydration, and complete collapse [18].

Studies with human volunteers have shown that the, acidity of the stomach is responsible for the large inoculum needed to initiate cholera. Although the ingestion of 10^8-10^9 cholera vibrios is generally required to cause cholera, human volunteers given bicarbonate to neutralize gastric acidity developed the disease when only 10^4 cells were administered. Cholera vibrios attach firmly to small intestinal epithelium and grow and release enterotoxin. The enterotoxin causes huge fluid losses of up to 20literes per day.

Control of cholera depends primarily on satisfactory sanitation measures, particularly in the treatment of sewage and the purification of drinking water [15].

4. *Escherichia coli*

E. coli is a Gram - negative, rod- shaped bacterium belonging to the family Enterobacteriaceae, non- spore - forming, growth is aerobic or facultatively anaerobic.

Metabolism is both respiratory and fermentative; acid is produced by the fermentation of glucose and lactose [4].

E.coli is a normal inhabitant of the intestinal tract of man and many other animal species. *E. coli* is also an important cause of gastrointestinal disease which is characterized by a profuse watery diarrhea with little mucus and no blood [4,9].

Four classes of pathogenic *E.coli* responsible for diarrhea are recognized enteropathogenic, enteroinvasive, enterotoxigenic, and verocytotoxin producing [26].

Enterohemorrhagic *E.coli O157:H7* is a pathogenic strain of *E.coli* that produces two potent toxins. This organism causes bloody diarrhea, and 2 to 7% of infections result in hemolytic uremic syndrome, in which the erythrocytes are destroyed and the kidneys fail [19].

Two waterborne out breaks 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

6. *Cyanobacteria:*

Blooms of *Cyanobacteria* occur in lakes and reservoirs used for potable supply. Three types of toxin can be produced, depending upon species:

- Hepatotoxins, produced by species of *Microcystis, Oscillatoria, Anabaena*, and *Nodularia* in brackish water.
- Neurotoxins, produced by species of *Anabaena, Oscillatoria, Nostoc, Cylindrospermum,* and *Aphanizomenon.*
- Lipopolysaccharides, from number of species [25].

Acute health effects in humans include gastroenteritis, liver damage, nervous system damage, pneumonia, 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 cyanobacterial hepatotoxins in drinking water. A 1976 outbreak of intestinal illness in Pennsylvania was associated with a cyanobacterial bloom in municipal water supply and affected 62% of the population [19].

Control of *Cyanobacteria* is problematic since several studies indicate that the toxins can remain potent for days after the organisms have been destroyed by copper sulfate or chlorination [19]. Only activated carbon and ozonation appear to remove or reduce toxicity. There are insufficient data allow guidelines to be recommended, but the need to protect impounded surface water sources from discharges of nutrient rich effluents is emphasized [25].

1.7.2 Viruses

The viruses of greatest significance in the water borne transmission of infectious disease are essentially those that 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 [26].

Viruses are the smallest obligate intracellular parasites known to man. They range in size from 20 to 300 nm. There are more than 100 types of enteric viruses that are excreted in faeces and infect the gastrointestinal tract and other parts of the human body [17].

1. *Hepatitis E virus*

HEV is a single serotype of virus that has single-stranded, positive –sense RNA. It is believed to be either a new RNA virus or a member of the *Calicivridae* family [19].

The virus that causes infectious hepatitis is the only documented waterborne viral pathogen. Numerous outbreaks of infectious hepatitis have been traced to faecal contamination of either drinking water supplies or shellfish bed [2,3].

Explosive outbreaks of infectious hepatitis are common, particularly where water treatment has failed. An epidemic in New Delhi, India in 1955 was traced to faecal contamination of the water supply. Between 20,000 and 200,000 cases of infectious hepatitis occurred. The virus is excreted in faeces and urine during and directly after the active phase of the disease [3].

HEV cause infection of the liver typically accompanied by lassitude, anorexia, weakness, nausea, vomiting, headache dark urine, and abdominal discomfort. When hepatitis, severe it may cause death from liver failure, or may result in chronic disease of the liver [26].

Control of infectious hepatitis is dependent on adequate treatment of sewage and water. Viruses are more resistant to chlorination than bacteria [2,3].

2. *Rota B virus*

Rota B virus was first reported in connection with a waterborne outbreak in china in 1984 and differs from the group A Rota virus strains that commonly cause pediatric diarrhea. Occur more frequently in adults than children, are associated with severe, cholera like illness, and have been reported mainly in china [19].

Rotaviruses have been detected in sewage, rivers, and lakes and in treated drinking -water in some, countries. Transmission occurs via the faecal-to-oral route. They infect and multiply in small intestine, and are

excreted in large numbers, as many as 1,000 virus particles may be present per gram of faeces for approximately 8 days after the onset of symptoms [26].

3. *Adenoviruses*

These viruses are found in small numbers in faeces of some patients with diarrhea, but occasionally in great numbers [4]. Waterborne transmission occurs by the faecal-to-oral rout, by inhalation of adenovirus aerosols into the lower respiratory tract, and by eye contact when the conjunctival surface is mildly irritated [26].

4. *Coronaviruses*

Are pleomorphic, enveloped RNA viruses that are well-established causes of diarrhea in animals. They were first observed in faeces of person with gastroenteritis by electron microscopy in 1975. Epidemiologic evidence suggests that faecal – oral transmission and personal hygiene may be key factors in transmission, since several studies noted that the highest prevalence, rates were among populations with low socioeconomic status and poor personal hygiene [19].

5. *Norwalk virus*

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, and various food items.

NV has been proposed as a member of the *Caliciviridae* family. NV and related viruses may be responsible for 23% of waterborne outbreak of acute gastroenteritis in the United States [19].

Norwalk virus infects the villa of the jejunum. Virus shedding in stools occurs during the first 72 hours after the onset of illness. The virus is transmitted by the faecal-to-oral route. Of all Norwalk-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. Infection result in delayed gastric emptying, nausea, vomiting, and abdominal cramps. Infection may be spread by municipal water systems, recreational swimming and stored water [26].

1.7.3 *Protozoa*

Drinking water plays a major role in the spread of three of the intestinal protozoa pathogenic for humans, namely *Giardia intestinalis, Cryptosporidium parvum*, and *Entamoeba histolytica. Balantidium coli* infection is uncommon, although the parasite has a worldwide 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, the *Naegleria*

fowleri and *Acanthamoeba spp.* Are associated primarily with recreation and the inhalation of warm soil, contaminated water [26].

1. *Giardia*

Giardiasis is an acute form of gastroenteritis caused by the protozoan parasite *Giardia lamblia. G.lamblia* is a flagellated protozoan that is transmitted to humans primarily by contaminated water, although food borne and even sexual transmission of giardiasis has been documented. The protozoal cells, called trophozoites produce a resting stage called a cyst, and this is the primary form, transmitted by water. Cysts germinate in the gastrointestinal tract and bring about the symptoms of giardiasis: An explosive, watery diarrhea and intestinal cramps, nausea, and malaise [15]. The time between 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 days with a median value of 8-15 days [26].

2. ***Cryptosporidiosis***

Human Cryptosporidiosis was first described in 1976, and the first reported waterborne outbreak in 1984.

Recent evidence indicates that *Cryptosporidium* species is the third most common enteric pathogen worldwide [2].

Human and other mammals are reservoirs for infection, and the contamination 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 – resistant pathogens known.

C. parvum is the major species responsible for clinical disease in humans and domestic animals, and the infective dose is thought to be small [26].

Cryptosporidiosis is characterized by severe diarrhea (80-90% of cases). Gastrointestinal symptoms, which may be accompanied by an influenza-like illness (20-40% of cases), including vomiting, and anorexia [26].

3. Entamoeba histolytica

E. histolytica is distributed worldwide and exists in trophozoite and cyst stages. Infection occurs by ingestion of cysts [26].

E. histolytica, the causative agent of amebiasis, is a common pathogenic protozoan transmitted to humans primarily by contaminated water and occasionally by the food borne rout.

The trophozoites of *Entamoeba* produce cysts. The cysts cause infestation, and cyst germination occurs in the intestine, where amebic cell grow both on and in intestinal mucosal cell [15]. The disease may manifest it self as either a mild diarrhea or chronic dysentery.

The amoeba produces cysts that can survive as long as 6 months in natural water. Thy are not resistant to chlorination. Amebiasis is eliminated in communities using chemically treated drinking water [2,15].

1.8 Current Status of Water Supply and Quality in Palestine

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

A study by Smith C. (1985), at cisterns of Abu Shkheidem village [23]. Indicates that the average quality of 75 cistern in village was 5.01 CFU of faecal coliform /100ml, the average of faecal coliform concentration in cisterns where electric pump was used was 2.61 CFU of faecal coliform/100ml, while in cisterns where buckets were used for removal of water was 7.61 CFU/100ml.

A study by Birzeit University Community Health Unit (1990) on water quality in the West Bank [21]. Presents a brief definition of clean drinking water, the concentration of faecal coliform is 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 that 100 faecal coliform/100 ml,

while cisterns which were fed from home yards had 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 at a shorter distance from soakage pits.

A study by Othman S. M. H. (2000) at cisterns and roof storage tanks in two villages (Beit-leed and Safarine) in the district of Tulkarm [21]. Indicates that the total coliform counts were 16.1 and 12 CFU/100ml water in cisterns and roof sorage tanks respectively, while average counts of thermotolerent coliform *E.coli* were 7.0 and 5.4 CFU/100ml water for cisterns and roof storage tanks respectively.

The results in this study indicats decrease in both indicators is clear up to adistance of 22 meters and above between cesspits and cisterns, variations up to 28 meters were of no significant values according to our finding with respect to total coliform count and thermotolerent coliform *E.coli*. Our findings regard cistern age indicate a significant increase in both indicators reflecting an increase degree of contamination and risk levels with increased cisterns age.

1.9 Objectives

The present work was aimed to determine:

1) Biological study of drinking water in Salfeet district to determine the sanitary quality of water by using total coliform and faecal coliform counts as indicators.

2) Isolation of pathogens *Salmonella, Escherishia coli 0157:H7* and *Pseudomonas aeruginosa* from water.

3) Searching for possible sources of water contamination.

4) Microscopy and culture of stool from people with gastroenteritis in the area to determine the type of the infecting pathogens.

5) An epidemiological study to determine if there is a relation between contamination of water and infection of people by pathogens isolated from the stool samples.

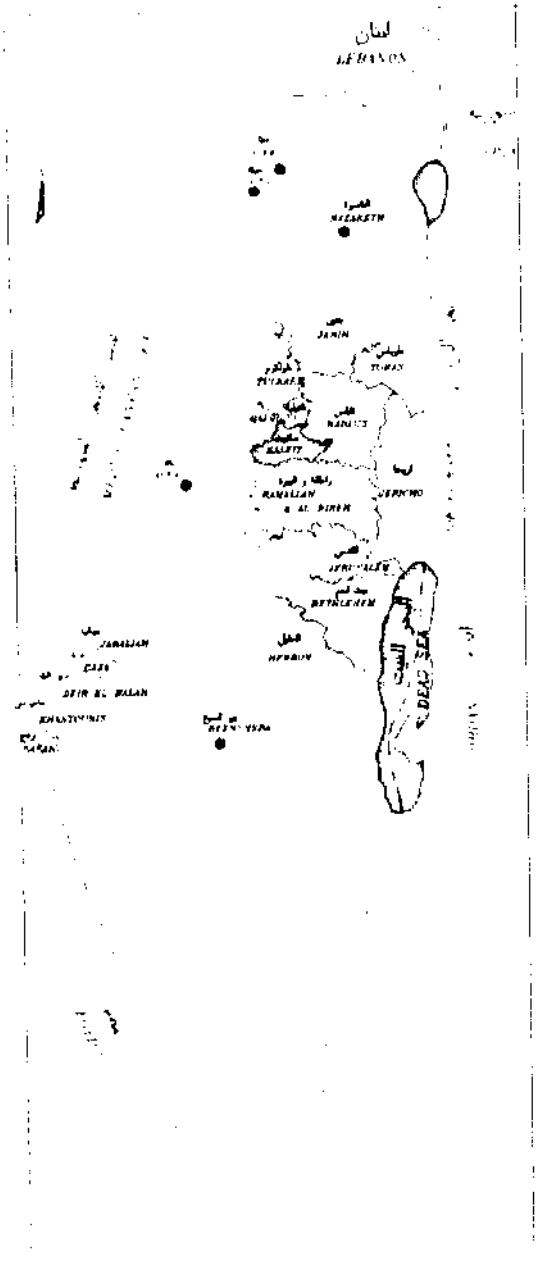

Fig.1.1. Palestine map including the study area (Salfeet district) [22].

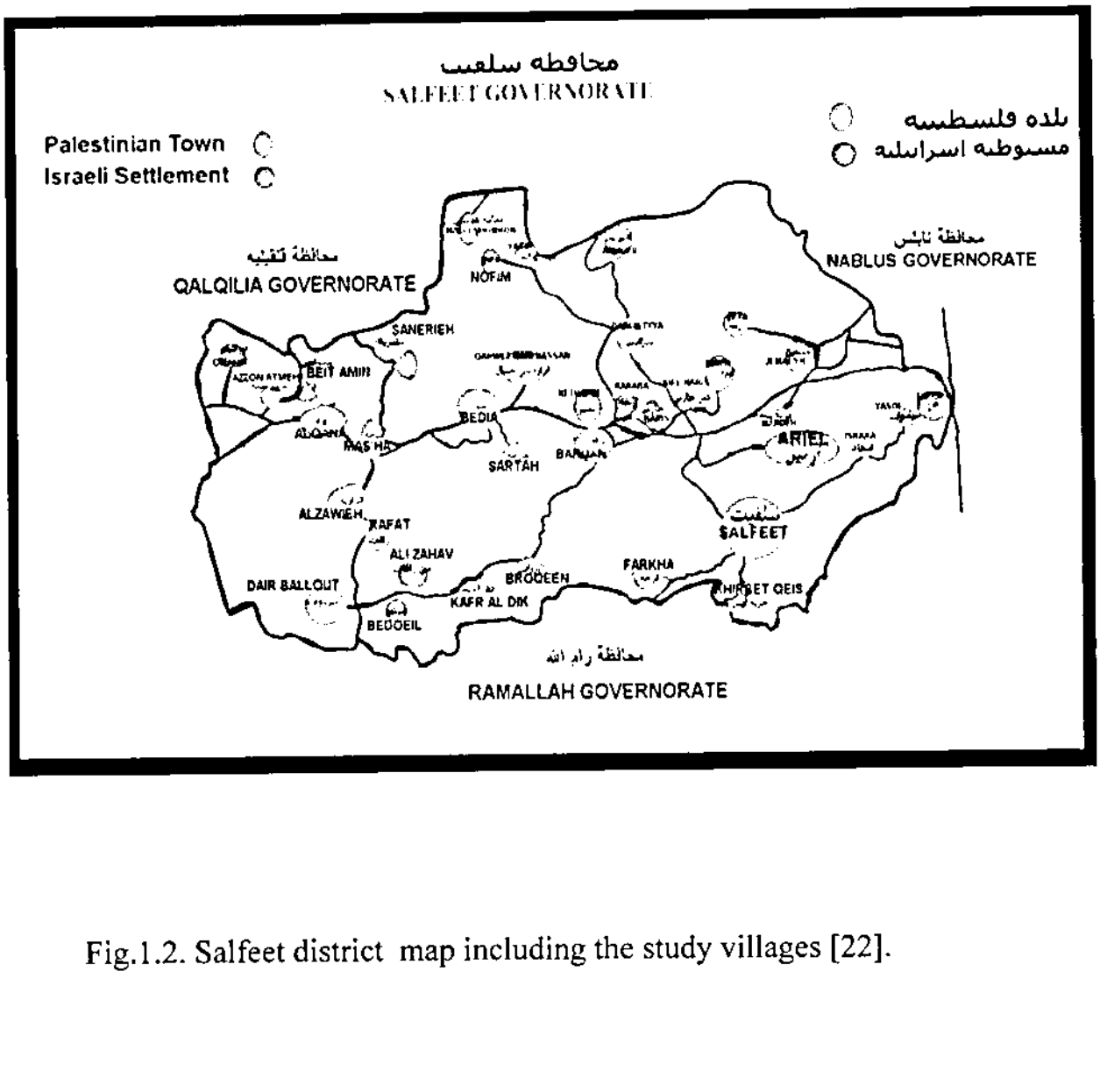

Fig.1.2. Salfeet district map including the study villages [22].

CHAPTER II

METHODOLOGY

2.1. Water sample collection

A total of 200 samples of water including 57 rainwater, 133 spring water and 10 municipality water, were collected randomly from the houses of three villages (Kafer ad Dik, Bruqin, Farkha) in Salfeet district, during the period from 13 June to 1 August, 1999.

The specimens were collected as follows:

2.1.1. Preparation of Sample bottles:

1. Screw capped glass bottles 400ml capacity were used for water collection.
2. After cleaning with hot water, the bottles were autoclaved at 121°C for 15min. under 15/in^2 pressure [12].

2.1.2 Samples collection

1. An indoor leak - free water faucet was cleaned for water samples collection in each house.
2. After removing faucet attachments such as screen or splash guard, water was allowed to run at full flow for 2-3 min to remove waste from pipelines.
3. Then after closing the faucet, it was wipped with a piece of cotton and sterilized by flaming.

5. After which water was allowed to run at full flow for 1-2 min.

6. The faucet was then closed to allow a stream of water having the size of a pencil.

7. After labeling the bottle was filled to 3/4 capacity, while holding the bottles cap in the other hand.

8. The bottle was closed immediately after sample collection.

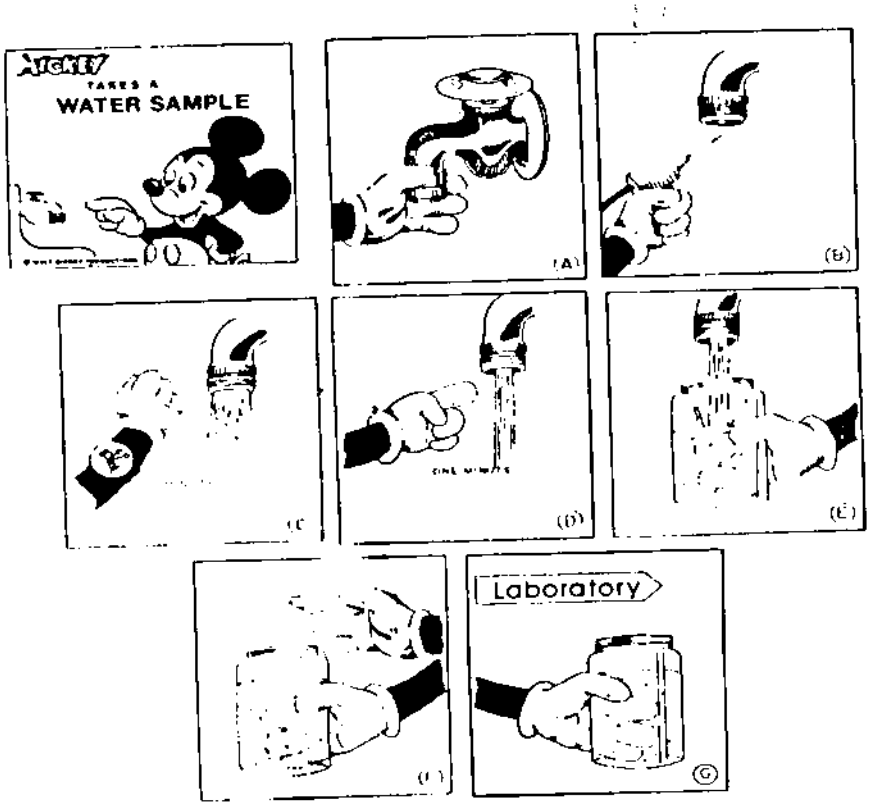

Fig.2.1. Checklist for collecting water sample [8].

The following information was obtained from the householder [8,28]:

1) Was the cistern cleaned this year?

2) Does the cistern have a trap door?

3) How far is the nearest latrine from the cistern?

4) Are animals kept in the household or its garden?

5) What is the source of water in the cistern? Rain full or spring water or municipality water?

6) What type of disinfectant if any do you use?

2.1.3 Water samples transport

Water sample bottles were sent to the microbiology laboratory at An - Najah University in icebox immediately after collection.

2.1.4 Water samples culture

As soon as samples arrived at the laboratory, each one was assigned an individual unit number and was analyzed as discrete unit using the membrane filter technique:

A - Preparation of petri dishes:

A total of 1.5-2 ml of membrane lauryl sulphate broth (Oxoid, MM615) was poured into the pad of Millipore specialized 47mm petri dish (PD1004700) [5].

A) Triple sugar iron (TSI) test [2]:

1.The very center of the colony to be picked was lightly touched with a sterile inoculating needle.

2.The TSI agar slant was aseptically inoculated by streaking the surface of 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 reactions (Table 2.1).

Table 2.1 Basic reactions of Enterobacteriaceae on TSI a gar.*

[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. rettgeri* *P.stuartii* *Serratia sp.*
+	-	+	+	+	*Citrobacter sp.*
+	-	+	-	-	*Enterobacter aerogenes* *E. cloacae*
+	+	-	+	-	*Salmonella sp.*
-	+	+	-	-	*Shigella sp.*
-	+	+	-	-	*Yersinia sp.*
-	-	+	-	-	*Klebsiella sp.*
-	-	-	+	+	*Proteus mirabilis* *p.vulgaris*
-	-	-	+	-	*Edwardsiella tarda*

"1" K/A: Alkaline slant (red) and acid butt (yellow).
"2" A/A: Acid slant (yellow) and acid butt (yellow).
*After: Al-kharraz [1].

B) Sulfide, Indole and Motility (S.I.M.) test [1]:

1. A sterile tube of S.I.M. media was aseptically inoculated with a portion of bacterial colony by stabbing the media once to depth of ½ - ¼ in with a sterile inoculating needle.

2. The tube was incubated overnight at 37°C.

Reactions on S.I.M. :

S: Blackening of tube - sulfide production present (sulfide positive), no color change in tube - sulfide negative [1].

M: Cloudiness throughout medium or brush like growth throughout medium or brush like growth around line of inoculation- positive motility [1].

I: Means that this medium was used for detection of indole production [2], the latter was achieved by:

1.Adding 1ml of xylene to 24 hr culture of organism in S.I.M. medium.

2.The tube was shaken well and allowed to stand for a few minutes until the solvent rised to the surface.

3.About 0.5ml 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 [2].

C) Citrate utilization test [2]:

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 Simon citrate agar slant by streaking the slant.

3. The tube was incubated for 24 hr at 37°C.

A positive reaction was, indicated by growth of the organism on the slant, with change of the color indicator from green to blue, where as, no growth or very little growth and no color change indicated a negative reaction [2]. Colonies of *Klebsiella pneumonia* (citrate positive) and *E.coli* (citrate negative) were used as control.

D) Urease test [2]:

1. With a sterile inoculating needle, the colony was lightly touched and inoculated aseptically into urea agar slant by streaking the slant only.

2. The tube was incubated at 37°C for 24 hr.

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.

2.1.6 Identification of *E.coli O157:H7*

E.coli strains isolated from water.were further identified for *E.coli 0157:H7.* The biochemical tests including T.S.I., S.I.M., Citrate

utilization and Urease test were done according to standard biochemical reactions. *E.coli O157: H7* strains show the same biochemical reactions as other *E.coli* strains. So all bacterial cultures which proved to be *E.coli* strains were further submitted for identification for *E.coli 0157:H7* by biochemical tests and serology as follows:

1. With a 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.
3. A positive reaction was indicated by green metalic sheen, on EMB was suspected to be *E.coli O157: H7.*
4. With a sterile inoculating loop, a portion of bacterial colony (metalic sheen colony) was streaked on to MacConkey sorbitol agar.
5. The plates were incubated over night at 37°C.
6. A positive reaction for *E.coli 0157:H7* was indicated by the appearance of colorless colonies.

Serological test:

E.coli O157:H7 latex test had been used for identification of *E.coli* serogroup *O157:H7.*

1. After the reagent had been brought to room temperature, vigorous shaking mixed the latex suspensions.

2. One drop of the test latex was dispensed 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 portion 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 1 minute.

Negative reaction was indicated by absence of agglutination where as positive reaction was indicated by agglutination with the test reagent within one minute and in this case a further portion of the colony was tested with the control latex reagent to ensure that the isolation was not an auto - agglutinating strain.

2.1.7 Isolation of *Salmonella*

Isolation of *Salmonella* was carried out as follow:

1. A sterile membrane filter was placed into a sterile holding apparatus.
2. The water sample was alloweded to pass through the filter under a vacuum pressure, and the bacteria were trapped on this filter.

3. The filter membrane was removed with sterile forceps and was placed onto 15-20ml of tetrathionat broth (Oxoid,CM29) enriched media.

4.The broth was incubated for 24hr at37°C.

A) Selective growth:

1. After incubation of tetrathionate broth, the cups were mixed by using a vortex.

2. Using a sterile loop, a loop full was taken aseptically from the tetrathionat broth and streaked on Xylose lysine desoxy cholate agar (X.L.D.) (Oxoid,CM469) and Salmonella shigella ager (S.S.) (Oxoid,CM99) [5].

3. The plates were incubated at 37c° for 24hr.

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 slant and stabbing butt.

3. The tube was incubated for 24hr at37°C.

Cultures, which produced alkaline (red) slant and acid (yellow) butt with or without production of H2s (blackening) in TSI agar, were retained as potential *Salmonella* isolates and were submitted for biochemical and serological tests.

2.1.8 Identification of *Salmonella*:

A) Urease test [2]:

The steps of urease test were mentioned previously.

All cultures that give negative urease test (no change in color of medium) were retained for further identification, where as all cultures giving positive urease test (pink color) were discarded.

B) S.I.M. test [2]:

S.I.M. test was carried out as mentioned previously.

All *Salmonella* are indole negative as indicated by absence of a brilliant red ring below the solvent layer after the addition of Ehrlich reagent. Most *Salmonella* are motile organisms as indicated by producing cloudiness in the medium. Some *Salmonella* species produce hydrogen sulphide, as indicated by blackening of the line of inoculation where as

others can not [2]. Suspected, colonies were further identified by Analytical Profile Index (API20E) system, followed by serology.

C) Serological test:

Salmonella latex test had been used for identification of suspected *Salmonella* colonies.

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.
2. One drop of the test latex was dispensed onto a circle on the reaction card close to the edge of the circle.
3. By using a sterile loop, the test latex and suspension were mixed together and spreaded to cover the reaction area.
4. 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.

2.2 Stool sample collection

During the period from 14 August to 19 October 1999, 102 stool specimens were collected from patients presenting with diarrhea at two Public Health Units located on Kafer ad-Dik and Bruqin villages in Salfeet district.

The specimens were collected as follows:

2.2.1 Collection &Transport of stool specimens

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

2.2.2 Microscopic examination

1. A smear was prepared on a slide in which one drop of normal saline was emulsified with a small amount of faecal material using wooden stick to make a thin film.
2. The slide was covered by a cover slip and examined microscopically under the low power objective (10x) and low light intensity, then examined under high - dry power (40x), to identify the possible presence of parasites, trophozoites cysts or ova of parasites.

2.2.3 Isolation of *Salmonella & Shigella*

Isolation of *Salmonella & Shigella* were carried out as follows:

A) Selective enrichment:

1. About one teaspoon of stool sample was transferred to 25ml of tetrathionate broth.
2. The broth was incubated at 37°C for 24hr.

B) Selective growth:

1. The following day, a loopful from the tetrathionat broth was streaked on (X.L.D.) and (S.S) using sterile inoculating loop.
2. The plates were incubated for 24hr at 37°C.
3. In the next day, the plates were examined for the presence of *Salmonella* or *Shigella* suspected colonies.

On S.S agar, *Salmonella* and *Shigella* appeared as transparent colonies usually with or with out black centers, while on X.L.D. a gar, *Salmonella & Shigella* appeared as pink- red colonies with or without black centers.

2.2.4 Identification of *Salmonella & Shigella*

Urease test:

All cultures that give negative urease test were retained for further identification, whereas all cultures giving positive urease test were discarded. The cultures were, further identified by biochemical tests and serology as mentioned previously.

CHAPTER III

RESULTS

3.1 Bacterial quality of drinking water in different water sources

Data presented in table 3.1 shows the average counts for total coliform and faecal coliform in Spring water, Rainwater, and Mekorote (network) water.

Table 3.1. Associations between average counts for bacterial indicators and water sources.

Water source	No. of samples	Percentage (%)	TC			FC		
			Average count	t	Sig.	average count	t	Sig.
Spring water	133	66.5	15.4	0.911	0.745	9.42	0.654	0.865
Rain water	57	28.5	19.4			11.43		
Mecorote water	10	5	0	0	0	0	0	0
Total	200	100	-	-	-	-	-	-

Sig. = significant t-critical=1.96.

The average counts for total coliform (CFU/100ml) in spring water was 15.4, in rainwater 19.4 and zero in mekorote water. The average counts of faecal coliform (CFU/100ml) in spring water was 9.4, in rainwater 11.4 and zero in mekorote water network. Figure 3.1 shows graphical presentation of these data.

From the table above we notice that, the computed t-test values of total coliform and faecal coliform according to water sources in every house are respectively 0.911 and 0.654, this means that, there is no significant differences between spring water and rainwater sources, but there is

significant differences between spring water and rainwater in one side and the mekorote water network in the other side at (α=0.05) with t- critical = 1.96.

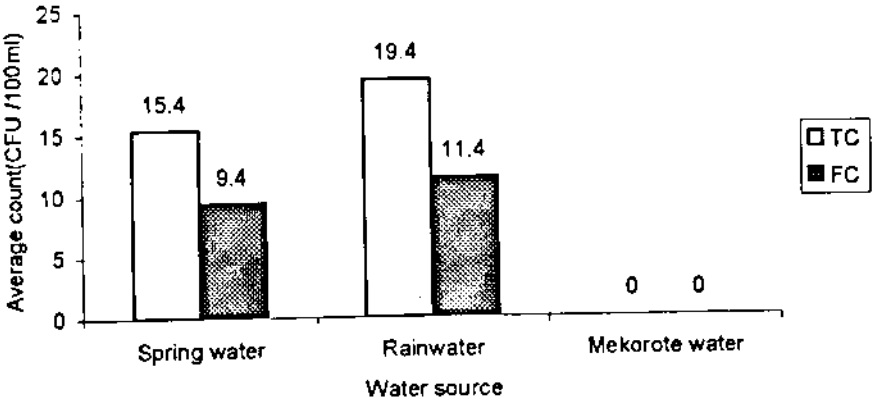

Fig.3.1. Average counts of bacterial indicator in spring water, rainwater and mekorot water network.

3.2 Bacterial quality of storage systems in Bruqin, Farkha and Kafr ad-Dik villages

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

Table 3.2. Results of ANOVA test for bacterial indicators in the three studied villages.

Village	No. of samples	Percentage (%)	TC			FC		
			Average count	F	Sig.	average count	F	Sig.
Bruqin	76	38	18	1.285	0.279	10.21	0.696	0.500
Farkha	64	32	9.71			7.43		
Kafr ad Dik	60	30	19.81			11.95		
Total	200	100	-	-	-	-	-	-

Sig.=Significant F-critical= 3.04

The average count of total coliform in roof storage tanks of Bruqin, Farkha and Kafr ad-Dik villages was 18, 9.71 and 19.81 CFU /100ml water respectively. The average count of faecal coliform in roof storage tanks of Bruqin, Farkha and Kafr ad-Dik villages was 10.21,7.43 and 11.95 CFU/100ml water, respectively. Figure 3.2 shows graphical presentation of these data.

Differences in mean quality of total coliform and faecal coliform in roof storage tanks in the three villages were of no significance with F- values of 1.285 & 0.696 respectively with F-critical =3.04 at (α=0.05).

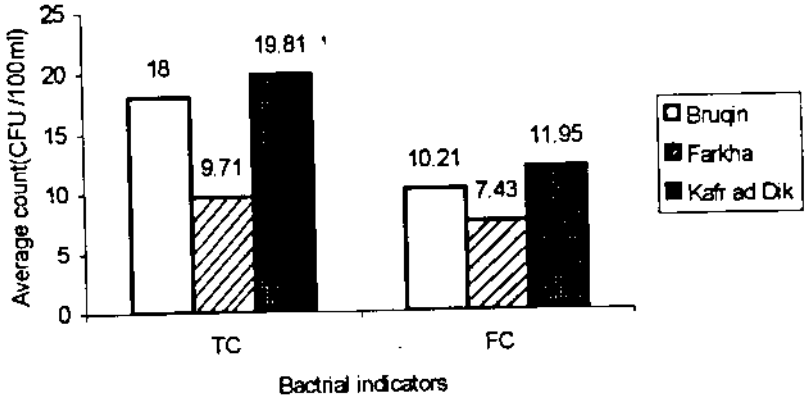

Fig.3.2.Average counts of bacterial indicators in storage system for Bruqin, Farkha and Kafr ad-Dik villages.

3.3 Degree of contamination in storage system

Table 3.3 shows the results in both rainwater and spring water, according to the WHO classification for the degree of contamination, with respect to total coliform counts.

Table 3.3. Distribution of rainwater, spring water and mekorote water according to contamination degree.

Total Coliform Count	Contamination degree	No. of rainwater samples	Percentage (%)	No.of spring water samples	Percentage (%)	No. of mekorote samples	Percentage (%)
0 - 3	0	12	21.0	14	10.5	10	100
3 - 50	1	40	70.0	100	75.2	0	0
50-50,000	2	5	9.0	19	14.3	0	0
>50,000	3	0	0	0	0	0	0
Total	-	57	100	133	100	10	100

Data presented in table 3.3 shows that 12 samples (21%) from rainwater, 14 samples (10.5%) from spring water and all samples of mekorote water network were free of contamination, (degree 0). While forty samples (70%) of rainwater and one hundred samples (75.2%) of spring water was in the first degree of contamination. Five samples (9%) of rainwater and 19 samples (14.3%) 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.

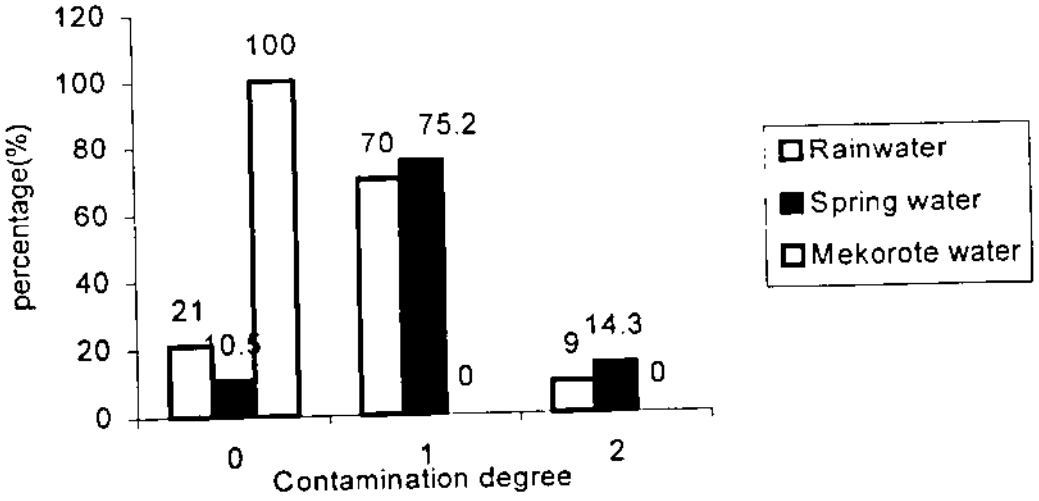

Fig.3.3. Distribution of rainwater, spring water and mekorote water according to contamination degree.

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

Table 3.4 shows the findings in both rainwater 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 rainwater and spring water with respect to *E.coli* count & risk levels.

E.coli Count/100ml	Risk*	Rainwater		Spring water	
		Number	Percentage(%)	Number	Percentage(%)
0	No risk	10	17.5	40	30
0-10	Low risk	31	54.3	65	48.8
10-100	Intermediate risk	16	28	28	21
100-1000	High risk	0	0	0	0
>1000	Very high risk	0	0	0	0
Total	-	57	100	133	100

*Risk as defined by WHO [21].

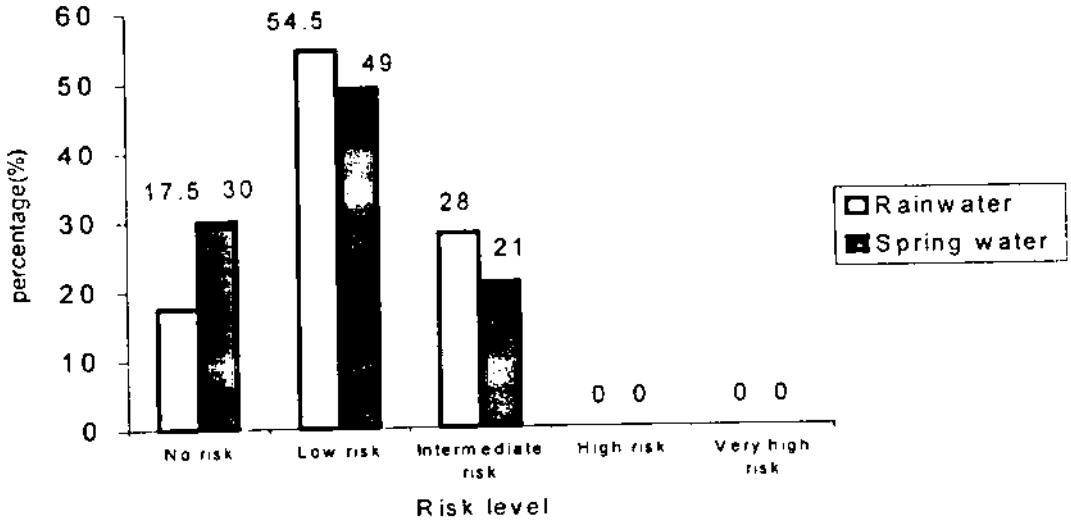

Fig.3.4. Distribution of rainwater and spring water with respect to *E.coli* count and risk level.

Data presented in table 3.4 shows that 10 samples (17.5%) of rainwater and 40 samples (30%) of spring water and all samples of mekorote water network were free of contamination (no risk). While 31samples (54.3%) of rainwater and 65 samples (48.8%) of spring water were with low risk level. 16 samples (28%) of rainwater and 28 samples (21%) of spring water were with an intermediate risk level, and none of these water sources show high or very high-risk level. Figure 3.4 shows graphical presentation of these data.

Data presented in table 3.5 shows the total of 46 water specimens positive for coliforms were cultured for *Salmonella, Shigella and E.coli O157:H7* in both rainwater and spring water.

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

Number	Frequency	TSI				Urea	S.I.M.			Simmon	Identification
		Slant	Butt	Gas	H_2S		Sulfide	Indole	Motility		
1	8	A	A	+	-	d	-	d	-	+	*Klebsiella*
2	1	K/A	A	+	+	+	+	-	+	-	*Proteus*
3	8	K	K	-	-	d	-	-	+	+	*Pseudomonas*
4	12	K/A	A	+	-	d	-	-	+	+	*Enterobacter*
5	16	A	A	+	-	-	-	+	+	-	*E.coli*
6	0	K	A	-	-	-	-	-	-	-	*Shiglla*
7	1	K	A	d	d	-	-	-	+	d	*Salmonella*

- TSI: Triple sugar iron agar.
- K: Alkaline reaction, which gives red color.
- A: Acid reaction, which gives yellow color.
- K/A: Some strains give alkaline results, others give acidic results.
- +: Positive result.
- - : Negative result.
- d : Different strains give different results.

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

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

Water Sources	No. of stool samples	*Giardia lamblia*		*Entamoeba histolytica*		*Hymenolepis nana*		χ^2
		N	%	N	%	N	%	
Rain	40	6	15	10	25	2	5	0.81
Spring	62	4	6.5	6	9.7	3	4.8	
Total	102	10	10.8	16	17.3	5	5	

χ^2 - critical = 5.99

Data presented in table 3.7 shows that 6 samples (15%) taken from persons depending on rainwater and 4 samples (6.5%) from persons depending on spring water were *G. lamblia* positive. Ten samples (25%) from persons depending on rainwater and 6 samples (9.7%) from persons depending on spring water were *E. histolytica* positive. Two samples (5%) from persons depending on rainwater and 3 samples (4.8%) from persons depending on spring water were *H. nana* positive. No clear association was detected between the sources of drinking water and numbers of intestinal parasitic infections encountered in this study at (χ^2 =0.81,df =2, P =0.05). Figure 3.6 shows, graphical presentation of these data.

Table 3.8 shows the different biochemical reactions for identification of bacteria in different stool samples.

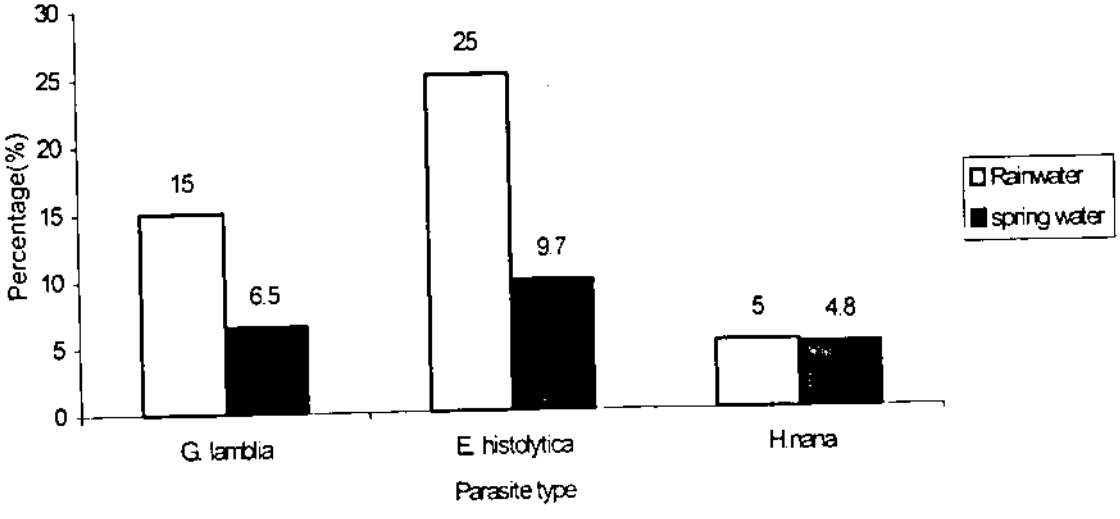

Fig.3.6. Distribution of intestinal parasites in persons depends on spring water and rainwater for drinking.

Table 3.8.Biochemical reactions for identification of bacteria in stool.

No.	Frequency	TSI				Urea	S.I.M.			Simmon	Identification
		Slant	Butt	Gas	H2S		Sulfide	Indole	Motility		
1	5	K	A	d	-	-	d	+	+	+	*Providancia*
2	11	K/A	A	+	+	+	+	-	+	-	*Proteus*
3	8	K/A	A	+	-	d	-	-	+	+	*Enterobactor*
4	12	K/A	A	+	-	-	-	-	+	-	*Hafina alvei*
5	15	A	A	+	-	d	-	D	-	+	*Klebsiella*
6	9	K/A	A	-	+	-	-	+	+	-	*Edwardsiella tarda*
7	3	K	A	-	-	-	-	-	+	+	N.I

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

3.5 Factors that affect water quality in rainwater and spring water

3.5.1 Rainwater

A. Animals raising

Data presented in table 3.9 shows average counts and computed t- test values of total coliform and faecal coliform according to animals raising in every household.

Table 3.9. Numbers, Percentages and computed t-test of rainwater cisterns according to animals raising in households.

Animals raising	No. of cisterns	Percentage (%)	TC			FC		
			average count	t	Sig.	average count	t	Sig.
Yes	32	56.2	29.2	3.341	0.002	19.50	3.98	0.000
No	25	43.8	7.4			1.96		
Total	57	100	-	-	-	-	-	-

Sig. = Significant t-critical=2.01

According to collected data, 32 cisterns (56.2%) were found near animal keeping areas. The average counts of total coliform and faecal coliform was 29.8 CFU/100 ml and 19.50 CFU/100 ml water, respectively. On other hand 25 cisterns (43.8%) were with no close animal keeping areas. The average counts of total coliform and faecal coliform was 7.4 CFU/100ml and 1.96 CFU/100ml water, respectively. Figure 3.7 shows graphical presentation of these data.

The computed t-test values of total coliform and faecal coliform according to animals raising in household are respectively 3.341 and 3.98, this means that, there is significant difference, between animals raising in households and no animals raising in households at (α=0.05) with t-critical=2.01.

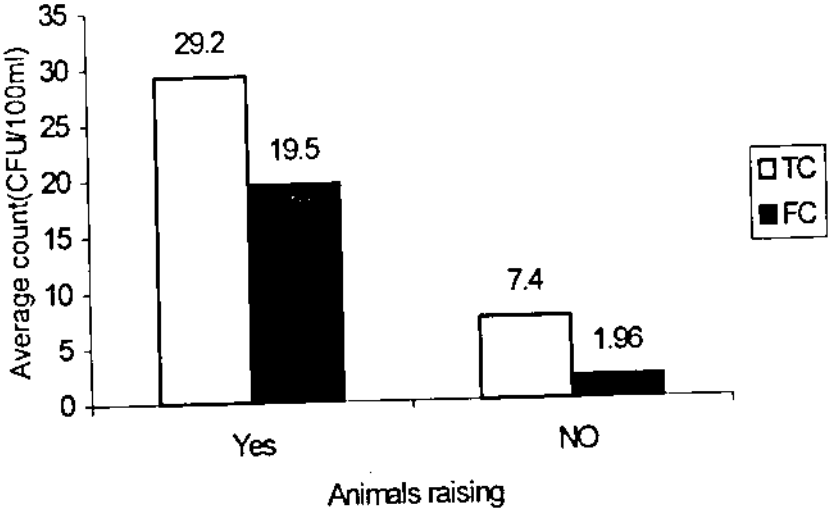

Fig.3.7. Average counts of bacterial indicators according to animals raising in house.

B. Cisterns location

Data presented in table 3.10 shows average counts and computed t- test values of total coliform and faecal coliform according to cistern location in every household.

Table 3.10. Numbers, Percentages and computed t-test of rainwater cisterns according to cisterns location in every house.

Cisterns Location	No. of cisterns	Percentage (%)	TC			FC		
			average count	t	Sig.	average count	t	Sig.
External	45	79	24.2	4.803	0.000	14.44	4.492	0.000
Internal	12	21	1.58			0.166		
Total	57	100	-	-	-	-	-	-

Sig. = Significant t-critical=2.01

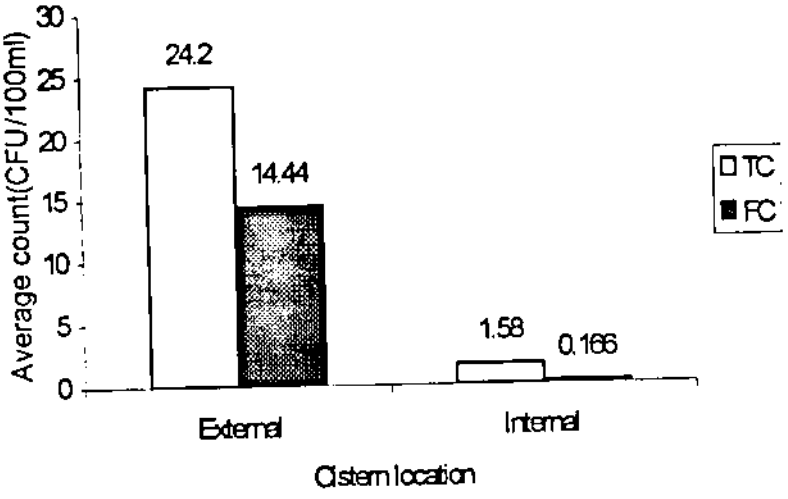

Fig.3.8. Average counts of bacterial indicators according to cistern location in every house.

According to collected data, 45 cisterns (79%) were located external the house. The average counts of total coliform and faecal coliform was 24.20 CFU/100ml and 14.44 CFU/100ml water, respectively. Twelve cisterns (21%) were location internal the house. The average counts of total coliform and faecal coliform was 1.58 CFU/100ml and 0.166 CFU /100 ml water. Figure 3.8 shows graphical presentation of these data.

The computed t-test values of total coliform and faecal coliform according to cisterns location in every house are respectively 4.803 and 4.492, this mean that, there is significant difference, between cisterns located external the house and cisterns located internal the house at (α=0.05) with t- critical = 2.01.

C. Distance between the septic tank and cisterns in every house

Data presented in table 3.11 shows the association between average counts for total coliform, faecal coliform and septic tank with respect to cisterns.

Table 3.11. Associations between average counts for bacterial indicators and distance between septic tanks and cisterns.

Cisterns Distance	No. of cisterns	Percentage (%)	TC			FC		
			average count	T	Sig.	average count	t	Sig.
Less than 20m	18	31.6	43.27	3.754	0.000	28.94	4.103	0.000
More than20m	39	68.4	8.43			3.35		
Total	57	100	-	-	-	-	-	-

Sig. = Significant t-critical=2.01

According to collected data, 18 cisterns (31.6%) were cisterns less than 20m from septic tank. The average counts of total coliform and faecal coliform was 43.27 CFU/100ml and 28.94 CFU/100ml water, respectively. While 39 cisterns (68.4%) were cisterns more than 20m from septic tank. The average counts of total coliform and faecal coliform was 8.43 CFU/100ml & 3.35 CFU/100ml water. Figure 3.9 shows graphical presentation of these data.

The computed t-test values of total coliform and faecal coliform according to distance between the septic tank and cisterns in every house are respectively 3.754 & 4.103, this means that, there are significant difference,

between cisterns located less than 20m from septic tank, and cisterns located more than 20m from septic tank, at (α=0.05) with t- critical = 2.01.

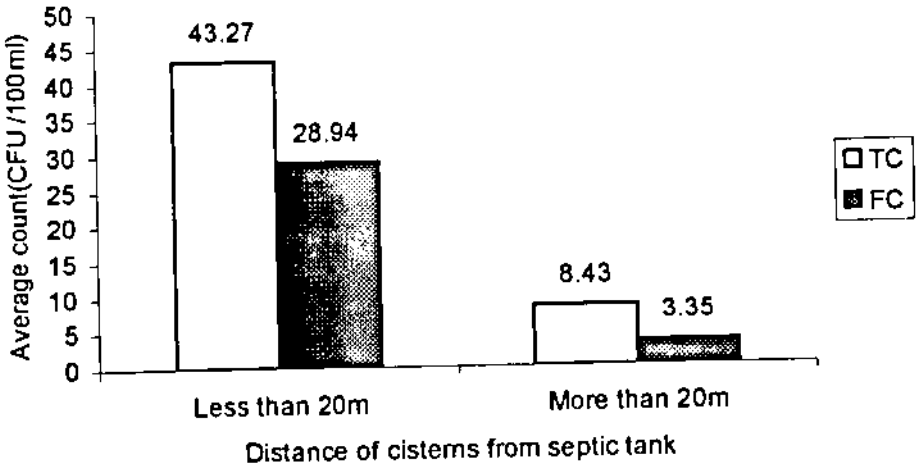

Fig.3.9. Average counts of bacterial indicators according to distance between the septic tank and cisterns in every house.

D. Cisterns cleaning

Data presented in table 3.12 shows the association between average counts for total coliform, faecal coliform and cleaning of cisterns the previous summer.

Table 3.12.Association between average counts for bacterial indicators and cleaning of cisterns.

Cistern Cleaning	No. of cisterns	Percentage (%)	TC			FC		
			average count	t	Sig.	average count	T	Sig.
Yes	33	58	8.57	3.203	0.004	3.42	3.575	0.001
No	24	42	34.37			24.58		
Total	57	100	-	-	-	-	-	-

Sig. = Significant t-critical=2.01

According to collected data, 33 cisterns (58%) were cisterns cleaning. The average counts of total coliform and faecal coliform was 8.57 CFU /100ml and 3.42 CFU/100ml water, respectively. Twenty four cisterns (43%) were cisterns not cleaned. The average counts of total coliform and faecal coliform was 34.37 CFU/100ml and 24.58 CFU/100ml water. Figure 3.10 shows graphical presentation of these data.

The computed t-test values of total coliform and faecal coliform according to cisterns cleaning are respectively 3.203 and 3.575 this means that, there is significant difference between cisterns cleaned and cisterns not cleaned at (α=0.05) with t- critical = 2.01.

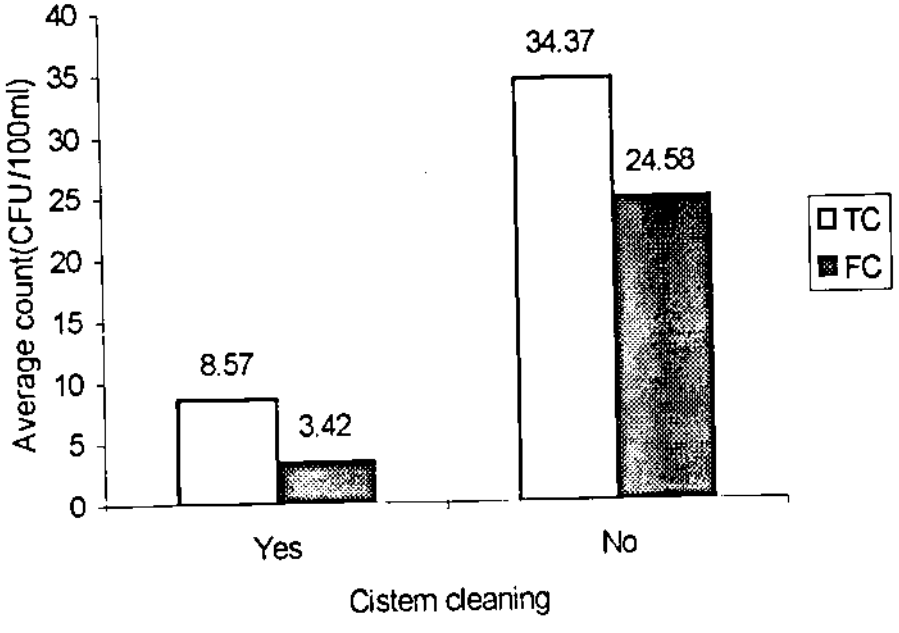

Fig.3.10. Average counts of bacterial indicators according to cleaning cisterns in every house.

3.5.2 Spring water

A. Animal raising

Data presented in table 3.13 shows average counts and computed t- test values of total coliform and faecal coliform according to animals raising in every household.

Table 3.13. Numbers, Percentages and computed t-test of rainwater cisterns according to animals raising in households.

Animals raising	No. of cisterns	Percentage (%)	TC			FC		
			average count	t	Sig.	average count	t	Sig.
Yes	47	35.3	35.063	6.203	0.000	24.17	5.80	0.000
No	86	64.7	3.744			1.66		
Total	133	100	-	-	-	-	-	-

Sig. = Significant t-critical=1.96

According to collected data, 47 cisterns (35.3%) were found near animal keeping areas. The average counts of total coliform and faecal coliform was 35.06 CFU/100 ml and 24.17 CFU/100 ml water, respectively. On other hand 86 cisterns (64.7%) were not close to animal keeping areas. The average counts of total coliform and faecal coliform was 3.744 CFU/100ml and 1.66 CFU/100ml water. Figure 3.11 shows graphical presentation of these data.

The computed t-test values of total coliform and faecal coliform according to animals razing in household are respectively 6.203 & 5.801, this means that, there are significant difference, between animals raising in households and no animals raising in households at (α=0.05) with t-critical=1.96.

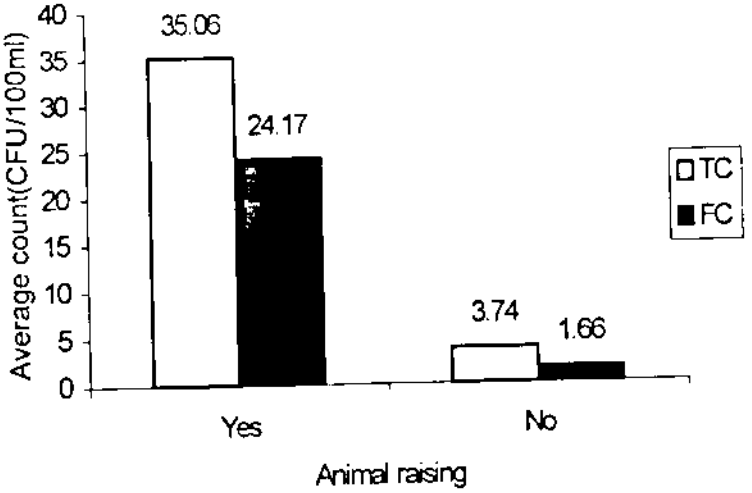

Fig.3.11. Average counts of bacterial indicators according to animals raising in house.

B. Cisterns location

Data presented in table 3.14 shows average counts and computed t- test values of total coliform and faecal coliform according to cistern location in every household.

Table 3.14. Numbers, Percentages and computed t-test of rainwater cisterns according to cisterns location in every house.

Cisterns Location	No. of cisterns	Percentage (%)	TC			FC		
			average count	T	Sig.	average count	t	Sig.
External	80	60	23.57	5.383	0.000	15.11	5.558	0.000
Internal	53	40	3.05			0.6604		
Total	133	100	-	-	-	-	-	-

Sig. = Significant t-critical=1.96

According to collected data, 80 cisterns (60%) were located external the house. The average counts of total coliform and faecal coliform was 23.57

CFU/100ml and 15.11 CFU/100ml water, respectively. Fifty three cisterns (40%) were location internal the house. The average counts of total coliform and faecal coliform was 3.05 CFU/100ml and 0.660 CFU/100 ml water. Figure 3.12 shows graphical presentation of these data.

The computed t-test values of total coliform and faecal coliform according to cisterns location in every house are respectively 5.383 and 5.558, this mean that, there are significant difference, between cisterns located external the house and cisterns located internal the house at (α=0.05) with t- critical = 1.96.

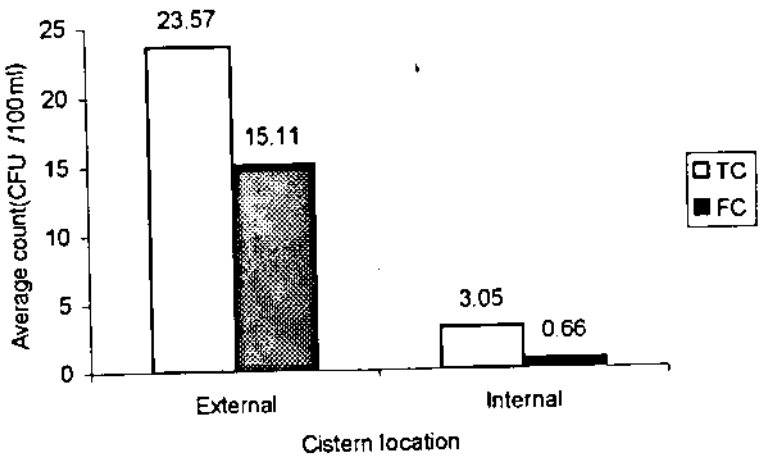

Fig.3.12. Average counts of bacterial indicators according to cistern location in every house.

C. Distance between the septic tank and cisterns in every house

Data presented in table 3.15 shows the association between average counts for total coliform, faecal coliform and cisterns with respect to septic tank.

Table 3.15. Associations between average counts for bacterial indicators and distance between cisterns and septic tank.

Cisterns Distance	No. of cisterns	Percentage (%)	TC			FC		
			average count	t	Sig.	average count	t	Sig.
Less than 20m	45	33.8	36.22	5.879	0.000	21.68	4.636	0.000
More than20m	88	66.2	4.75			3.21		
Total	133	100	-	-	-	-	-	-

Sig. = Significant t-critical=1.96

According to collected data, 45 cisterns (33.8%) were cisterns less than 20m from septic tank. The Average counts of total coliform and faecal coliform was 36.22 CFU/100ml and 21.68 CFU/100ml water, respectively. Eighty eight cisterns (66.2%) were cisterns more than 20m from septic tank. The average counts of total coliform and faecal coliform was 4.75 CFU/100ml and 3.21 CFU/100ml water. Figure 3.13 shows graphical presentation of these data.

The computed t-test values of total coliform and faecal coliform according to distance between the septic tank and cisterns in every house are respectively 5.879 & 4.636, this means that. there are significant difference,

between cisterns located less than 20m from septic tank, and cisterns located more than 20m from septic tank, at(α=0.05) with t- critical = 1.96.

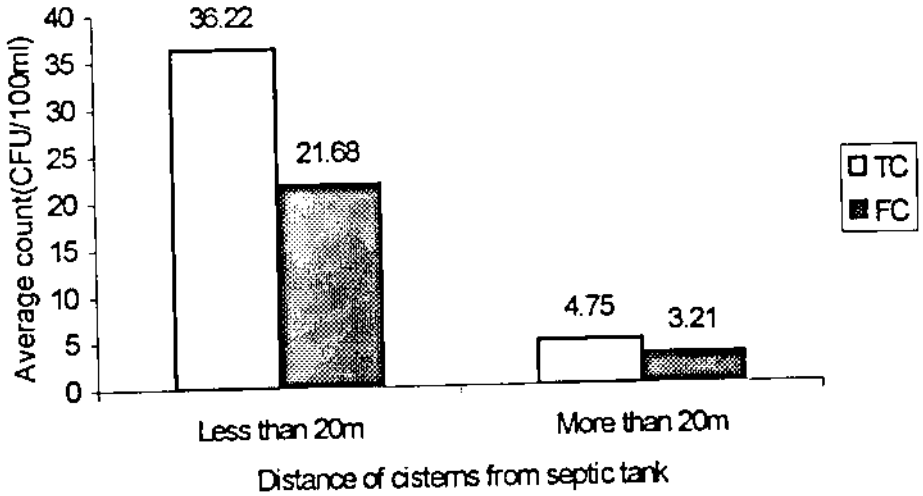

Fig.3.13. Average counts of bacterial indicators according to distance between the cisterns and septic tanks in every house.

D. Cisterns cleaning

Data presented in table 3.16 shows the association between average counts for total coliform, faecal coliform and cleaning of cisterns the previous summer.

Table 3.16.Association between average counts for bacterial indicators and cleaning of cisterns.

Cistern Cleaning	No. of cisterns	Percentage (%)	TC			FC		
			average count	t	Sig.	average count	t	Sig.
Yes	70	52.6	3.67	5.647	0.000	1.600	5.280	0.000
No	63	47.4	27.63			18.23		
Total	133	100	-	-	-	-	-	-

Sig. = Significant t-critical=1.96

According to collected data, 70 cisterns (52.6%) were cisterns cleaned. The average counts of total coliform and faecal coliform was 3.67 CFU /100ml and 1.600 CFU/100ml water, respectively. While Sixty three cisterns (47.4%) were cisterns not cleaned. The average counts of total coliform and faecal coliform was 27.63 CFU/100ml and 18.23 CFU /100ml water. Figure 3.14 shows graphical presentation of these data.

The computed t-test values of total coliform and faecal coliform according to cisterns cleaning are respectively 5.647 and 5.280 this means that, there is significant difference between cisterns cleaned and cisterns not cleaned at (α=0.05) with t- critical = 1.96.

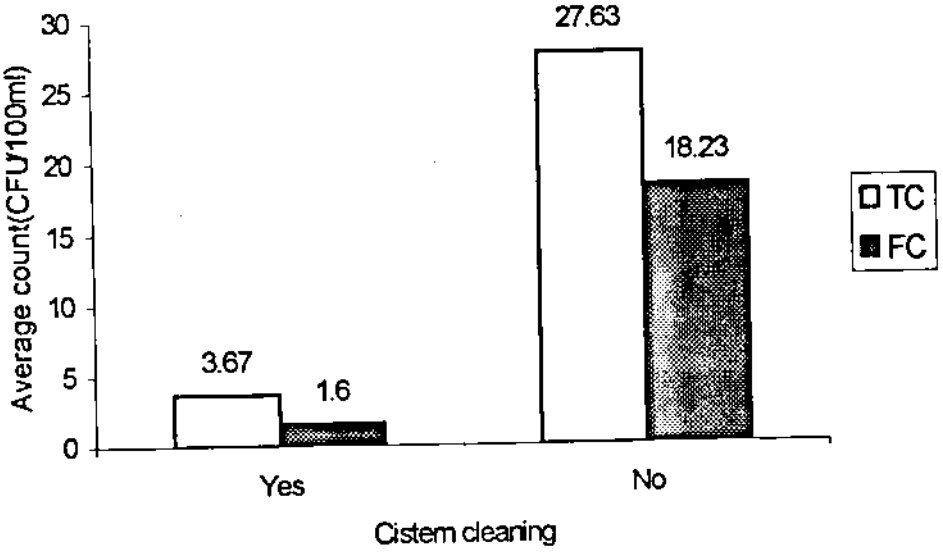

Fig.3.14. Average counts of bacterial indicators according to cleaning cisterns in every house.

CHAPTER IV
DISCUSSION, CONCLUSIONS and RECOMMENDATIONS

4.1 Average counts of Total Coliform and Faecal Coliform in storage system

Average counts of total coliform in spring water and rainwater was 15.4 and 19.4 CFU /100ml water respectively (Table 3.1).

According to international standards set by WHO, these values were higher than the safe limit, which is 0-3 CFU/100ml water. Such findings could be due to the fact that, non-of these water sources were treated by any of the used disinfectants for this purpose. Average counts of total coliform in water network (mekorote water) was zero CFU /100ml this value located in the safe limit (contamination degree zero), because this water source use disinfectant.

Average counts of faecal coliform *E.coli* in cisterns of spring water and rainwater were 9.4 and 11.4 CFU/100ml water respectively (Table3.1).

According to the international standards set by WHO, these values were higher than the safe limit, which is, zero. The finding of higher average counts of faecal coliform *E.coli* in the studied water sources (spring water & rainwater), strongly indicate that such sources were exposed to faecal contamination. Behind such findings in the studied villages is the improper sewage disposal. All included households do not have sewage

networks for sewage water disposal and they rely on septic tanks that are usually designed to serve single household. These septic tanks required digging into grounds which increase 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 hyper saturation and flooding will occur, while in permeable and high drainage capacity soil rapid filtration through the soil will occur, especially during the rainy seasons [21]. Both hyper-saturation of septic tanks and rapid filtration of wastewater result in contamination of these cisterns.

Differences for both indicators were not significant between rainwater and spring water in roof storage tanks water, but differences for both indicators were significant between water network and both water sources (spring water & rainwater). Both indicators were with very much lower values in water network compared to rainwater and spring water (Table 3.1). This is an expected observation, because rainwater collected from roof top of the house, and storing in the house well, and spring water collected from a near by spring by tanker trucks and storing in the well house, then using electric pumps, to transfer water from the cistern to a roof top metal 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 water network connected to the roof storage tank directly

from water network. On the other hand water network supply is chlorinated, from this, normally mekorote drinking- water is safety.

4.2 Bacterial quality of water storage systems in Bruqin, Farkha and Kafer ad-Dik villages

Table 3.2 represents a comparison between different roof storage tanks in the studied villages. The findings for both indicators were similar and differences in average counts were with no significant values. Our finding strongly indicates that the three villages are suffering from contamination of these water sources. Economical, cultural, life styles, sewage systems and geographical nature similarities are behind the finding of 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 (table 3.3), which strongly indicates that (70%) of the rainwater and (75.2%) of spring water were with first degree of contamination. Only (21%) of rainwater, (10.5%) of spring water and all houses which depend on mekorote network (100%) were free of contamination. According to WHO recommendation, such contamination degree is hazardous to human health and therefore, both spring and rainwater require treatment that involve the regular use of disinfectant

(chlorine). Spring water and rainwater with second degree of contamination (9%, 14.3%) can be treated with filtration and disinfecting.

4.4 Contaminated water sources as potential risk factor for human health

Based upon WHO classification, the majority of our storage systems are within low and intermediate risk levels as shown in table 3.4. An increase in the risk level is an indicator of increase levels of faecal contamination, thus our findings indicate that the majority of our storage systems were exposed to faecal contamination. Whether this situation is mainly due to septic tanks or other sources require further investigation. Annual reports by the Palestinian Ministry of Health (see appendix II) regarding water-borne diseases show an alarming number of cases indicative of low water quality.

4.5 Bacterial quality in spring water and rainwater

Table 3.5 represents a comparison between the bacterial quality of the two water sources. The findings for both indicators were similar and differences in percentage were with no significant values except in one sample of rainwater, which contain *Salmonella*. Our finding strongly indicates that both sources are suffering from contamination. Economical,

animals, sewage systems and geographical nature similarities in most houses studied are 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.

The findings indicate that no association between drinking water source and intestinal parasite in this study. However, *E.histolytica*, *G.lamblia* and *H.nana* were the dominant intestinal parasites in persons depending on rainwater source. These three parasites were also infected persons depending on spring water but with small percent. These parasites were found in the small intestine of humans and other mammals, and this cause prolonged diarrheal disease [7].

Behind such findings in the studied households are drinking water contamination by sewage, poor hygiene, lack of sanitation, improper sewage disposal, and during the rainy season flooded floors of houses are observed in these villages leads to transmission of these pathogens.

Both indicators showed higher average counts in household with cisterns having external trap doors compared to those with internal trap doors (Table 3.10).

4.7.3 Septic tanks distance

Septic tanks (improper sewage systems) are usually accused in water contamination. Thus, septic tanks should be located at a safe distance from all surrounding septic tanks and other sources of pollution. The safe distance should be determined from the time taken by contaminants 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 matters likely to be discharged and land use and ownership. Our findings with respect to distance and level of contamination based on average counts of both indicators show clearly that high average counts for both indicators were associated with distance up to 20 meters away from septic tanks. Both indicators showed noticeable decrease on their average counts in cisterns far away from septic tanks 20 meters and above (Table 3.11). This findings are in agreement with results in a study by Othman (2000) at cisterns and roof storage tanks in Beit-leed and Safarine villages and a study by Birzeit University Community Health Unit (1990) on water quality in the West

Bank [21]. As we mentioned earlier it seems to be, that, it is very difficult to specify a safe minimum distance, as this will depend on the slope of the land, septic tanks levels as well as the soil texture.

4.7.4 Cisterns cleaning

A comparison between cisterns cleaned and cisterns not cleaned for both indicators was in favor of cisterns cleaned as both indicators were with lower average values in cisterns cleaned compared with that in the cisterns not cleaned. These differences were statically significant (Table 3.12).

4- Cistern should be emptied and cleaned completely from the water of the previous year to remove the sediments.

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

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

7- Roof storage tanks should be provided with a tap for withdrawal of water.

8- Roof storage tanks should be inspected, cleaned and disinfected at least once a year if the water comes from a protected source, but if the water is not cleaned the tank will require more frequent cleaning, the frequency depends on the water quality.

9- The septic tanks and other pollutants sources should be located down hill of cisterns where ever possible and this is based on the topography, subsurface geology, land ownership and land use.

10- The sewage disposal systems (septic tanks) should be replaced by sewage network systems.

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

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

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

14- Environmental 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.

15- Comprehensive hygiene educational programs should be developed and implemented, to ensure that the community:

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 contamination of water.

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

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الملخص

الممرضات المحمولة في الماء وعلاقتها بالأمراض المعوية في منطقة سلفيت - دراسة وبائية

أجريت هذه الدراسة خلال فصل الصيف بين شهري حزيران وآب لعام 1999 حيث جمعت 200 عينة ماء شرب عشوائيا من الخزانات السطحية في ثلاث قرى من منطقة سلفيت وهي(بروقين، فرخة، وكفر الديك).

احتوت العينة على 57عينة مصدرها ماء المطر و133 عينة مصدرها ماء النبع وعشر عينات مصدرها مياه الشبكة القطرية، حيث تم تحليل هذه العينات باستخدام طريقة الترشيح الغشائي، وذلك عن طريق فحص مؤشرات التلوث المستخدمة في فحص المياه وهي بكتيريا القولونيات وبكتيريا القولونيات الغائطية (الاشريكيه القولونيه)، والعينات التي أظهرت نتيجة ايجابية للمؤشرين السابقين الذكر، تم زراعتها مرة أخرى لعزل كل من *Salmonella Shigella, E.coli 0157:H7* .

دلت نتائج كل من مؤشرات الفحص البكتيري أن معدلات التلوث في الخزانات السطحية ذات قيمة أعلى مما هو موصى به من قبل منظمة الصحة العالمية للمياه الآمنة للشرب . حيث كانت معدلات القراءة لبكتيريا القولونيات في المياه التي مصدرها نبع، والمياه التي مصدرها المطر، والمياه التي مصدرها الشبكة القطرية 15,4 و19,4 وصفر مستعمرة بكتيرية لكل 100مل ماء على التوالي .في حين كانت معدلات القراءة لبكتيريا القولونيات الغائطية (الاشريكيه القولونيه) في المياه التي مصدرها النبع، والمياه التي مصدرها الشبكة القطرية 9,4 و11,4 وصفر مستعمرة بكتيرية لكل 100 مل ماء على التوالي. نلاحظ من هذه النتائج أن المياه التي مصدرها الشبكة القطرية كانت غير ملوثة صفر في جميع العينات، بينما كانت المياه التي مصدرها النبع أو المطر عالية التلوث.

عند مقارنة درجات التلوث بين الثلاث قرى الموجودة في الدراسة باستخدام المؤشرات السابقة تبين أن الفرقا بين مستويات التلوث في الثلاث قرى متشابهة وليست ذات دلالة إحصائية.

فيما يتعلق بدرجة التلوث المقدرة بالاعتماد على بكتيريا القولونيات في كل من الماء الذي مصدره أو المطر كانت درجات التلوث متشابهة، حيث كانت غالبية الخزانات السطحية ضمن الدرجة الأولى من التلوث حسب معايير منظمة الصحة العالمية، بينما كانت جميع العينات المأخوذة من مياه الشبكة القطرية خالية من التلوث أي أن درجة التلوث صفر، أما فيما يتعلق بعامل الخطورة المقاس بالاعتماد على القولونيات الغائطية (الاشريكيه القولونيه) فقد صنفت كل من ماء النبع وماء المطر على أنها ضمن درجات الخطورة المتدنية والمتوسطة، وذلك حسب معايير منظمة الصحة العالمية.

بينت الدراسة فيما يتعلق بدرجة التلوث والمنازل التي تمتلك أماكن لتربية الحيوانات وباب البئر بها خارجي والبعد بين آبار الجمع وحفر الامتصاص أقل من20 متر والمنازل التي لا تنظف آبارها سنوياً، أن معدلات قراءة كلا المؤشرين مرتفع بشكل واضح مما يدل على أن هذه الأسباب لها دور في زيادة تلوث الماء، في حين لوحظ انخفاض واضح في معدلات كلا المؤشرين في البيوت التي لا يوجد بها أماكن لتربية الحيوانات، وباب البئر في داخل المنزل، والمسافة بين آبار الجمع والحفر الامتصاصية تزيد عن 20 متر، والمنازل التي تنظف آبار الجمع سنوياً.

وفي هذه الدراسة أيضا تم خلال الفترة الواقعة بين شهري آب و تشرين الأول جمع 102 عينة براز من أشخاص مصابين بالإسهال من قرى كفر الديك وبروقين بمساعدة الوحدة الصحية التابعة لوزارة الصحة الفلسطينية، لدراسة الطفيليات المعوية السائدة وعلاقتها بمصدر الماء.

أظهرت الدراسة أن أكثر الطفيليات شيوعا هي على النحو التالي

rdia lamblia 10.8%, Entameba histolytica 17.3%, Hymenolepis nana

أظهرت نتائج هذه الدراسة أن هناك علاقة بسيطة وضعيفة بين الطفيليات المعوية وم
الشرب.