Prevalence of *Cryptosporidium* in Children with Diarrhea in the West Bank

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Thesis

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Birzeit-Palestine

نسبة انتشار *Cryptosporidium* في الأطفال المصابين بالإسهال في الضفة الغربية

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This thesis was successfully defended and approved on …….

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Dedication

To my parents for their patience and support
To my dear brothers and sisters for their encouragement
To my dear friends for their great help and support

…With respect and love …

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S. M. Abu Alrub

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Abstract

The prevalence of *Cryptosporidium* was assessed among 760 children with diarrhea in seven districts of the West Bank. Sixty two fecal samples were collected from northern, central and southern regions from children without diarrhea as the control group. Fecal specimens were concentrated by sedimentation technique and stained by modified acid fast staining. Data on each child was obtained and documented.

Eighty eight (11.6%) of the 760 fecal specimens examined were positive for *Cryptosporidium*. The prevalence rate in females was 12.4% (38/307), and 11.0% (50/453) in males. The highest prevalence rate of *Cryptosporidium* in Hebron was 15.2% (28/184) , followed by Jenin 14.3% (9/63), Nablus 11.1% (7/63), Qalqilya 10.8% (16/148), Toulkarm 10.7% (6/56), Ramallah 9.3% (12/129), and Bethlehem 8.5% (10/117). The prevalence rate was found to be slightly higher in camps (12.9%) than villages (12.2%) and cities (9.8%). This can be due to the poor hygienic and sanitary conditions. The prevalence rate of *Cryptosporidium* in the control group was 3.2% (2/62) which is consistent with other previous studies.

Significant difference in the prevalence rate of *Cryptosporidium* was clearly detected among different age groups. The age group (<5 years) is extremely higher (14.4%) than other groups; (5-10 years) age group (7.7%) and (10-15 years) age group (5.9%).. This may be due to lack of sanitary practice, self awareness and personal cleanliness in (0-5 years) group of children because they tend to put every thing in their mouths without awareness.

The data suggest that there is a need to implement routine testing for *Cryptosporidium* on all diarrheal stool specimens obtained from children. This study necessitates the re-evaluation of the current standards for safe drinking water. The Ministry of Health should develop new strategies to prevent the transmission of cryptosporidiosis.

نسبة انتشار *Cryptosporidium* في الأطفال المصابين بالإسهال في الضفة الغربية

ملخص الدراسة

لقد تم دراسة نسبة انتشار هذا الطفيل في 760 طفلا (453 ذكور، 307 اناث) مصابا بالإسهال في سبع مناطق من الضفة الغربية. تم جمع 62 عينة براز لأطفال غير مصابين بالاسهال من المنطقة الشمالية والمركزية والجنوبية كعينة ضابطة. تم تركيز عينات البراز بواسطة تقنية الترسيب Sedimentation Technique.صبغت العينات بواسطة Modified Acid Fast Staining. تم الحصول على المعلومات اللازمة عن كل طفل ووثقت.

إن نسبة انتشار هذا الطفيل كانت 11.6% (760/88)، حيث كانت نسبة الإصابة في الذكور 11% (453/50)، والنسبة في الإناث كانت 12.4% (307/38). كان أعلى معدل انتشار للطفيل في منطقة الخليل 15.2%، حيث تبعها على التوالي،منطقة جنين 14.3%، منطقة نابلس 11.1%، منطقة قلقيلة 10.8%، منطقة طولكرم 10.7%، منطقة رام الله 9.3% ، منطقة بيت لحم 8.5%. كانت نسبة انتشار الطفيل في العينة الضابطة 3.2% (62/2).

لقد كانت نسبة انتشار الطفيل أعلى في المخيمات 12.9% بالمقارنة مع القرى 12.2% والمدن 9.8%، ويعزى ذلك إلى تدني الظروف الصحية ومستوى النظافة والازدحام في المخيمات. لقد وجد فرق ذات دلالة إحصائية على نسبة انتشار هذا الطفيل بين الفئات العمرية الثلاث للأطفال المصابين بالإسهال، حيث سجلت الفئة العمرية (0–5) أكثر إصابة (14.4%) ، بالمقارنة مع الفئة العمرية (5–10) التي بلغت نسبة الإصابة فيها 7.7%، فيما كانت نسبة الإصابة في الفئة العمرية (10–15) متدنية (5.9%). يمكن أن يعزى ذلك الفرق إلى نقص الممارسات الصحية و الوعي الذاتي والنظافة الشخصية عند الفئة(0–5)، خاصة أن الأطفال في هذه المرحلة يميلون إلى وضع الأشياء في أفواههم دون تمييز.

إن هذه النتائج التي تم التوصل إليها تبين مدى الحاجة إلى فحص هذا الطفيل وبشكل دوري في كل المختبرات والمستشفيات خاصة أن نسبة الانتشار كانت عالية (11.6%). إضافة إلى أن هذه الدراسة يمكن أن تساعد في إعادة تقييم مدى صلاحية المياه المستخدمة في الشرب عن طريق وزارة الصحة، من اجل تبني استراتيجيات وخطط جديدة لمنع انتقال هذا الطفيل.

CHAPTER ONE

1.1 Introduction

Diarrheal diseases are extremely common in developing and developed countries. They are responsible for morbidity and mortality of millions of individuals each year (Verweij, *et al*., 2004). The intracellular parasite, *Cryptosporidium* is considered to be one of the most important enteric pathogens with world-wide distribution (Current, 1994; Mclauchlin, *et al.*, 2000).

Many studies indicate that *Cryptosporidium* oocysts are present in 65-97% of surface water in the U.S.A and oocysts are found in relatively smaller numbers in the range of 27%-54% in fully treated (disinfected and filtered) municipal water (Juranek, 1995) .

Accurate data are not yet available on the extent of *Cryptosporidium* world distribution; however estimates indicate that 5-10% of reported diarrheal disease in developing countries compared to 1-3% in the developed countries is caused by *Cryptosporidium* species (Kehl, *et al.,* 1995).

In children with diarrhea, *Cryptosporidium* is one of the most common enteric pathogens recovered (Current, 1994). The rate of infection is predicted to be higher in malnourished children (Sallon, *et al.,* 1988; Current, 1994; Hunter and Nichols, 2002). A study was carried out among 240 Iraqi children (under five years of age) presenting with a primary diagnosis of diarrhea. They found that *Cryptosporidium* oocysts were excreted by 8.8 % (21/240) of children (Mahdi, *et al.,* 1996).

A study has been conducted on 265 children under five years of age in Irbid-Jordan (Youssef, *et al.,* 2002). They found that the prevalence of *Cryptosporidium* is 1.5% compared to 32.5% rotavirus and 12.8% enteropathogenic *Escherichia coli* (Youssef, *et al.,* 2002).The prevalence of *Cryptosporidium* in Egypt, in children with diarrhea was reported to be 16.6% in 1986, 11.6% in 1987 and 27.9% in 1995 (Michel, *et al.,* 2000).

A study has been conducted in Rawalpindi, Pakistan, to investigate the prevalence of *Cryptosporidium* among 475 young children with acute diarrhea and 150 children as control group. They found that the prevalence of the parasite in children with acute diarrhea is 10.3% compared to 3.3% in the control group (Iqbal, *et al.,* 1999). A hospital based study was carried out on 2095 patients of all ages in north eastern India. The prevalence of *Cryptosporidium* was found to be 7.2% (Nath, *et al.,* 1999).

A three-year survey has been conducted in Taiwan to examine water samples and fecal specimens for *Cryptosporidium* and *Giardia.* They found that the percentage of these two parasites in 10 large water plants are 70% and 75% respectively. Fecal specimens from 9 species of animals indicate a prevalence of 20% (22/110) for *Cryptosporidium* and 10% (11/110) for *Giardia* (Hsu, *et al.,* 2002).

Additional reports have further documented the ability of *Cryptosporidium* to cause infection even when ingested in relatively small amounts of fully chlorinated water

that follows the standard methods for safe water drinking (DuPont, *et al.,* 1995; Carpenter, *et al.,* 1999).

1.2 Significance of the Study

This study can be considered as a comprehensive study on the prevalence of *Cryptosporidium* in the West Bank, Palestine. No records were detected on the prevalence of the parasite in this part of Palestine except for the work by (Sallon, et al, 1988).

The study would help in the following points:

• Data obtained from this study can serve as a starting point for future studies.

• Development of new strategies by the Ministry of Health to control the transmission of *Cryptosporidium* by evaluating the current standards for safe drinking water.

1.3 Objectives

The main objective of this study is to increase the awareness among health care givers about *Cryptosporidium*. It also signifies the need for the routine examination of all diarrheal fecal specimens for the presence of oocysts of this parasite

Specifically:

• To screen for *Cryptosporidium* in fecal samples of children with diarrhea in the West Bank.

• To investigate the prevalence of *Cryptosporidium* by district geographical locations in the West Bank.

• To investigate the prevalence of *Cryptosporidium* by gender.

• To determine if the prevalence of *Cryptosporidium* is age related.

• To determine the prevalence of *Cryptosporidium* in the control group (children without diarrhea)

1.4 Characteristics of *Cryptosporidium*

Cryptosporidium is an intracellular protozoan parasite that has emerged as an important cause of diarrhea in humans and animals (Dalle *et al.*, 2003). It belongs to the Phylum, Apicomplexa and Coccidia subclass (Liu, *et al.,* 1999).

Since 1982, *Cryptosporidium* has been increasingly recognized as a cause of severe and life-threatening diarrhea in patients with AIDS (Juranek, 1995; Hellard, *et al.*, 2003). The disease is severe, prolonged and chronic in immunocompromised patients and malnourished children but is a self -limiting disease in immunocompetent individuals (Current, *et al.*, 1983; Newman, *et al.*, 1994; Clark, 1999; Fayer *et al*., 2000).

Cryptosporidium establishes a compartment within the host cell, which is morphologically different from the setting used by the related parasites. So this unique parasitophorous vacuole may shelter the parasite from antimicrobial drugs (Clark, 1999).

Cryptosporidium oocysts are the infective stages for the transmission and survival of the organism in the environment. The oocyst is not a static structure, but it is able to incorporate antigens by a mechanism involving vesicle fusion with the intestinal wall and the incorporation of the antigen to the outer oocyst wall (Entrala, *et al*., 2001).

Cryptosporidium oocysts are remarkably resistant to many common disinfectants, including chlorine-based compounds. Oocysts are heat sensitive; a temperature of 65 ˚C

inactivates oocysts in 5-10 minutes. Desiccation over a period of 2 hours or more is lethal to oocysts. Oocysts can remain viable for about 18 months in a cool, damp or wet environment (Robertson, *et al.,* 1992). They are quite common in rivers and lakes, especially where there has been sewage or animal contamination. They are generally susceptible to freezing, although this varies by onset of freezing; snap freezing destroys oocysts reliably, but with slow freezing, such as that found in natural environment, oocysts have been reported to survive temperatures as low as -22 °C (Juranek, 1995).

Transmission of the parasite occurs by the fecal-oral route through the ingestion of oocysts present in water or food that shed in feces of infected hosts (Dalle, *et al*., 2003). The small size of the *Cryptosporidium* oocyst and its resistance to many chemical disinfectants, pose a challenge for standard filtration and disinfection procedures. Moreover, the low dose for infection and the prolonged excretion of high numbers of oocysts make *Cryptosporidium* ideal for waterborn transmission (Carpenter, *et al*., 1999).

1.5 *Cryptosporidium* Taxonomy:

Members of the genus *Cryptosporidium* are placed taxonomically in the phylum Apicomplexa, class Conoidasida, subclass Coccidiasina, order Eucoccidiorida, suborder Eimeriorina, and family Cryptosporidiidae (Bulter and Mayfield, 1996). Thirteen *Cryptosporidium* species are currently identified on the basis of oocyst morphology, natural host specificity, and genetic characterizations (Xiao, *et al.,* 2004); (Table 1.1).

Table 1.1 Currently identified species of *Cryptosporidium*

***Cryptosporidium* species**	**Origin of isolate**
C. parvum	Cattle, Human and other mammals
C. hominis	Humans
C. andersoni	Cattle
C. wrairi	Guinea pigs
C. muris	Rodents
C. felis	Cats
C. canis	Dogs
C. meleagridis	Birds
C. galli	Birds
C. baileyi	Birds
C.molnari	Fish
C. saurophilum	Snakes and Lizards
C. serpentis	Snakes and Lizards

(Xiao, *et al.,* 2004)

At least five different species of *Cryptosporidium* have been recognized to infect human, although each *Cryptosporidium* species or genotype has specific host specificity. These types are: human genotype *C. parvum*, bovine genotype *C. parvum*, *C. meleagridis*, *C. felis,* and *C. canis*, respectively in the order of their prevelance in human (Limor, *et al.,* 2002).

There are two distinct genotypes of *Cryptosporidium parvum,* Genotype I which was recognized in human isolates and Genotype II in calves isolates or humans isolates who were exposed to calf feces (Fleming, *et al.* 1999).

Anthroponotic and zoonotic life cycles could occur in human infections with *Cryptosporidium* (CDC, 2003; Alves, *et al.,* 2003), because human cryptosporidiosis is mainly caused by *C.parvum* and *C. hominis* (C. parvum human genotype). *C. parvum* is

found in human, wild animals, and domestic livestock, whereas *C. hominis* is found almost exclusively in human (Alves, *et al.,* 2003).

1.6 Life Cycle

The life cycle of *Cryptosporidium* is completed within one host. The resistant stage found in the environment is the thick-walled oocyst, containing four sporozoites (Bulter and Mayfield, 1996). The mature and sporulated oocysts (5 μm) are shed in the feces of infected humans or animals, each containing 4 sporozoites (Clark, 1999). The oocysts are ingested by other suitable hosts through contamination of environment, water or food (CDC, 2003).

Sporozoites excyst from the oocyst (excystation process) and parasitize epithelial cells of the gastrointestinal tract, then the sporozoites differentiate into a spherical trophozoite (Bulter and Mayfield, 1996), and asexual multiplication occurs, forming two types of meronts (schizonts), type I meronts contain 6-8 nuclei, which become incorporated into 6 to 8 merozoites (CDC, 2003).

When the meront is mature, each merozoite is able to infect a new host cell and then develops either into type I meront or type II meront, which contains 4 merozoites when mature (Clark, 1999).

The merozoites from type II meronts also invade new host cells but they initiate sexual multiplication by differentiating into either microgamont (male) or macrogamont (female) stages (CDC, 2003). Upon fertilization of the macrogamonts by microgamonts, the fertilized macrogamon (zygote) then develops into an oocyst that sporulates within the infected host by undergoing mitosis (Chen, *et al.,* 2002; CDC, 2003).

When meiotic sporozoite formation (sporogony) is completed, each oocyst becomes containing 4 potentially infective sporozoites (Chen, *et al.,* 2002). There are two types of oocysts produced during the cycle , the thick-walled oocysts (80%) which are commonly excreted from the body in the feces, and the thin-walled (20%) which are involved in autoinfection because they excyst within the gut, they release merozoites and infect new host cells (Bulter and Mayfield, 1996; CDC, 2003).

Each generation of the parasite can develop and mature within 12-14 hours, so the rapid life cycle and the autoinfection cycles together may lead to the production of huge numbers of parasitic cells in the gut and to secondary infection sites in the duodenum and large intestine (Bulter and Mayfield, 1996; Figure 1.1).

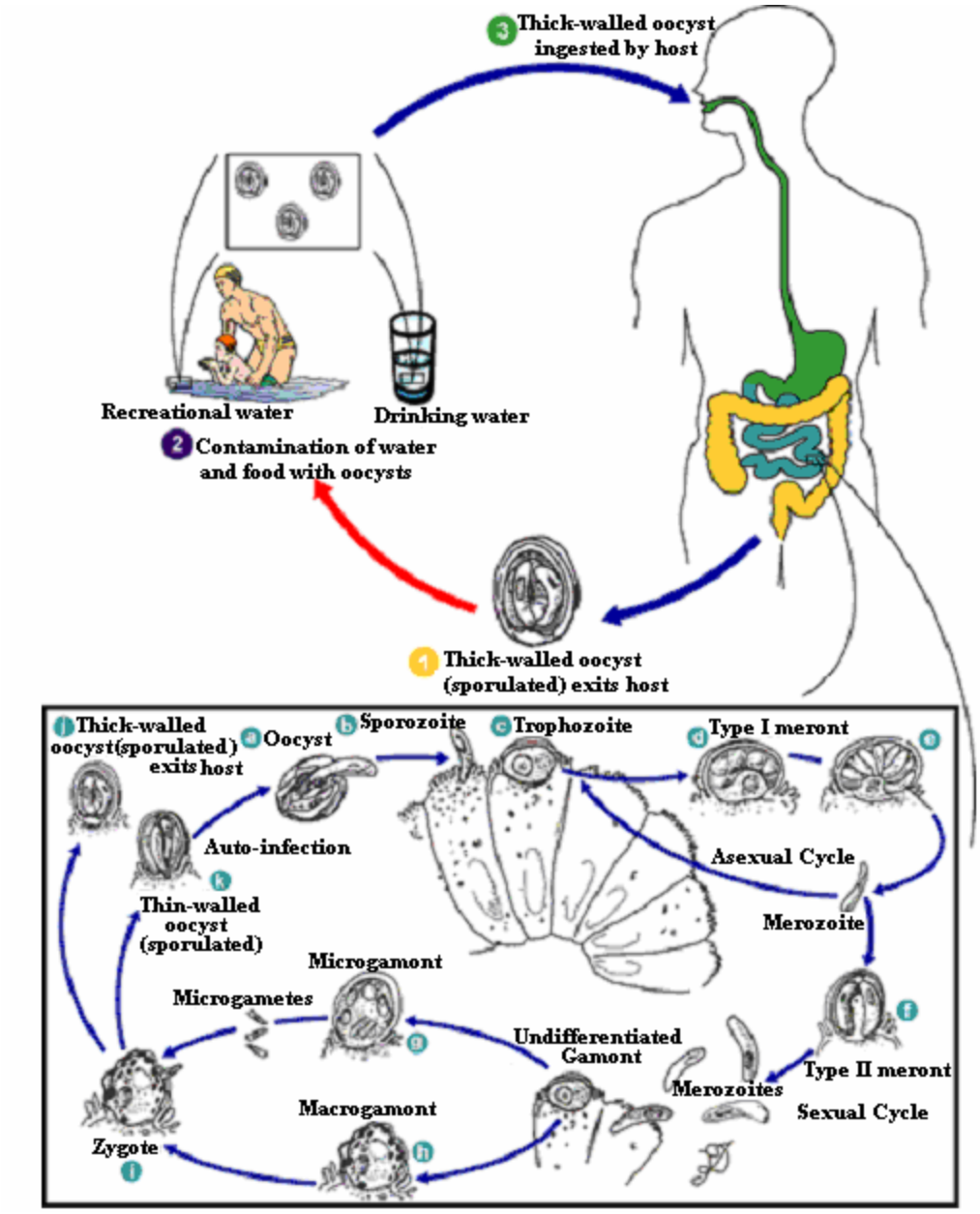


Figure 1.1 Life cycle of *Cryptosporidium parvum* and *C. hominis* (CDC, 2003).

1.7 Mode of transmission:

The infective stage is the sporulated oocyst that is shed in feces of infected people. The infection may be acquired in a number of ways such as contaminated water, animal-contact, person to person contact especially in children day care centers, and

contaminated food such as vegetables, fruits, raw meat and unpasteurized milk (Juranek, 1995).

In a study that has been conducted to demonstrate the infectivity of *Cryotosporidium* on 29 health volunteers, they found that 20% (6/29) became infected after receiving a dose of 30 oocysts. The infectivity became 88% (14/16) when 16 healthy volunteers had received 300 oocysts and 100% of the healthy volunteers became infected after receiving a dose of 1000 oocysts (DuPont, *et al.,* 1995).

1.8 Diagnosis

Cryptosporidial oocysts can be examined by wet mount preparation stained with iodine, especially specimens containing moderate to high numbers of oocysts (CDC, 2003). The modified acid-fast stain is usually used to detect the presence of cryptosporidial oocysts in fecal samples (Alles, *et al.,* 1995; Verweij, *et al.,* 2004).

Diagnosis of *Cryptosporidium* can be done also using immunofluorescent antibody (IFA) and enzyme immuno assay (EIA) (Guerrant, 1997; Fleming, *et al.,* 1999). However, the microscopic identification of *Cryptosporidium* requires well-trained and experienced microscopists (Kehl, *et al*., 1995; Alles, *et al.,* 1995; Morgan *et al.,* 1998 ; Johnston, 2003). Immunodetection of antigens on the surface of the organism in fecal specimens using monoclonal antibodies is the gold standard for the diagnosis of *Cryptosporidium* (CDC, 2003; Johnston, 2003).

Using direct immunofluorescent antibody (DFA) resulted in a significantly increased detection rate of *Cryptosporidium* by 69.6% (39 positive samples, p=0.005)

compared with conventional staining methods (23 positive samples) from a total number of 2696 fresh stool specimens examined in the routine practice of parasitology section in Massachusetts general hospital, Boston (Alles *et al.,* 1995).

Another study has been performed on 511 fecal specimens to compare between conventional methods (modified acid fast stain) and a recently developed polymerase chain reaction technique (PCR), they found that 36 positive cases of *Cryptosporidium* were identified using PCR, while 29 positive cases were identified using routine microscopy, therefore conventional methods exhibited 98.9% specificity and 83.7% sensitivity compared to 100% specificity and sensitivity to PCR method (Morgan, *et al.,* 1998).

Many genotyping methods have been developed to improve the understanding of the route of transmission of *Cryptosporidium* isolates, such as PCR-restriction fragment length polymorphism (RFLP) and light cycler PCR for the real-time detection and differentiation of *Cryptosporidium* (Limor, *et al*., 2002).

An optimized method to maximize the DNA extraction of *Cryptosporidium* parvum from small numbers of purified and partially purified oocysts present in mineral water sources by 15 cycles of freezing with liquid nitrogen and thawing at 65 ˚C in a lysis buffer containing sodium dodecyl sulfate (Nichols and Smith , 2004).

The initial amplification of *Cryptosporidium* DNA using a conventional thermocycler followed by real-time PCR using a lightCycler with SYBR Green I is a novel approach for the identification of *Cryptosporidium* species, especially *C. parvum*

and *C. hominis*, in environmental samples with heterogeneous mixtures of *Cryptosporidium* species (Amar, *et al*., (2004).

1.9 Clinical Features

Infection with *Cryptosporidium* species results in a wide range of clinical manifestations, from asymptomatic infections to severe and life-threatening illness (CDC, 2003). In immunocompetent individuals, cryptosporidiosis is accompanied by watery diarrhea, dehydration, weight loss, abdominal pain, nausea, vomiting, and fever. symptoms are usually resolved within 1-2 weeks (Juranek, 1995); but the symptoms can be chronic and more severe in immunocompromised patients (Clark, 1999).

Symptomatic *Cryptosporidium* infection have also been recognized in other organs including other digestive tract organs, lungs, but the small intestine is the site most commonly affected with the parasite (CDC, 2003). Cryptospopridiosis is widely prevalent in malnourished children, patients following solid-organ transplantation, malignant diseases, primary immunodeficiency diseases and to a lesser extent in diabetic patients (Hunter and Nichols, 2002).

1.10 Epidemiological Aspects of *Cryptosporidium*:

The parasite was first recognized in 1907 by Clarke and Tyzzer (Hunter and Nichols, 2002). During the 1970s, this organism became well known to Veterinary workers, and was reported as the causative agent of human cryptosporidiosis at the Johns Hopkins School of medicine in 1976 (Guerrant, 1997).

Seven cases of cryptosporidiosis were reported in humans in the period between 1976 until 1982, five of them were in immunsuppressed patients (Guerrant, 1997). Since

1982, as the prevalence figures began to rise, *Cryptosporidium* has been increasingly implicated as a cause of severe and life-threatening diarrhea in AIDS patients (Current, 1983). By 1984, 58 cases of cryptosporidiosis were reported, 40 cases were found in immunocompromised patients and 33 cases were detected in patients with AIDS (83%). Among the immunocompramised patients, 55% of the 40 patients died (Guerrant, 1997).

By the early 1990s, *Cryptosporidium* was identified as a major cause of community gastroenteritis causing outbreaks associated with drinking water, swimming pools, and exposure to animals (Hellard, *et al.,* 2003).

Seroepidemiologic studies showed a prevalence of IgG antibodies to *Cryptosporidium parvum*. In Brazil, 90% of children had serologic evidence of *Cryptosporidium* infection in their first year of life. 17%-32% by adulthood in the U.S.A had serologic evidence of *Cryptosporidium* infection by adulthood. More than 50% of children in China had serologic evidence by 5 years of age (Guerrant, 1997).

The seropositivity of *Cryptosporidium*-specific antibodies (IgM and IgG) was 94.6% in a study conducted on 223 members of 31 housholds with children younger than 3 years of age in an urban slum in Fortaleza, Brazil; and who were diagnosed as positive for *Cryptosporidium* oocysts in stool examination (Newman, *et al.,* 1994).

A review of 100 reports of more than 133,175 patients with diarrhea showed infection of *Cryptosporidium* in the range of 0.26% -22% of immunocompetent persons in developed countries and 1.4% - 40.9% in developing countries (Guerrant, 1997; Chen, *et al.,* 2002). The rate of *Cryptosporidium* infection among HIV infected persons ranges from 6%-70% in developed countries and 8.7-48% in developing countries (Guerrant, 1997); Table1.2)

Table 1.2 Rates of *Cryptosporidium* infection among immunocompetent and HIV-positive patients in developing and developed countries (Guerrant, 1997).

Countries	Patients with diarrhea	Controls without diarrhea
1-HIV-Positive:		
a. Developed countries	14 %(6%-70%) [n=148/1074]	0 %(0%-0%) [n=0/35]
b. Developing countries	24 %(8.7%-48%] [n=120/503]	5 %(4.9%-5.3%) [n=5/101]
2- Immunocompetent:		
a. Developed countries	2.2 %(0.26%-22%) [n=2232/107329]	0.2 %(0%-2.4%) [n=3/1941]
b.Developing countries	6.1 %(1.4%-40.9%) [n=1486/24269]	1.5 %(0%-7.5%) [n=61/4146]

1.11 *Cryptosporidium* outbreaks

Numerous well-documented outbreaks of cryptosporidiosis have occurred. Most of these were waterborne outbreaks. They resulted from problems in the filtration and flocculation processes associated with water treatment plants. Numerous waterborne *Cryptosporidium* outbreaks have been described, mainly in the United States of America, European countries, Japan, and Canada (Dalle, *et al.*, 2003).

The most serious outbreak was recognized in April, 1993 in Milwaukee, Wisconsin, U.S.A. An estimated 403,000 persons were infected by drinking water contaminated with *Cryptosporidium* (Chen, *et al.,* 2002). The suspected cause was due to errors in treatment, possibly contamination of filtered water with raw, unfiltered water.

Other operation-related problems were also suspected such as inadequate monitoring of water turbidity and inoperable equipment (Mackenzie, et *al.,* 1994).

This outbreak is the largest waterborne outbreak in the United States history. The incidence rate was 52% among those served by the South Milwaukee Water Works Plant. Consequently, many immunocompromised patients died and many healthy persons become ill. The mean duration of illness was 12 days with a range of 1 to 55 days, and the average maximum number of watery diarrheal stools was 19 per day at the peak of the illness. Watery diarrhea was the predominant symptom among 93% of confirmed cases (Mackenzie *et al.,* 1994).

Chlorinated recreational water facilities have been implicated in many outbreaks of cryptosporidiosis during the last decade. Standards for water purity in the United States have been strengthened in response to cryptosporidiosis outbreaks; however small numbers of oocysts can still breach water filtration in a 25-50% the communities with strengthened standards (Carpenter, *et al*., 1999).

Additional outbreaks in public swimming pools and wade pools have further documented the ability of *Cryptosporidium* to cause infection even when ingested in relatively small amounts of fully chlorinated water that follows the standard methods for safe drinking water (Guerrant, 1997).

1.12 Pathogenesis and Immunologic Features

Subsequent to oocyst excystation, the released sporozoites usually adhere to the surface of intestinal mucosa via a sporozoite specific-lectin adherence factor (Bulter and Mayfield, 1996). The watery nature of the diarrhea associated with *Cryptosporidium* infections has suggested the presence of an enterotoxin; however, no enterotoxin has been purified from fecal extracts (Guerrant, 1997).

Experimental evidence suggests that glucose-coupled Na^+ absorption is decreased and Cl^- secretion is increased, therefore, the diarrhea associated with *Cryptosporidium* appears to be primarily osmotic in nature (Clark, 1999). Epithelial mucosal cells release cytokines that activate phagocytic cells. These activated cells start releasing large amounts of soluble factors (histamine, serotonin, adenosine, prostaglandins, leukotriens and platelet activating factor) that increase intestinal secretion of water and chlorine and also inhibit intestinal absorption (Chen, *et al*., 2002).

The soluble factors act on different substrates including enteric nerves leading to epithelial cell damage through T cell mediated inflammation and producing villus atrophy and crypt hyperplasia which are accompanied by mixed inflammatory cell filtrate within the lamina propria, consequently, intestinal absorption is impaired and secretion is enhanced (Bulter and Mayfield, 1996; Figure 1.2)

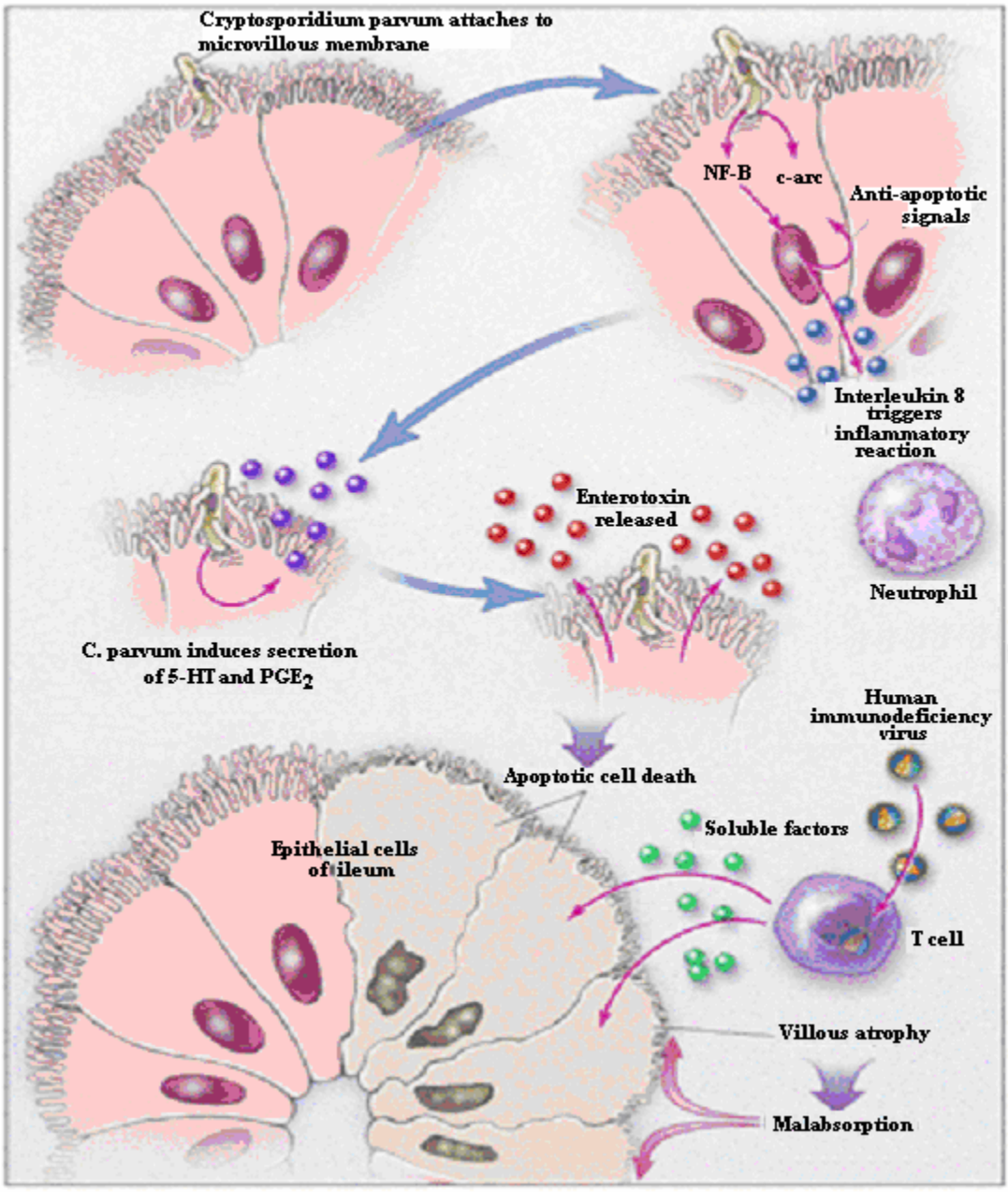


Figure 1.2 Pathogenesis of Cryptosporidial Enteropathy and Cholangiopathy:

The organism activates a second-signal pathways, such as the nuclear factor-B (NF-B) and c-src systems. Activation of NF-B induces the production of cytokines and chemokines, such as interleukin-8, which triggers an inflammatory reaction and

stimulates anti-apoptotic survival signals in directly infected cells. *C. parvum* induces secretion of 5-hydroxytryptamine (5-HT) and prostaglandin E_2 (PGE_2) into the lumen of the small intestine (Chen, *et al.,* 2002).

Macrophages produce tumor necrosis factor (TNF) in the lamina propria, but TNF doesn't directly affect epithelial transport. Adding a fibroblast monolayer, an indomethacin-inhibtable secretory effect with TNF was noted. Researchers suggested a prostaglandin-dependent secretory effect through a bumetanide –inhibitable chlorine secretory pathway (Guerrant, 1997; Figure 1.3).

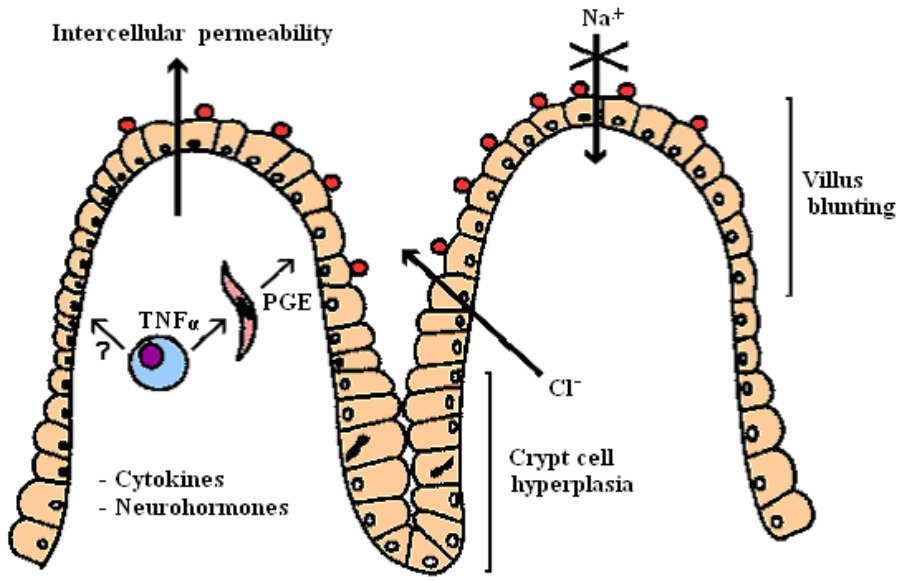


Figure 1.3 Schematic representation of *Cryptosporidium* pathogenesis:

An increased intercellular permeability and inflammation in the submucosal layer has been associated with *Cryptosporidium* infection. Macrophages secreting tumor necrosis factor-alpha (TNF-α) or other cytokines may stimulate fibroblasts and other cells in the

lamina propria to secrete prostoglandins (PGE). So, this promotes secretion and impair absorption (Clark and Sears, 1996).

Cryptosporidium parvum activates a nuclear factor B (NF-B) system which infects biliary epithelial cells. So the release of NF-B associated cytokines and chemokines has a critical role in the pathogenesis of inflammation associated cryptosporidiosis. *C. parvum* induces epithelial cell apoptosis in biliary infection and this was appeared to be associated with the Fas receptor-Fas ligand death pathway, but this mechanism of cell death has not been confirmed in vivo (Farthing, 2000) .

1.13 Cryptosporidiosis in Palestine

Epidemiological data on the prevalence of *Cryptosporidium* infections seem to be very sparse in most of the developing countries including Palestine. Caritas Baby Hospital-Bethlehem, Palestine; is the only hospital that routinely tests for the presence of *Cryptosporidium* in children's fecal samples. Oocysts are detected microscobically both in wet mounts and stained fecal preparations.

There is a high probability for mis-diagnosed cases with *Cryptosporidium.* This may be due to lack of experience of the technicians to detect the small size *Cryptosporidium*'s oocysts.

A study has been conducted at Caritas Baby Hospital on 221 children with gastroenteritis, showed that the prevalence of *Cryptosporidium* to be 13.5% compared to 7.2% *Entamoeba histolytica*, 3.6% *Giardia,* 11.3% *Salmonella*, and 0.9% Shigella (Sallon, *et al.*,1988).

A one year perspective study was carried out in Gaza on 1225 children with diarrhea; the major pathogens were 18.5% *Salmonella*, 14.6% *Cryptosporidium,* 6.8% rotavirus, and 8.3% *Campylobacter* species (Sallon, *et al*., 1994). So this percent of *Cryptosporidium* in stool samples indicates a very high prevalence rate, but it needs to be examined further in all over Palestine.

1.14 Prevention

The effective control measures should be aimed toward preventing the transmission of the oocysts. While keeping in mind the resistant nature of oocysts to many chemical disinfectants and antiseptics (Chen *et al*, 2002; Carpenter, et al. 1999). However Ozone is probably the most effective chemical agent in inactivating *Cryptosporidium* oocysts (Guerrant, 1997). The maintenance of immune system functions by the use of HAART is the best way to prevent cryptosporidiosis in AIDS patients (Clark, 1999).

1.15 Treatment

Although there is no reliable therapy for cryptosporidiosis. There are many agents used to reduce the infection (Chen, et al., 2002). The treatment options depend mainly on the immunologic conditions of the patient (Guerrant, 1997). In immunocompetent individuals, no specific therapy is indicated because the disease is self-limiting. Oral or intravenous fluids and electrolytes replacement may correct the dehydration and acute diarrhea (Chen *et al.,* 2002; Clark, 1999). Nitazoxanide has provided some encouraging results in the management of diarrhea in immunocompetent patients (CDC, 2003), but the most widely used one is paromomycin (Clark, 1999; Guerrant, 1997), however it doesn't eradicate the parasite but it reduces oocysts number and decreases the frequency of the

diarrhea (Guerrant, 1997).

In AIDS patients, the ideal treatment is strengthening the immune function of the body by administering Highly Active Antiretroviral Therapy (HAART), to help in resolving the infection (Chen et al 2002; Clark, 1999). If HAART therapy is not effective, several antibiotics can be used (paromomycin, nitazoxanide, and azithromycin).

CHAPTER TWO

Material and Methods

2.1 Study design

This study comprises 760 fecal specimens of human origin associated with diarrhea, in addition to 62 samples as control group in children without diarrhea. These samples were collected in the period between Septemper 2003 and November 2004 from hospitals, in Ramallah (n=129)-Ramallah Governmental Hospital, Bethlehem (n= 117) - Caritas Baby Hospital, Hebron (n= 184) -Alia Hospital, Jenin (n= 63) - Governmental Hospital, Nablus (n= 63) - AL-Watani Hospital, Qalqylia (n= 148) - Qalqylia Hospital, and Tulkarm (n= 56) - Governmental Hospital.

The fecal samples of the control group were collected from southern, central and northern regions of the West Bank. Twenty one samples were collected from Alia Hospital-Hebron (southern region). Twenty one samples were collected from Ramallah Governmental Hospital-Ramallah (central region) and twenty samples were collected from the Governmental Hospital-Jenin (northern region).

2.2 Study population

The target group for this study was children between ages of less than one year to 15 years of age. The samples collected were representative of the population in terms of gender, region and age as described in the following table.

Table 2.1 Distribution of samples by district.

District	Region			Total
	city	village	Camp	
Bethlehem	25	73	19	**117**
Hebron	45	116	23	**184**
Jenin	23	21	19	**63**
Nablus	24	23	16	**63**
Qalqilya	60	88	-	**148**
Ramallah	42	53	34	**129**
Tulkarm	15	20	21	**56**
Total	**234**	**394**	**132**	**760**

Table 2.2 Distribution of samples by gender.

District	Gender		Total
	Female	Male	
Bethlehem	54	63	**117**
Hebron	61	123	**184**
Jenin	29	34	**63**
Nablus	27	36	**63**
Qalqilya	56	92	**148**
Ramallah	55	74	**129**
Tulkarm	25	31	**56**
Total	**307**	**453**	**760**

Table 2.3 Distribution of samples by age categories.

District	Age Categories			Total
	>0-5	>5-10	>10-15	
Bethlehem	117	--	--	**117**
Hebron	104	47	33	**184**
Jenin	28	24	11	**63**
Nablus	35	22	6	**63**
Qalqilya	73	49	26	**148**
Ramallah	69	41	19	**129**
Tulkarm	38	12	6	**56**
Total	**464**	**195**	**101**	**760**

2.3 Sample Collection and Processing

Fecal samples were collected in a dry, clean, leakproof plastic container. Each sample was labeled with the child's name, gender and age. Additional information about each sample was obtained from the hospital (place of residency and patient's hospital number).

2.3.1 Formalin fixation

One volume of the fecal sample was mixed thoroughly using wooden applicator stick, with 3 volumes of 10% formalin. The sample was mixed again, and the specimen containers were sealed well. All samples were reinforced with parafilm, the container was inserted in a plastic bag, and samples were stored at 4 °C (CDC, 2003; Johnson, *et al.,* 2003).

2.3.2 Formalin-ethyl acetate sedimentation technique:

To maximize the recovery of oocysts, fixed-fresh stool samples were concentrated prior to microscopic examination using ethyl acetate sedimentation method as recommended by the Center of Disease Control and Prevention (CDC, 2003). Concentration procedures separate parasites from fecal debris and increase the chances of detecting oocysts (Garcia, 1999).

Procedure:

- The specimen was mixed well.
- 5ml of the fecal suspension were strained through wetted cheesecloth-type gauze placed over a disposable paper funnel into a 15 ml centrifuge tube.
- 0.85% saline was added through the debris on the gauze to bring the volume in the centrifuge tube to 15ml.

- Sample was centrifuged at 500 x g for 10 minutes.

- Supernatant was decanted. Then 10 ml of 10% formalin were added to the sediment and mixed thoroughly with wooden applicator stick.

- 4 ml of ethyl acetate were added, the tube was stoppered, and shook vigorously in an inverted position for 30 seconds.

- Each sample was centrifuged again at 500 x g for 10 minutes.

- The plug of floating debris was removed from the top of the tube by ringing the sides with an applicator stick. The top part of supernatant was decanted.

- A cotton-tipped applicator was used to remove debris from the sides of the centrifuge tube.

- Five drops of 10% formalin were added to resuspend the concentrated specimen.

2.3.3 Modified acid fast staining:

I- 50% Ethanol:

50 ml of absolute ethanol were added to 50 ml of distilled water and the solution was stored at room temperature; the solution is stable for 1 year (Garcia, 1999).

II- Kinyoun's Carbol Fuchsin:

4 g of basic fuchsin were dissolved in 20 ml of 95% ethanol (Solution A) and 8 gram of phenol crystals were dissolved in 100 ml of distilled water (Solution B), the two solutions A and B were mixed, and the solution was stored at room temperature; the solution is stable for 1 year (Garcia, 1999).

III- 1% Sulfuric Acid:

One ml of concentrated sulfuric acid (98%) was added to 99 ml of distilled water and the solution was stored at room temperature; the solution is stable for 1 year (Garcia, 1999).

IV- Loeffler Alkaline Methylene Blue:

0.3 g of methylene blue was dissolved in 30 ml of 95% ethanol and the solution was stored at room temperature; the solution is stable for 1 year.

Procedure:

The procedure by (Garcia, 1999) was followed in staining fecal specimens:

- A thin smear of 1 to 2 drops of specimen prepared on a slide and allowed to air dry.

- Specimens were fixed with absolute methanol for 1 minute.

- Specimens were stained with Kinyoun's carbol fuchsin for 5 minutes, then rinsed briefly with distilled water and drained.

-The slide was rinsed briefly (3 to 5 seconds) with 50% ethanol.

-The slide was rinsed thoroughly with distilled water and drained.

- Decolorization by using 1% aqueous solution of sulfuric acid for 2 minutes or until color stopped running off the slide.

- The slide was rinsed with distilled water and drained.

- The slide was counterstained with methylene blue for 1 minute.

- The slide was rinsed with distilled water, drained, and air dried.

- Specimens were examined microscopically using the 100x oil immersion objective lens.

- The slide was considered positive if red oocysts are seen and negative if red colored oocysts are not seen.

2.4 Statistical analysis:

Chi square (χ^2) test was used to establish the p value using SPSS program.

CHAPTER THREE
Results

A total of 760 children with diarrhea, 307 females and 453 males aged (> 0-15) years old were surveyed for *Cryptosporidium* infection in seven districts (Bethlehem, Hebron, Jenin, Nablus, Qalqilia, Ramallah and Toulkarm) in the period between September 2003 and November 2004. Out of 760 fecal samples, 88 samples were positive, being infected with *Cryptosporidium* spp. a prevalence rate (11.6%, 88/760); Table 3.1).

Cryptosporidial oocysts stained red with visible sporozoites with light blue background under light microscope; Figure 3.1 and 3.2.

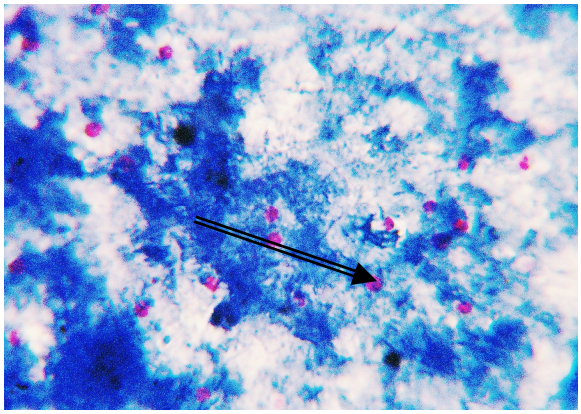


Figure 3.1 Cryptosporidial oocysts under light microscope (100X) stained by modified acid fast stain.

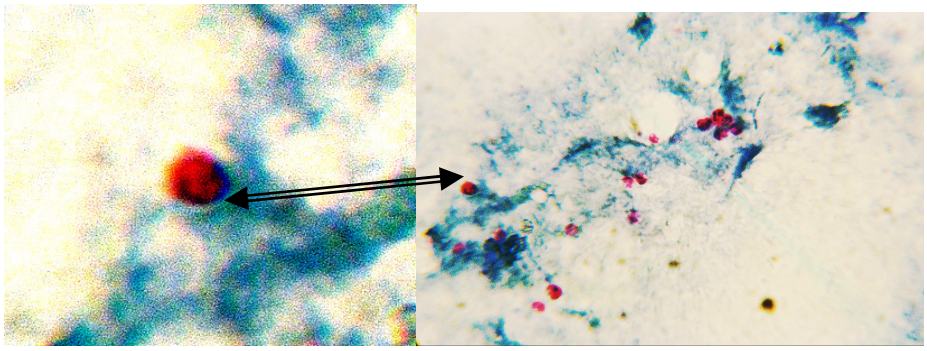


Figure 3.2 Cryptosporidial oocysts with visible sporozoites under light microscope (100X), stained by modified acid fast stain.

3.1 Prevalence of *Cryptosporidium* in the seven assayed districts

The Prevalence rate was found to be comparable among the seven districts and there is no significant difference in the prevalence rate of *Cryptosporidium* (P>0.05) but it was slightly higher in Hebron and Jenin districts than other districts. The prevalence rate was as follows; Hebron district (15.2%, 28/184), Jenin district (14.3%, 9/63), Nablus (11.1%, 7/63), Qalqilia district (10.8%, 16/148), Toulkarm (10.7%, 6/56), Ramallah (9.3%, 12/129) and Bethlehem district (8.5%, 10/117; Figure 3.3).

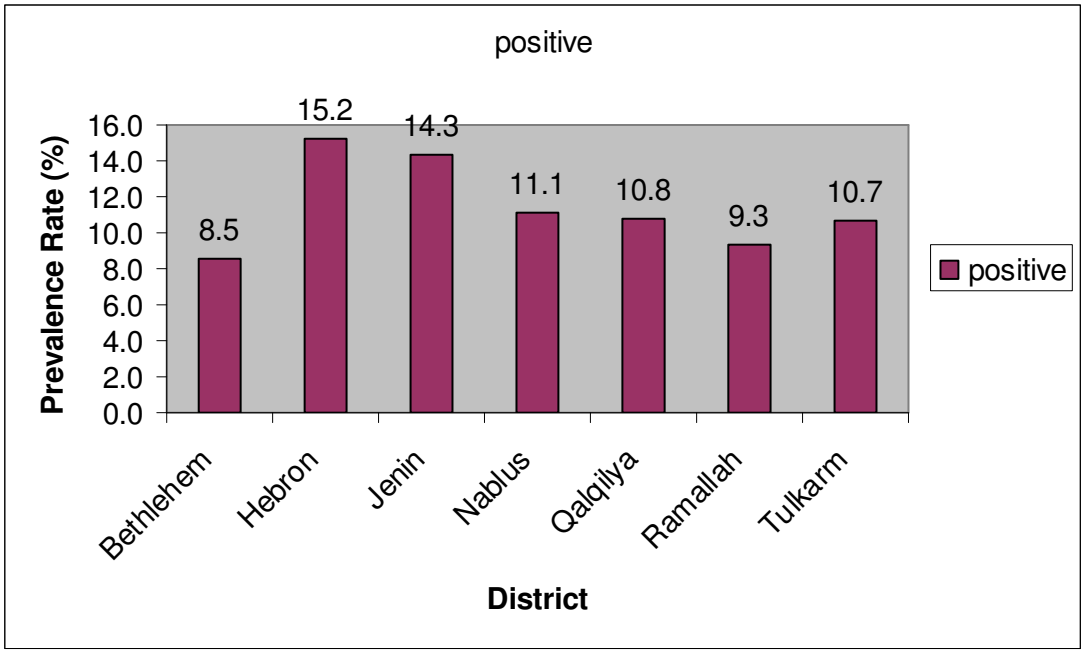


Figure 3.3 Prevalence of *Cryptosporidium* in the seven assayed districts.

3.2 Prevalence of *Cryptosporidium* in the seven districts by region (city, village and camp)

The prevalence rate was not found to be significantly different between the three regions ((P>0.05) in all districts, but the prevalence of the parasite was found to be higher in camps than villages and cities. The prevalence rate of *Cryptosporidium* in the different regions was as follows; camps (12.9%, 17/132), villages (12.2%, 48/394) and cities (9.8%, 23/234; Figure 3.4).

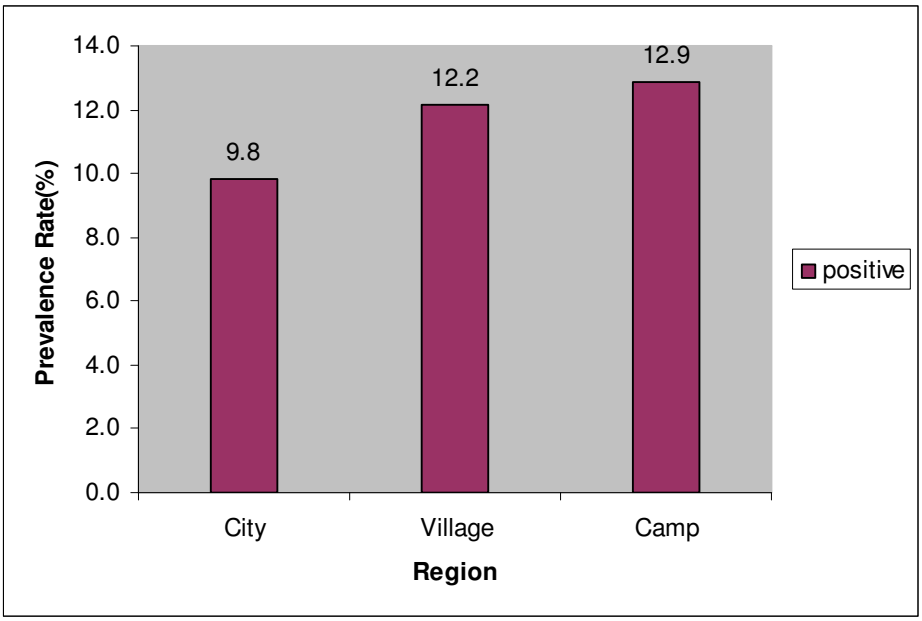


Figure 3.4 Prevalence of *Cryptosporidium* in the seven districts by region (city, village and camp).

3.3 Prevalence of *Cryptosporidium* in the seven districts by gender

The prevalence rate of *Cryptosporidium* infection was slightly higher in females (12.4%, 38/307) than in males (11.0%, 50/453), but there was no significant statistical difference between males and females on the prevalence rate of the parasite ($p>0.05$); Figure 3.5).

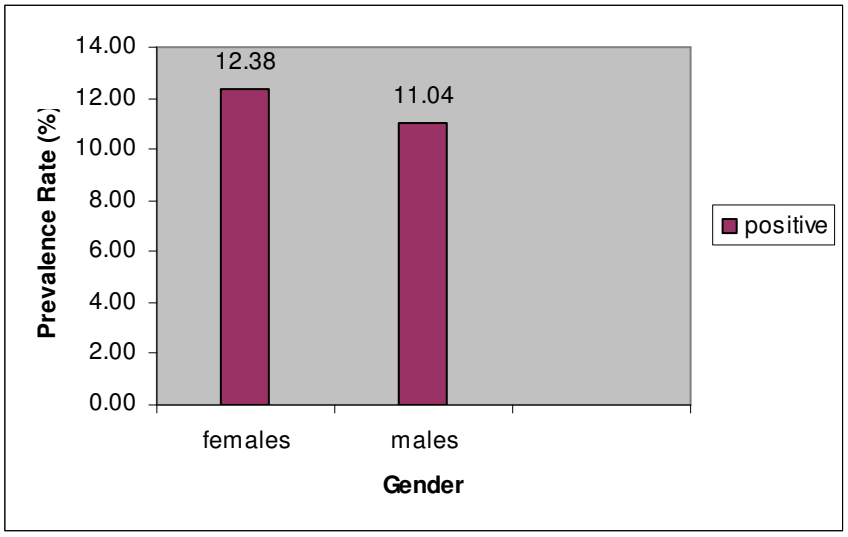


Figure 3.5 Prevalence of *Cryptosporidium* in the seven districts by gender.

3.4 Prevalence of *Cryptosporidium* in the seven districts by age categories

The prevalence rate of *Cryptosporidium* infection was found to be significantly different between age categories, and the prevalence rate was extremely higher in the age category (>0-5) than other categories ($P<0.05$); the prevalence rate of the parasite in the different age categories was as follows; 0-5 age category (14.4%, 67/464), >5-10 age category (7.7%, 15/195) and >10-15 age category (5.9%, 6/101; Figure 3.6).

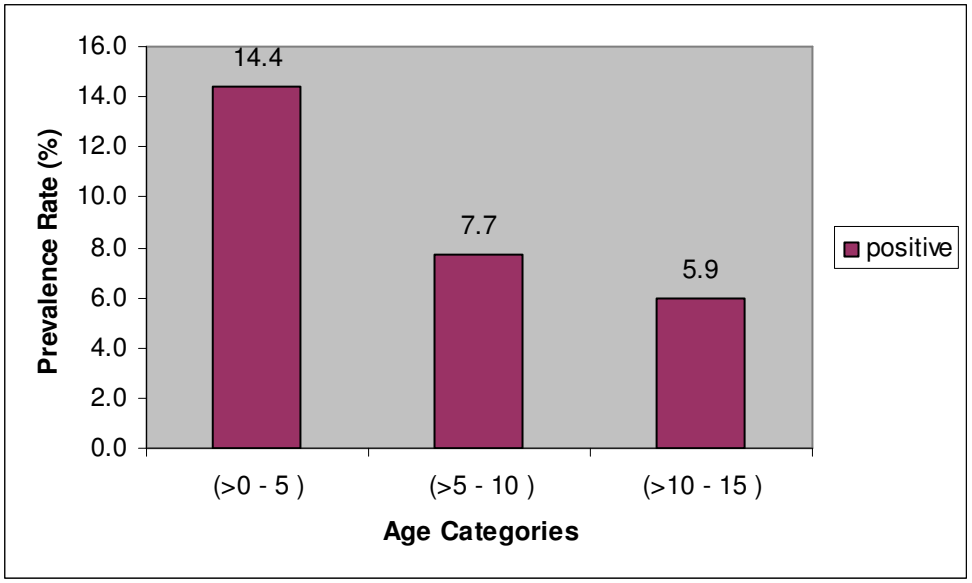


Figure 3.6 Prevalence of *Cryptosporidium* in the seven districts by age categories.

3.5 The prevalence rate of *Cryptosporidium* in each district by city

The prevalence rate of *Cryptosporidium* infection was moderately higher in Hebron and Jenin in comparison with other cities, but there was no significant difference between cities of different districts on the prevalence rate (P>0.05). The prevalence rate was as follows; Hebron city (15.6%, 7/45), Jenin city (13.0%, 3/23), Qalqilya city (10.0%, 6/60), Nablus city (8.3%, 2/24), Bethlehem city (8.0%, 2/25), Tulkarm city (6.7%, 1/15) and Ramallah city (4.8%, 2/42)(Figure 3.7).

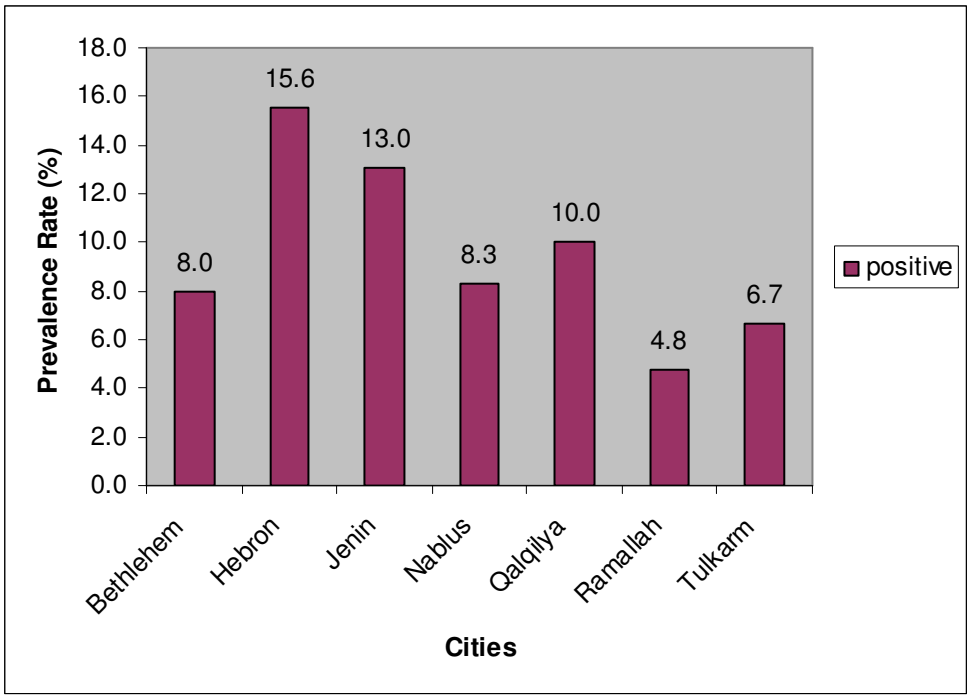


Figure 3.7 The prevalence of *Cryptosporidium* in each district by city.

3.6 The prevalence rate of *Cryptosporidium* in each district by camps

The prevalence rate was found to be comparable between the camps in the different districts, and there was no significant difference between the camps on the prevalence rate of *Cryptosporidium* (P>0.05), but it was slightly higher in Jenin camp than other camps in different districts. The prevalence rate was as follows; Jenin camp (15.8%, 3/19), Tulkarm camps (14.3%, 3/21), Hebron camps (13.0%, 3/23), Nablus camps (12.5%, 2/16), Ramallah camps (11.8%, 4/34) and Bethlehem camps (10.5%, 2/19) (Figure 3.8).

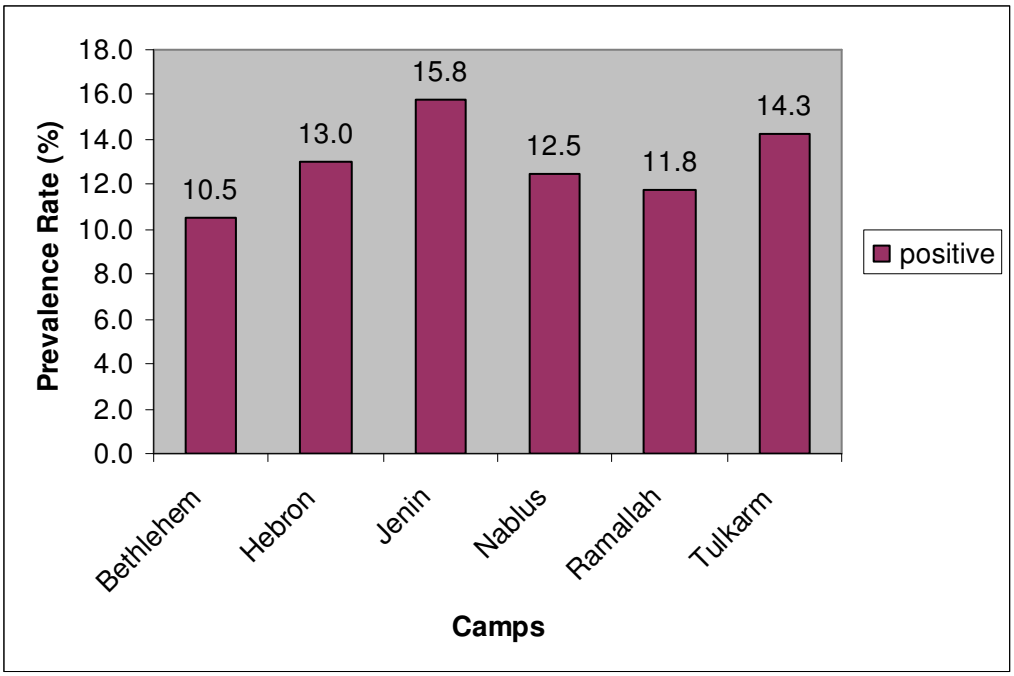


Figure 3.8 The prevalence of *Cryptosporidium* in each district by camp

3.7 The prevalence rate of *Cryptosporidium* in each district by villages

The prevalence rate of *Cryptosporidium* infection was not found to be significantly different between the villages of different districts (P>0.05), but it seemed to be clear that prevalence rate was slightly higher in Hebron and Jenin villages. The prevalence rate was as follows; Hebron villages (15.5%, 18/116), Jenin villages (14.3%, 3/21), Nablus villages (13.0%, 3/23), Qalqilya villages (11.4%, 10/88), Ramallah villages (11.3%, 6/53), Tulkarm villages(10.0%, 2/20) and Bethlehem villages (8.2%, 6/73) (Figure 3.9) .

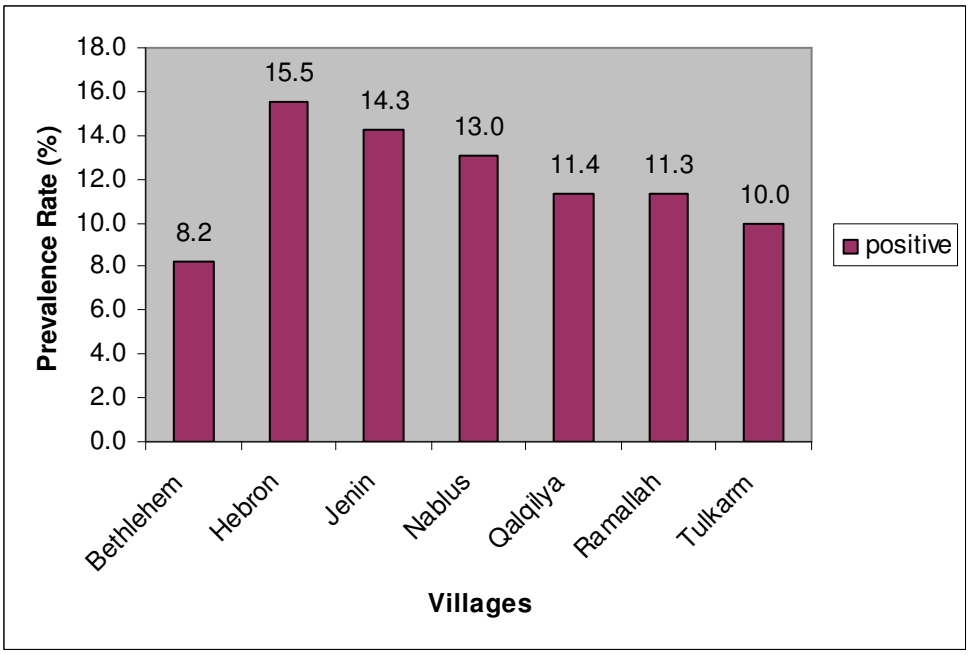


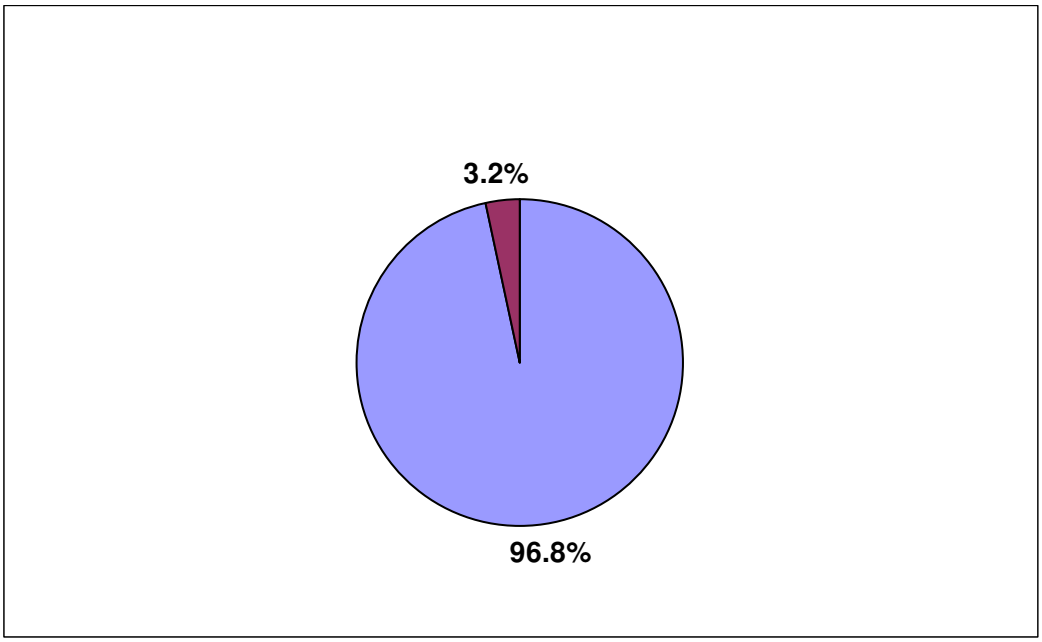
Figure 3.9 The prevalence of *Cryptosporidium* in each district by villages.

3.8. Prevalence of *Cryptosporidium* in the control group

The prevalence rate of *Cryptosporidium* in the control group is 3.2% (2/62); (Table 3.2) and (Figure 3.10).

3.2 Prevalence of *Cryptosporidium* in the control group.

Region	negative	positive	Total
South	20	1	**21**
Central	20	0	**20**
North	20	1	**21**
Total	**60**	**2**	**62**
Percentage	**96.8%**	**3.2%**	**100%**



3.10 Prevalence of *Cryptosporidium* in the control group.

CHAPTER FOUR

Discussion

4.1 Prevalence rate of *Cryptosporidium* in the West Bank, Palestine.

This study demonstrates clearly that the prevalence rate of *Cryptosporidium* in children with diarrhea in the West Bank is relatively high (11.6%) compared to 3.2% in the control group. So, this result is approximately close to other previous results like the the study which has been conducted at Caritas Baby Hospital with a prevalence rate of 13.5% (Sallon, *et al*., 1988). Moreover, the prevalence rate of this study is relative to the one in Gaza Strip, where the prevalence rate of *Cryptosporidium was* (14.6%). The results are also consistent with those obtained in Egypt in 1987 with a prevalence rate (11.6%) (Michel, *et al.,* 1987).

This study has similar results to those obtained in Rawalpindi, Pakistan, with a prevalence rate of 10.3% in diarrheic children and 3.3% in the control group (Iqbal, *et al*., 1999). Also, the study conducted in Iraq is not far from this result where the prevalence rate was 8.8% for children under five years old with a primary diagnosis of gastroenteritis (Mahdi, *et al*., 1996).

The results obtained in this study are within the range (1.4%-40.9%) extracted from 100 reports with more than 133,175 diarrheic patients infected with *Cryptosporidium* in immunocompetent individuals in developing countries (Guerrant, 1997).

A study conducted in Irbid, Jordan showed different results where the prevalence rate of *Cryptosporidium* was 1.5% among children under five years of age (Youssef, *et al.*, 2000). This difference could be attributed to the children socioeconomic class and

regional distribution. In addition to the hygienic condition and current methods of filtration and flocculation of water supplies done in Jordan which may be different from our localities in the West Bank.

The high prevalence rate of *Cryptosporidium* in the West Bank may be attributed to wastewater disposal because 71.2% of people in southern part of the West Bank use porous cesspit compared to 61.7% and 56.1% in northern and central part of the West Bank respectively (Palestine Central Bureau of Statistics, 2004). Also, the sewage network is not well developed, yet it is used by 40.5% of the people in the central part of the West Bank compared to 26.3% and 30.6% in southern and northern parts of the West Bank respectively. Moreover domestic rain wells are deeper than cesspit levels in nearly 80% of all regions of the West Bank (Palestine Central Bureau of Statistics, 2004). Ultimately, drinking water supplies will be contaminated and become a health hazard where transmission of pathogens is enhanced.

4.2 The prevalence rate of *Cryptosporidium* in different districts

The prevalence rate of *Cryptosporidium* is moderately higher in Hebron district (15.2%) than other districts. This might be attributed mainly to contamination of drinking water supplies. The Annual report conducted by the Central Public Health Laboratory(CPHL), Ministry of Health; in 2004 about contamination of drinking water; on 5698 samples of drinking water from different districts showed that 35.8% (177/494) of drinking water in Hebron is contaminated compared to 30.9% (138/447) in Toulkarm, 24.7% (330/1338) in Jenin, 22.8% (313/1374) in Nablus, 22.3%(72/323) in Qalqilia, 21.5% (123/572) in Ramallah, and 13.4% (67/501) in Bethlehem.

The mid-year report of 2005 about contamination of drinking water which has been conducted by (CPHL) on 3066 samples of drinking water also indicates that drinking water in Hebron is more contaminated (38.2%, 87/228) compared to 28.2% (188/667) in Nablus, 25.1% (106/423) in Ramallah, 24.9% (67/269) in Toulkarm, 22.6% (51/226) in Bethlehem, 18.8% (37/197) in Qalqilia, and 16.5% (118/715) in Jenin.

A household environmental survey conducted by Palestine Central Bureau of Statistics in 2004 showed that 97.6% of people in the central part of West Bank use public water network for drinking water, 1.7% use domestic wells, 0.3% of people use springs and 0.4 use water tanks. While 77.6% of people in northern part of West Bank depend on public water network for drinking of water, 18.6% use domestic wells, 3.4% use water tanks and 0.4% use springs. Also 78% of people in southern part of the West Bank depend on public water network, 17.6% depend on domestic wells, 3.5% depend on water tanks, 0.3% depend on springs (Palestine Central Bureau of Statistics, 2004). These results show clearly that domestic wells almost are not used in the central part of West Bank compared to 17.8% and 18.6% in south and north of West Bank respectively.

From these results it can be concluded that using domestic wells may contribute to contamination of drinking water if they are not cleaned periodically. The use of dumping sites as a method of solid wastes disposal without environmental control in the southern part of the West bank mainly in Hebron is very high (57.6%) compared to 14.3% and 0% in the central and the northern parts of the West Bank, respectively. Consequently, this will increase contamination and enhances the transmission of pathogens into human (Palestine Central Bureau of Statistics, 2004).

4.3 The Prevalence rate of *Cryptosporidium* in different regions

The prevalence rate of *Cryptosporidium* seemed to be slightly higher in camps than in cities and villages and this could be the result of poor hygienic and sanitary conditions in camps in general, in addition to problems in water supplies and methods of wastewater disposal (Abu Mourad, 2004).

4.4 The Prevalence rate of *Cryptosporidium* in males and females

There was no significant difference between males and females on the prevalence rate of the parasite. However, females (12.4%) have a slightly higher prevalence rate than males (11.0%). This slight difference may be related to differences in social and hygienic habits practiced by both sexes. But the reason is unknown, because data on gender, race and ethnicity are incomplete currently. So conclusions could not be drawn about differences in the epidemiology of cryptosporidiosis based on gender (CDC, 2005).

4.5 The Prevalence rate of *Cryptosporidium* by age categories of children

The highest prevalence rate was encountered in >0-5 years age category (14.4%) followed by > 5-10 years category (7.7%) and finally with >10-15 years age category (5.9%). This significant difference ($P<0.05$) could be attributed to the incomplete maturation of the immune system of the first category (>0-5) in addition to the lack of self awareness, personal hygiene and cleanliness at this critical age, therefore, they are most easily and frequently exposed to parasitic infections such as *Cryptosporidium*

infection (Hunter and Nichols, 2002; Abu Mourad, 2004, CDC, 2005), meaning that they are considered to be as the highest risk group in the study.

Recommendations

● This study shows that the Prevalence rate of *Cryptosporidium* in the West Bank is relatively high in comparison with other countries. So there is a need for further researches in the future to identify the parasite in more details such as detection of *Cryptosporidium* in water supplies and sources of infection. Screens for the parasite by other confirmatory methods such as immunofluorescent antibody (IFA), enzyme immunoassay (EIA) and Polymerase Chain Reaction (PCR) are needed.

● Detection of the parasite should be done routinely in public and private laboratories. The modified acid fast method, which can be done within few minutes, may be adopted. Technicians should be trained to test for *Cryptosporidium* in public and private hospitals in the West Bank and Gaza Strip.

● Help in the development of new strategies by the Ministry of Health to control the transmission of cryptosporidiosis.

● Prevention of any future outbreaks of cryptosporidiosis and the identification of *Cryptosporidium* genotypes to determine the origin of the species of the parasite.

● Awareness campaign should be conducted throughout the country about the importance of sanitary and hygienic practices in preventing parasitic infections including *Cryptosporidium* infection.

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Table 3.1 Distribution of *Cryptosporidium* prevalence rate (%) in children with diarrhea in the seven surveyed districts by gender, region and age categories.

District	Result	Gender		Region			Age Categories			Total
		Female	Male	City	Village	Camp	>0 - 5	>5 - 10	>10 - 15	
Bethlehem	Positive	4(8.9%, 4/54)	6(9.5%, 6/63)	2(8%, 2/25)	6(8.2%, 6/73)	2(10.5%, 2/19)	10(8.5%, 10/117)	-	-	117
	Negative	50	57	23	67	17	107	-	-	
	Total	**54**	**63**	**25**	**73**	**19**	**117**	**-**	**-**	
Hebron	Positive	13(21.3%, 13/61)	15(12.2%, 15/123)	7(15.6%, 7/45)	18(15.5%, 18/116)	3(13%, 3/23)	22(21.2%, 22/104)	4(8.5%, 4/47)	2(6.1%, 2/33)	184
	Negative	48	108	38	98	20	82	43	31	
	Total	**61**	**123**	**45**	**116**	**23**	**104**	**47**	**33**	
Jenin	Positive	4(13.8%, 4/29)	5(14.7%, 5/34)	3(13%, 3/23)	3(14.3%, 3/21)	3(15.8%, 3/19)	6(21.4%, 6/28)	2(8.3%, 2/24)	1(9.1%. 1/11)	63
	Negative	25	29	20	18	16	22	22	10	
	Total	**29**	**34**	**23**	**21**	**19**	**28**	**24**	**11**	
Nablus	Positive	2(7.4%, 2/27)	5(13.9%, 5/36)	2(8.3%, 2/24)	3(13%, 3/23)	2(12.5%, 2/16)	6(17.1%, 6/35)	1(4.5%, 1/22)	0(0%, 0/6)	63
	Negative	25	31	22	20	14	29	21	6	
	Total	**27**	**36**	**24**	**23**	**16**	**35**	**22**	**6**	
Qalqilya	Positive	6(10.7%, 6/56)	10(10.9%, 10/92)	6(10%, 6/60)	10(11.3%, 10/88)	-	10(13.7%, 10/73)	4(8.1%, 4/49)	2(7.7%, 2/26)	148
	Negative	50	82	54	78	-	63	45	24	
	Total	**56**	**92**	**60**	**88**	**-**	**73**	**49**	**26**	
Ramallah	Positive	6(10.9%, 6/55)	6(8.1%, 6/74)	2(4.7%, 2/42)	6(11.3%, 6/53)	4(11.7%, 4/34)	8(11.6%, 8/69)	3(7.3%, 3/41)	1(5.2%, 1/19)	129
	Negative	49	68	40	47	30	61	38	18	
	Total	**55**	**74**	**42**	**53**	**34**	**69**	**41**	**19**	
Tulkarm	Positive	3(12%, 3/25)	3(10.7%, 3/28)	1(6.6%, 1/15)	2(10%, 2/20)	3(14.3%, 3/21)	5(13.1%, 5/38)	1(8.3%, 1/12)	0(0%, 0/6)	56
	Negative	22	25	14	18	18	33	11	6	
	Total	**25**	**28**	**15**	**20**	**21**	**38**	**12**	**6**	
Total		**307(12.4%, 38/307)**	**453(11%, 50/753)**	**234(9.8%,23/234)**	**394(12.2%, 48/394)**	**132(12.9%, 17/132)**	**464(14.4%, 67/464)**	**195(7.7%, 15/195)**	**101(5.9%, 6/101)**	760