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Prevalence of *Cryptosporidium* Species Among Children ≤ 5 Years Old in North West-Bank, Palestine/ Cross Sectional Study

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Dedication

This thesis is dedicated to my late father, my beloved mother, brothers, sisters and all my family members especially my dearest uncle Adli for his continuous encouragement throughout the course of my research.

In addition I would like to express my love and gratitude to my husband Bashar for his endless assistance and limitless effort.

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Haneen Da'as

الإقــرار

أنا الموقعة أدناه مقدمة الرسالة التي تحمل العنوان:

Prevalence of *Cryptosporidium* Species Among Children ≤ 5 Years Old in North West-Bank, Palestine/ Cross Sectional Study

اقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء مــا تمــت الإشارة إليه حيثما ورد، وان هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أية درجة علمية أو بحث علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالبة:**

Signature: **التوقيع:**

Date: **التاريخ:**

Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
C.	*Cryptosporidium*
CDC	Centre of Disease Control and Prevention
DNA	Deoxyribonucleic Acid
E. histolytica	*Entamoeba histolytica*
EIA	Enzyme Immuno Assay
ELISA	Enzyme-Linked Immunosorbant Assay
G. lamblia	*Giardia lamblia*
HAAT	Highly active antiviral therapy
HIV	Human Immunodeficiency Virus
IFA	Immunofluorescent Antibody
PCBS	Palestinian Central Bureau of Statistics
PCR	Polymerase Chain Reaction
Spp.	Species
WB	West Bank
ZN	Ziehl Neelsen

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Prevalence of *Cryptosporidium* Species Among Children ≤ 5 Years Old in North West-Bank, Palestine/ Cross Sectional Study

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Abstract

Background: Cryptosporidiosis is an infection caused by *Cryptosporidium*; a protozoan parasite that infects the gastrointestinal tract. The infection is of major public health concern in both developed and developing countries. The disease is potentially life-threatening in immunocompromised persons. Younger children are more susceptible to infection than others. Human get infection either from food or water. Objectives: The current study aims to explore the prevalence of cryptosporidiosis and other related intestinal parasitic infection in children of age ≤5 years old. Methodology: A cross-sectional study was conducted by collecting fecal materials from 500 children from regions of North West Bank. Fecal samples were analyzed microscopically and positive samples with *Cryptosporidium* were used for molecular diagnosis using polymerase chain reaction. Results: The prevalence of cryptosporidiosis in Palestinian children ≤5 years old was 13.6%. Prevalence rates for amoebiasis and giardiasis were 11% and 4.4% respectively. Refugee camps showed higher infection, although not significantly different ($p>0.05$). Data analysis revealed no statistically significant association between infection with cryptosporidiosis and predictor variables. Conclusion: Prevalence of cryptosporidiosis among children ≤ 5 years old is 13% and thus, measures to prevent parasitic infection are recommended.

Chapter One
Introduction

1.1 Introduction

Intestinal parasitic infections remain a serious public health problem globally [1]. They have been associated with human malnutrition [2].

Intestinal parasites are organisms that live in the gastrointestinal tract of humans and animals; they are the common cause of human diarrhoeal disease worldwide leading to significant morbidity and mortality in the world, particularly in developing countries.

There are over 100 different types of parasites that can live in human hosts. Since the world's population is becoming more mobile, parasitic infections may reach epidemic levels in most countries in the world.

The major groups of intestinal parasites include parasitic worms (helminthes) and protozoans. Helminths are multicellular worms that are generally visible to the naked eye in their adult stages. Tapeworms, pinworms, and roundworms are among the most common helminths. Infection by these worms (helminths) is widespread throughout the world, affecting hundreds of millions of people. Children are particularly susceptible and typically have the largest number of worms, and it is possible to be infected with more than one kind of worms.

Protozoans have only one cell, and can multiply inside the human body, which contributes to their survival and enables serious infections to develop. *Cryptosporidium*, *Microsporidia* and *Isospora* are the most common protozoans in HIV patients. Many protozoan parasites can survive for long periods in soil and water; others normally thrive and multiply in the environment.

Infection by intestinal parasites is orally through contaminated food or water. People in developing countries are at particular risk due to drinking water from sources that may be contaminated with parasites that colonize the gastrointestinal tract.

Among intestinal parasites; helminthes, hookworms, *Ascaris lumbricoides* and *Trichuris trichiura* are considered as major intestinal nematodes. These parasites cause 39.0 million infections as compared to malaria at 35.7 million [3].

Parasitic protozoa that infect intestinal tract include *Entamoeba histolytica* that is considered as the causative agent of amebiases, *Giardia lamblia* that causes giardiasis and *Cryptosporidium Spp.* that causes cryptosporidiosis .

E. histolytica is the most common of the intestinal amoebae that cause disease; it is the agent of amoebic dysentery. This parasite not only causes severe diarrhea but can cause abscesses in the intestine, liver, and other organs. Around 500 million people are infected worldwide while 75,000 die of the disease annually, and it ranks third on the list of parasitic causes of death worldwide behind malaria and schistosomiasis [4]. *E. histolytica* can easily contaminate food crops as well as water supplies.

Giardia lamblia was the first protozoan parasite described, its role as pathogenic organisms was not recognized until the 1970s [5]. Prior to that time, the organism was thought to be harmless commensal of the intestine. It is found in surface waters all over the earth and spread in the feces of both humans and animals. The prevalence of the disease varies from 2.0% - 5.0% in developed to 20.0% - 30.0% in developing countries [6], and now it turned out to be one of the most common causative agents of epidemic and endemic diarrhoeal illness throughout the world [5].

Cryptosporidium is an intracellular protozoan parasite that is associated with gastrointestinal diseases in all classes of vertebrates including mammals, reptiles, birds and fish [7].

Cryptosporidiosis is a diarrheal disease caused by microscopic parasites of the genus *Cryptosporidium.* Once an animal or person is infected, the parasite lives in the intestine and passes in the stool. The parasite is protected by an outer shell that allows it to survive outside the body for long periods of time and makes it very resistant to chlorine disinfectants. Both the disease and the parasite are commonly known as "Crypto." [8, 9].

Along with *Giardia*, *Cryptosporidium* is among the most common parasitic enteric pathogens in humans. The organisms infect and reproduce in the epithelial cells of the digestive or respiratory tracts [7].

There are more than ten named species of *Cryptosporidium* including species that infect mammals, birds, reptiles and fish [10]. *C. parvum* (4 µm diameters) is the main species responsible for disease in human. *C. parvum* has been divided into two separate species: *C. homonis* (previously *C. parvum* genotype 1), and *C. parvum* (genotype 2). *C.*

hominis apparently infect only humans, while *C. parvum* is found in humans and a number of other animals [11, 12].

C. felis , *C. muris* , *C. canis*, *C. suis*, and *C. meleagridis* have also been identified in some individuals. Additional heterogeneity within species may lead to variations in infectivity and clinical expression in different hosts [13, 14].

Cryptosporidiosis is most common in children aged between 1 and 5 years, although outbreaks occur worldwide in all age groups [15]. People with weak immune systems (those with AIDS, or persons who have undergone transplantation or are receiving chemotherapy) are likely to be most seriously affected [16].

1.2 Characteristics of *Cryptosporidium*

Cryptosporidium oocysts are highly resistant to many common disinfectants such as aldehyde-, ammonia-, alcohol-, chlorine-, and alkaline based commercial disinfectants.

Oocysts are heat sensitive, a temperature of 65º C inactives oocysts within 5-10 minutes. Over a period of 2 hours or more, desiccation is lethal to oocysts. Oocysts can remain viable for about 18 months in a cool, damp or wet environment [17], so they are common in rivers and lakes especially where there has been sewage or animal contamination. Since these oocysts are generally susceptible to freezing, this varies by onset of freezing; snap freezing will destroy oocysts reliably, but with slow freezing, such as that found in natural environment, oocysts have been reported to survive temperatures as low as -22º C [18] .

The small size of *Cryptosporidium* oocysts and its resistance to disinfectants facilitates transmission of disease and challenges the standard filtration and disinfecting procedures. In addition to the high probability for mis-diagnosed cases due to it's small size [19].

In a study that has been conducted to demonstrate the infectivity of *Cryptosporidium* on volunteers, they found that the infective dose can be as low as one oocyst [20]. Thus, low dose for infection and the prolonged excretion of high numbers of oocysts make *Cryptosporidium* ideal for waterborne transmission since infected individuals can excrete up to a billion oocysts per infection [21], and *Cryptosporidium* oocysts are present in 65.0% to 97.0% of surface waters and are difficult to eradicate and also can survive in the environment for months [18].

1.3 Epidemiologic features

Since the first reported cases of human infection in 1976, *Cryptosporidium* has become one of the most commonly reported enteric pathogens in both immunocompetent and immunocompromised persons worldwide [22], and has been reported from 3 days to 95 years old. It is responsible for both epidemic as well as endemic levels of intestinal diseases [23].

The proportion of the general population excreting oocysts is 1.0% to 3.0% in developed countries and 10.0% in developing countries [24].

Cryptosporidial infection accounts for 2.2% (range, 0.26% to 22.0 %) of cases of diarrhea in immunocompetent persons in developed countries and 6.1% (range, 1.4% to 41.0%) of cases of diarrhea in immunocompetent persons in developing countries [8].

It occurs in up to 7.0% of children with diarrhea in developed countries and up to 12.0% of children with diarrhea in developing countries [8]. Cryptosporidial infection is more common in immunocompromised persons, especially those with AIDS. In developed countries, it occurs in 14.0% of patients with AIDS and diarrhea; in developing countries, it occurs in 24.0% of such patients [8].

Cryptosporidiosis remains a clinically significant problem in patients without access to highly active antiretroviral therapy (HAAT) and in malnourished children, as well as in people in developed countries who have undergone transplantation or are receiving chemotherapy.

The general epidemiologic and biologic features of *Cryptosporidium parvum* are explained in table 1.1.

Table (1.1): Biologic and epidemiologic features of *Cryotosporidium parvum* that favor transmission to humans [22].

Outbreaks of water-borne infection occur.
Organism has ubiquitous geographic distribution and wide host range.
Oocysts are resistant to disinfectants.
Transmission can occur by direct fecal–oral (person-to-person or zoonotic) route.
As few as 10 to 100 oocysts can cause infection.
Oocysts are excreted in fully infective form with no external maturation required and with the capacity to complete their entire life cycle in a single host.
Oocysts are excreted in very large numbers, which are increased by autoinfective stage of production.
Many asymptomatic infections exist.
Specific and fully effective treatment is lacking.

1.4 Mode of transmission

Cryptosporidium is shed in the feces of infected humans and animals. People become infected by ingesting the organism [25]. Infected individuals can shed the organism in their stool for 6-18 days in immunocompetent individuals but may be prolonged in immunosuppressed patients, (some individuals shed oocysts but appear asymptomatic) [26]. Because cryptosporidiosis is transmitted by the fecal-oral route, the greatest potential to transmit the organism comes from infected people who have diarrhea, people with poor personal hygiene and diapered children [9, 8].

Cryptosporidium can be spread by person-to-person or animal-to-person contact, contaminated food such as vegetables, fruits, raw meat and unpasteurized milk and by drinking contaminated water (rivers, lakes, streams) [27]. Cryptosporidial oocysts may be found in all types of water, including untreated surface water, filtered swimming-pool water, and even chlorine-treated or filtered drinking water [22]. Contamination of untreated surface water and filtered public water supplies is a growing concern, since water-borne outbreaks have been reported worldwide [22].

Person-to-person spread of *C. parvum* is one of the most common modes of transmission.

Children still wearing diapers who attend day care centers are at high risk for this form of transmission through intimate play or careless diaper changing practices. Infections acquired by children in the day care are transmitted to care-givers at the facility and to older children and adults who come in contact with the infected child at home [28].

Health care workers, users of communal swimming pools and travelers to regions of highly endemic disease are also at increased risk. *Cryptosporidium* was present in 2.8 % of 795 international travelers with diarrhea in one report [29].

1.5 Clinical features

After two to ten days of infection by the parasite, symptoms will appear (the incubation period is typically approximately one week, but can be as long as 28 days). The number of oocysts ingested appears to be related to the time and duration of infection, but not the severity of illness [25]. Some persons may not have symptoms, others have self-limited watery diarrhea (more than 90%) lasting 5-10 days, headache, abdominal cramps, nausea, vomiting, and 36% also have fever, that may lead to weight loss and dehydration [30,31]. There is also evidence that specific species or subtype families are associated with different clinical manifestations. For example, in a cross-sectional study of 230 HIV-infected patients in Peru, infection with *C. hominis* was associated with diarrhea alone while infection with *C. parvum* was associated with diarrhea and vomiting [32].

In healthy persons these symptoms usually last 1 to 2 weeks so that the immune system is able to stop the infection [30, 31]. In persons with suppressed immune systems, such as persons who have AIDS or have an organ or bone marrow transplant or receiving chemotherapy, the infection may be chronic and become life-threatening [3, 19].

The level of host immunity primarily account for the variable course of infection in different people, so the severity and duration of diarrhea and the other clinical manifestations of the infection differ in immunocompetent and immunocompromised patients. Cryptosporidiosis in patients with AIDS have a wide spectrum, from asymptomatic to a fulminant illness, (Table 1.2) [7] shows the clinical features of *Cryptosporidium* in immunocompetent and immunocompromised persons.

Table (1.2): Clinical features of cryptosporidiosis [7].

Characteristic	In immunocompetent persons	In immunocompromized persons
Susceptible population	Children, especially those under 1 year of age, and adults of any age	Immunocompromised persons of any age, especially those with AIDS
Site of infection	Usually intestinal	Intestinal or extraintestinal
Enteric presentation	Asymptomatic, acute, or persistent	Asymptomatic, transient, chronic, or fulminant
Common clinical symptoms	Diarrhea, fever, abdominal cramps, vomiting, nausea, and weight loss	Diarrhea, fever, right-upper-quadrant pain, jaundice, weight loss, and vomiting
Clinical duration	Up to 2 wk	From 2 days to lifetime
Severity according to CD4+ count >200 cells/mm3 <100 cells/mm3 <50 cells/mm3		 Spontaneous resolution Chronic and extraintestinal Fulminant
Estimated outcome	High mortality in infants and young children in developing countries	Transient or asymptomatic (5–184 wk), chronic (3–127 wk), or fulminant (2–28 wk)†
Treatment	No specific treatment necessary	Highly active antiretroviral therapy alone or with antiparasitic agents

†Ranges indicate time to death after infection.

1.6 Life cycle

Cryptosporidium is capable of completing all stages of its development (asexual and sexual) within a single host as shown in figure 1.1[7].

The thick-walled oocyst is the resistant stage found in the environment. When mature oocysts (5 µm) are ingested, the host body temperature, the interaction with stomach acid and bile salts triggers excystation and releasing four banana-shaped motile sporozoites that attach to the epithelial cell wall of the gastrointestinal tract [13,33].

These sporozoites differentiate into a spherical trophozoite and mature asexually into two types of meronts, type I meronts contain 6-8 nuclei which release 6-8 merozoites intraluminally [34]. 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 [13].

Merozoites from type II meronts also invade new host but undergo sexual maturation by differentiating into either microgamont (male) or macrogamont (female) stages [34]. After fertilization of the macrogamont by microgamonts, the fertilized macrogamon (zygote) then develops into an oocyst that sporulates within the infected host by undergoing mitosis [7].

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 oocysts (20%) that are involved in autoinfection because they excyst within the gut, they release merozoites and infect new host cells [33,34].

The new generation of the parasite can develop and mature within 12-14 hours helping it to produce huge numbers of parasitic cells in the gut and to secondary infection sites in the duodenum and large intestine [33].

Oocysts are infectious and can remain viable for many months at a wide range of temperatures.

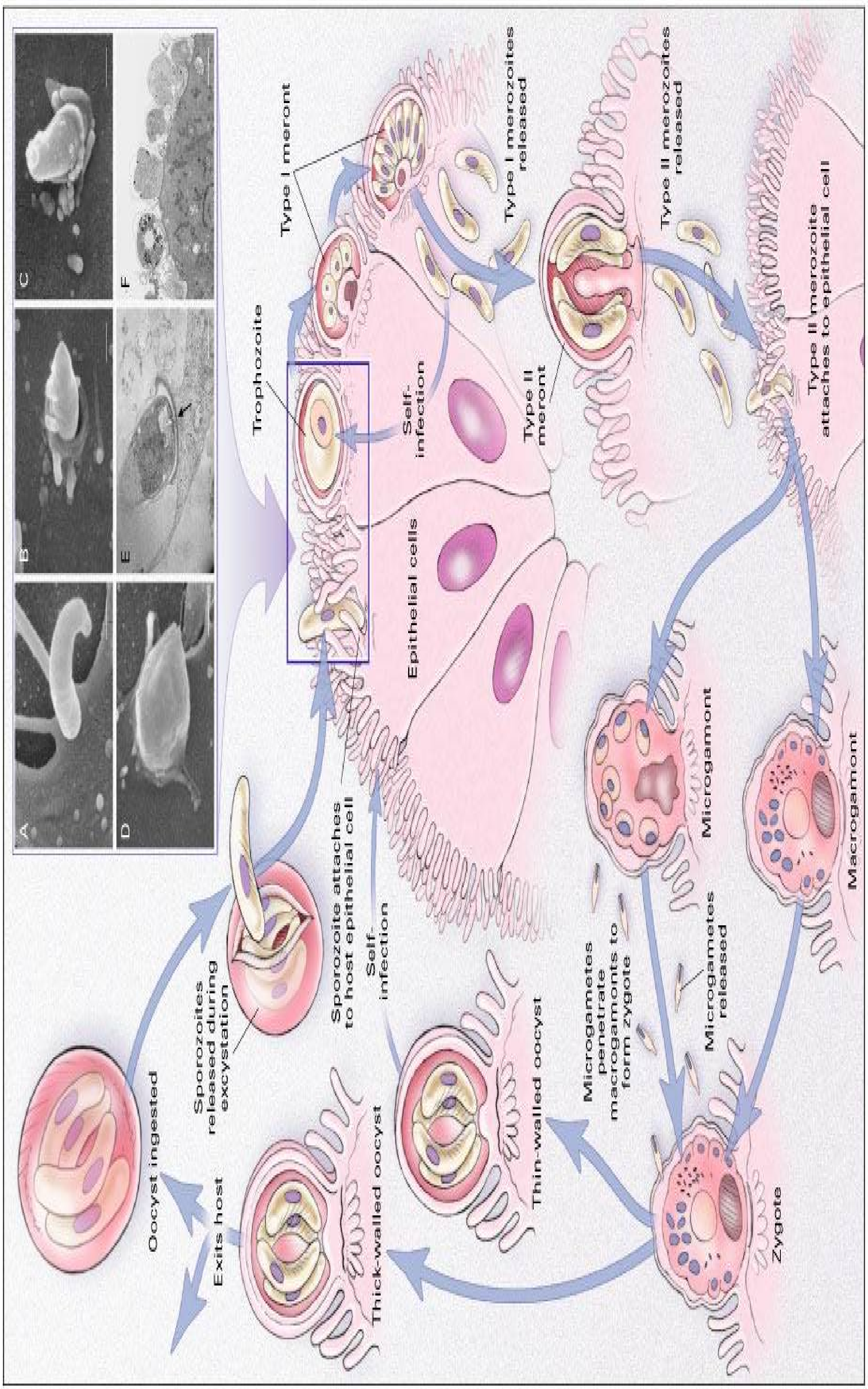

Figure (1.1): Life cycle of *Cryptosporidium* [7].

1.7 Diagnosis

Diagnosis of cryptosporidiosis is made mainly microscopically by examination of stool samples for the prescence of oocysts. Because detection of *Cryptosporidium* is difficult, patients may be asked to submit several stool samples over several days and the microscopic identification of *Cryptosporidium* requires well-trained and experienced microscopists.

Stool specimens are examined microscopically using different techniques (e.g. acid-fast staining, malachite green staining, immunofluorescent antibody (IFA) and enzyme immuno assay (EIA)).

Molecular method (e.g. polymerase chain reaction – PCR) is increasingly used, since they can be used to identify *Cryptosporidium spp.* at the species level. But since *Cryptosporidium*'s tests are not routinely done in most laboratories, health care providers should specifically request testing for this parasite.

Serologic tests are of limited value, because many healthy persons have antibodies to *Cryptosporidium*.

When biliary disease is suspected, ultrasonography is the best initial diagnostic test. It will show thickening of the biliary-duct wall, dilatation of the gallbladder, or both and in ruling out associated conditions such as stones, compression, and tumors. Also percutaneous liver biopsy is rarely helpful and has no important role in diagnosis.

1.8 Conclusion and prevention

Cryptosporidium is a highly infectious cause of diarrheal illness around the world. The human host range is broad and includes people with AIDS, children in various countries, and outbreaks among immunocompetent individuals. Within many of these groups, the manifestations of disease are diverse, ranging from asymptomatic infections to life-threatening illness.

Therefore, effective control measures must aim to reduce or prevent oocyst transmission, since *Cryptosporidium* infection occurs by the ingestion of oocysts excreted in the feces of infected host individuals.

Good hygiene, such as handwashing (washing hands with soap can reduce the risk of diarrhoeal diseases by 42.0% to 47.0%, and the promotion of hand washing might save a million lives per year) [35] after using the toilet and before eating or preparing food and proper disposal of contaminated material such as diapers, are the most important ways to prevent infection. Thus, avoid swimming in recreational water if you have cryptosporidiosis and for at least 2 weeks after the diarrhea stops, since people can pass *Cryptosporidium* in their stool and contaminate water for several weeks after symptoms have ended. They do not need to have a fecal accident in the water. Infected people may have *Cryptosporidium* on their skin in the anal and genital areas. Therefore immersion in the water may wash the parasites off the body. As a result of infected people swimming, many outbreaks of cryptosporidiosis among recreational water users have occurred.

Also people should avoid close contact with anyone who has a weakened immune system. And we must exclude children with diarrhea from child care settings until the diarrhea has stopped.

In people with AIDS and in others whose immune system is weakened (cancer chemotherapy, kidney dialysis), crypto can be serious, long-lasting and sometimes fatal so an extra precautions are needed for them [8, 9].

Boiling or filtering water may decrease the risk of infection in immunosuppressed patients. It is also suggested that immunocompromised patients who are at high risk for severe infection limit their exposure to *Cryptosporidium* by minimizing oral exposure to water from lakes, streams, and public swimming pools. They should avoid the stool of all animals and wash their hands thoroughly after any contact with animals or the living areas of animals. Also they should wash, peel, or cook all vegetables. And extra care must be taken when traveling.

In locations such as hospitals, laboratories, and day-care centers, management should minimize contact with sources of infection. This means isolation of infected persons, careful handling and disposal of biohazardous waste, washing hands after changing diapers, and boiling of water before consumption.

1.9 Treatment

Recovery from *Cryptosporidium* infection depends largely upon the immune status of the host.

Immunocompetent hosts usually have a spontaneous recovery within a few weeks, and no treatment is necessary unless symptoms persist. Supportive care with oral or intravenous fluids and electrolyte replacement helps correct the dehydration that accompanies acute diarrhea.

When therapy is required, nitazoxanide, a nitrothiazole benzamide, is the preferred drug in children 1 to 12 years of age and also in adults [36]. Clinical improvement at 3-day course of nitazoxanide was significantly obvious in nonimmunodeficient patients in a multicenter, randomized, double-blind, placebo-controlled study that conducted in 90 outpatients 12 years of age and older from the Nile Delta region of Egypt [36].

In patients with AIDS, the best treatment is improvement of immune function with highly active antiretroviral therapy [13]. If (HAAT) is not possible or effective, combination therapy with an antimicrobial agent and an antidiarrheal agent will continue to be standard treatment for cryptosporidial diarrhea [37].

1.10 Literature review

On global scale, acute diarrhea and dehydration are major causes of children mortality, causing about 3.3 million deaths yearly [38].

Cryptosporidiosis is a common cause of childhood diarrhea, especially in developing countries, while sporadic cryptosporidiosis transmission occurs worldwide [39, 8].

The initial reports of *Cryptosporidium* infection was published by Tyzzer in 1907 [40], his finding was not regarded as important at that time.

In turkeys, Slavin described the parasite as a potential cause of diarrhea in 1955 [41]. Later its importance increased in 1970s when cryptosporidiosis was recognized in calves [42].

Although the first case of human cryptosporidiosis was reported in 1976 [43], more awareness of the organism really came to the fore in the 1980s, due to its association with HIV infection [44].

By the year 1984, 58 cases of cryptosporidiosis were reported, 40 cases were found in immunocompromized patients and 33 (82.5%) cases were detected in patients with AIDS, 22 (55.0%) of these 40 patients died [45].

During 1990s, *Cryptosporidium* was titled as a major cause of community gastroenteritis causing outbreaks associated with drinking water, swimming pools, and exposure to animals [46].

In February 1994 cryptosporidiosis was added to the list of reportable diseases in New York State, and since that date 400-500 cases are reported in New York State each year [47].

Waterborne *Cryptosporidium* outbreaks occurred in both large and small communities, mainly in the United States of America, European countries, Japan, and Canada, with the largest outbreak occurring in Milwaukee, Wisconsin in 1993, affecting an estimated 403,000 people [48]. The suspected cause was due to errors in treatment, possibly contamination of filtered water with raw, unfiltered water or inadequate monitoring of water turbidity. The mean duration of illness was 12 days with a range of 1 to 55 days, and the average maximum number of watery diarrheal stool was

19 per day at the peak of the illness. Watery diarrhea was the predominant symptom among 93.0% of confirmed cases [49].

Then, *Cryptosporidium* rapidly became recognized as one of the most serious and difficult to control waterborne pathogens to date.

Cryptosporidiosis became a significant cause of diarrheal diseases in both developing and industrialized nations [50], but several epidemiologic studies have demonstrated that *Cryptosporidium* is more prevalent in developing countries (5.0% to >10.0%) than in developed countries (<1.0% to 3.0%) [45-51]. It is present in 1 to 3 percent of immunocompetent patients with diarrhea in industrialized countries and 7.0% to 10.0 % in developing countries [24, 29]. In a 2005 survey from FoodNet of laboratory-confirmed causes of acute foodborne illnesses in ten states in the US, *Cryptosporidium* occur in 8.0 %, an annual incidence of 3 cases per 100,000 persons [52].

About 2.2% of all patients whose cases of AIDS are reported to Centers for Disease Control and Prevention (CDC) have cryptosporidiosis, 3.5% of children whose cases of AIDS are reported to the CDC have cryptosporidiosis [53].

1.11 Previous studies

In Saudi Arabia and neighboring countries a study was conducted to examine the epidemiology and disease burden of *Cryptosporidium* by reviewing 23 published studies of *Cryptosporidium* and the etiology of diarrhea between 1986 and 2006 [54]. The prevalence of *Cryptosporidium* infection in humans ranged from 1.0% to 37.0% with a median of 4.0%, while in animals it was different for different species of animals and geographic locations of the studies[54]. Most cases of cryptosporidiosis occurred among children less than 7 years of age, and particularly in the first two years of life. The seasonality of *Cryptosporidium* varied depending on the geographic locations of the studies, but it was generally most prevalent in the rainy season [54]. The most commonly identified species was *C. parvum* while *C. hominis* was detected in only one study from Kuwait[54]. The cumulative experience from Saudi Arabia and four neighboring countries (Kuwait, Oman, Jordan and Iraq) suggest that *Cryptosporidium* is an important cause of diarrhea in humans and cattle [54].

In Egypt in 2005, the prevalence and clinical characteristics of *Cryptosporidium* associated diarrhea was studied over a 2 years period by conducting a study titled by "Diarrhea Associated with *C. parvum* among Young Children of the Nile River Delta in Egypt" [55]. After a stool sample was obtained from each of the 1275 children with diarrhea evaluated, 214 (17.0%) were found to be infected with *Cryptosporidium*. Younger age was a risk factor for developing *Cryptosporidium*-associated diarrhea. Children <12 months of age were 2.4 times more likely to be infected with *Cryptosporidium* ($p<0.01$) and children 12 to 23 months were 1.9 ($p<0.05$) times more likely to be infected with the organism as compared to older children. Breastfeeding had a trend towards protection against *Cryptosporidium*-associated diarrhea ($p = 0.07$) [55].

In Jordan, a study entitled "Cryptosporidiosis in children in a north Jordanian pediatric hospital" was conducted in 2004 to investigate the rate of infection by *C. parvum* among children from birth to 12 years attending

Princess Rahma Teaching Hospital in Irbid [56]. Single stool specimens were collected from 300 children, 7 specimens were from children undergoing chemotherapy treatment for cancer. Diagnostic methods used for detection of infection were direct wet mount preparation, flotation concentration, cold Kinyoun Ziehl–Neelsen stain and direct immunofluorescence. As a result *C. parvum*_oocysts were detected in 112 samples (37.3%) using direct immunofluorescence, which showed the highest sensitivity. Source of drinking water appeared to be an important risk factor for transmission of infection [56].

Natural History of *G. lamblia* and *Cryptosporidium* Infections in a Cohort of Israeli Bedouin Infants was conducted by taking 164 Bedouin children, from a population not previously studied, which is in transition from nomadism to a settled life style [57]. Stools were sampled monthly from birth to two years of age and at all diarrhea episodes. The risk of infection with *G. lamblia* and *Cryptosporidium* infection by the age of two years old was 91.5% and 48.8%, respectively. *Cryptosporidium* prevalence was 3.0% to 4.0% at all ages and the asymptomatic detection rate was 1.6% being an important cause of diarrhea in young children in the community [57].

At the national level, there are a few unpublished reports mentioned the infection status of *Cryptosporidium* in Gaza strip. The results of these reports revealed the prevalence of this parasite in Gaza and Palestine, and showed the interventions needed for its control and management [58].

A study was conducted in Gaza Strip to determine the prevalence of intestinal *Cryptosporidium* among patients with diarrhea who attended Al-

Nasser Pediatric Hospital in Gaza [58]. So stool specimens from each of 416 children aged less than 1 year to more than 5 years old attending Al-Nasser Hospital were examined by Ziehl Neelsen (ZN) stain and ELISA for the presence of *Cryptosporidium* oocysts from January to May 2005. As a result *Cryptosporidium* oocysts were found in 62 (14.9%) specimens using modified ZN [58].

Moreover, a prospective survey of children admitted with gastro-enteritis to the Nasser Children's Hospital, Gaza revealed that 19.0% were excreting *Cryptosporidium,* a significantly ($p<0.05$) greater percentage than that (7.0%) observed in children admitted for other reasons and the detection of *Cryptosporidium* decreased when the change from hot dry to colder weather occurred [59].

Also a follow-up study of a cohort of the children with cryptosporidiosis indicated that over three-quarters were dehydrated and all were below their expected weight-for-age. There was a statistically significant association between *Cryptosporidium* gastro-enteritis and evidence of respiratory tract infection [59].

Concerning *Cryptosporidium* in West bank, a study has been conducted in Bethlehem at Caritas Baby Hospital on 221 children with diarrhea and gastroenteritis, showed that the prevalence of *Cryptosporidium* was 13.5% compared to 7.2% *E. histolytica*, and 3.6% *G. lamblia* [60] .

In another study a total of 760 children with an age less than one year to 15 years of age with diarrhea, were surveyed for *Cryptosporidium* infection in seven districts (Ramallah, Bethlehem, Hebron, Jenin, Nablus, Qalqilia, and Tulkarm districts), in the period between September 2003 and November 2004 [61].

The prevalence rate was found to be comparable among the seven districts but it was slightly higher in Hebron and Jenin districts than other districts. The prevalence rate was as follows: Ramallah 9.3%, Bethlehem 8.5%, Hebron 15.2%, Jenin 14.3%, Nablus 11.1%, Qalqilia 10.8%, and Tulkarm district 10.7%. And the prevalence of the parasite was found to be higher in refugee camps than villages and cities, in addition to the significant difference among the different age groups, where children aged >0-5 years old was the higher risk group [61].

1.12 Significance of the study

Cryptosporidiosis has been studied in West Bank. However, these studies were either on patients or on age groups other than children aged less than 5 years. And since the age group less than 5 years are the most susceptible as shown in different countries; the current work aims at exploring the prevalence rate among this age category based on classical as well as molecular diagnosis.

1.13 Purpose of study

The aim of this study was to determine the prevalence of *Cryptosporidium spp.* among children aged ≤ 5 years old in North West Bank districts, Palestine.

The specific objectives were:

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

- To determine the prevalence of other intestinal parasites namely *Entamoeba histolyticaldispar* and *Giardia spp.* among these children.

- To identify the factors that contribute to the spread of the intestinal parasites.

- To quantify the public health impact of this disease.

- To evaluate the need for routine examination of all diarrheal fecal specimens for the presence of *Cryptosporidium*.

Chapter Two
Materials and methods

2.1 Materials and methods

The Study was approved by Ethics Committee at An-Najah National University and carried out in the Genetic Laboratory/Faculty of Medicine.

A cross-sectional study was conducted to investigate the prevalence of *Cryptosporidium spp*. in districts of North West Bank.

2.2 Study area

`The study was carried out in districts of Nablus, Tulkarm, Qalqiliah and Tubas. These regions lie in North of West Bank (Figure 2.1).

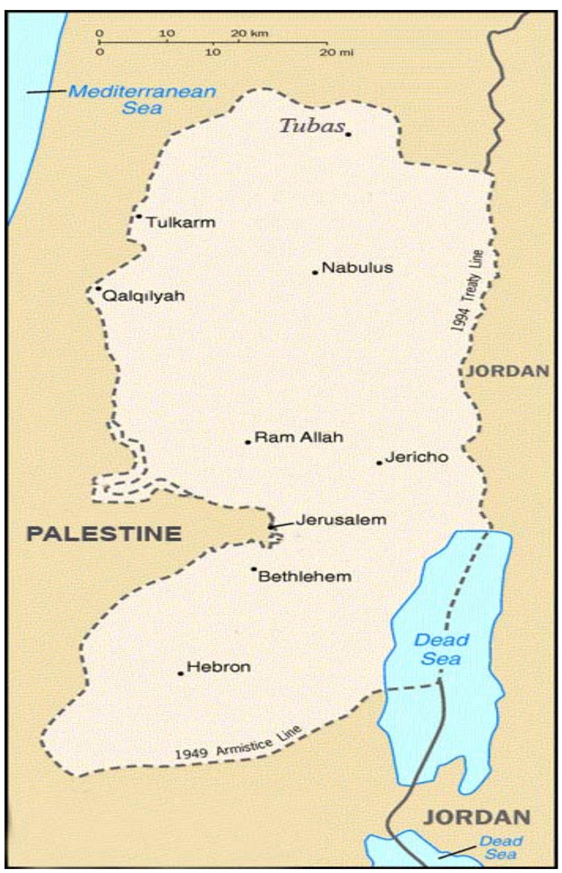

Figure (2.1): Districts locations on the Palestinian map

2.3 Study population and sampling

The total population of North West Bank districts is 707,817 [62]. The total number of children ≤5 years old is 81643 children [62]. The total number of children aged ≤ 5 years old for different regions (city, village, camp) in each district was taken from the Palestinian Central Bureau of Statistics (PCBS) [62], table (2.1).

Table (2.1): Number of children ≤ 5 years old in each region

	Nablus	Tulkarm	Qalqiliah	Tubas	Total
City	22916	13121	7410	4299	47746
Rural	15540	4370	5031	1593	26534
Camps	4279	2243	0*	841	7363
Total	42735 (52.4%)	19734 (24.2%)	12441 (15.2%)	6733 (8.2%)	81643 (100%)

* No camps in Qalqiliah district

2.4 Sampling procedure:

In the present study a total of 500 children were selected in the period of August 2008 to February 2009. The sample size was determined based on the equation:

$$\text{Sample size (SS)} = \frac{(1.96)^2\,(1\text{-}P)}{\varepsilon^2\,P}$$

Where:

ε is known as the relative precision = 0.2

P is the estimated prevalence rate according to neighboring countries =0.15

The number of children that has been selected from each district was determined according to the total number of children ≤ 5 years old in each district, i.e Nablus district $\frac{500 \times 42735}{81643} = 262$ children from Nablus district and by using the same way for the other districts the number of children that has been selected was as follows: 121 from Tulkarm district, 76 from Qalqiliah district and 41 from Tubas district.

Samples distribution for different regions in the four districts was determined based on the total number of children ≤ 5 years old in each region, i.e Nablus city: $\frac{500 \times 22916}{81643} = 140$. Table (2.2).

Table (2.2): Distribution of samples in each district

	Nablus	Tulkarm	Qalqiliah	Tubas	Total
City	140	80	45	26	291
Rural	95	27	31	10	163
Camps	27	14	0*	5	46
Total	262 (52.4%)	121 (24.2%)	76 (15.2%)	41 (8.2%)	500 (100%)

* No camps in Qalqiliah district

In the present study, 266 (53.2%) males and 234 (46.8%) females aged ≤ 5 years old were selected from Nablus, Tulkarm, Qalqiliah, and Tubas districts.

2.5 Sample selection

Children participants in this study were stratified randomly from health clinics and kindergartens. In each stratum, samples were selected randomly.

2.5.1 Inclusion criteria

Children of both sexes aged ≤ 5 years old, resident in any district of Nablus, Tulkarm, Qalqiliah or Tubas.

2.5.2 Exclusion criteria

Children aged more than 5 years old, or children from districts out of specified regions.

2.6 Stool collection

A single fecal sample from each child was collected in a dry, clean, leak-proof plastic container. Half of each sample was stored in 10% formalin and the other half was stored in 10% ethanol.

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 concentrated directly or stored in refrigerator at 4 ºC temperature for further use. Each sample was labeled with the child's name, age and place of collection.

2.7 Stool analysis

Each sample was concentrated by formalin-ethyl acetate sedimentation method prior to microscopic examination. The concentration procedures maximize the recovery of oocysts by separating parasites from fecal debris increasing the chances of detecting oocysts.

Formalin-ethyl acetate sedimentation technique:

The technique was performed as recommended by the Centre of Disease Control and Prevention (CDC) [34]:

- 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 15ml 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 10ml of 10% formalin were added to the sediment and mixed thoroughly with wooden applicator stick.
- 4ml 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.

After samples were concentrated, fecal materials were analyzed microscopically for intestinal parasites at 4x and then on 40x or 100x with oil. Each fecal sample was stained with iron heamatoxylin for protozoa e.g. *Amoeba* or *Giardia* and malachite green for *Cryptosporidium*. Samples showed *Cryptosporidium* were used for DNA extraction and polymerase chain reaction (PCR).

2.8 Parasite staining

2.8.1 Iron heamatoxylin stain

Iron heamatoxylin stain has been performed according to Johnson [63].

2.8.2 Malachite green negative staining

Malachite green negative staining has been used as described by Elliot et al [64], and performed as follows: (See figure 2.2)

1. Dry stool slide was fixed in methanol for 1 min.
2. Incubated in malachite green for 5 min.
3. Washed shortly with 50% ethanol.
4. Then washed shortly with water.

5. And distained for 2 min in 0.5% aq. Acetic acid (Thick smears for 3 min.).
6. Finally dried on warm surface.

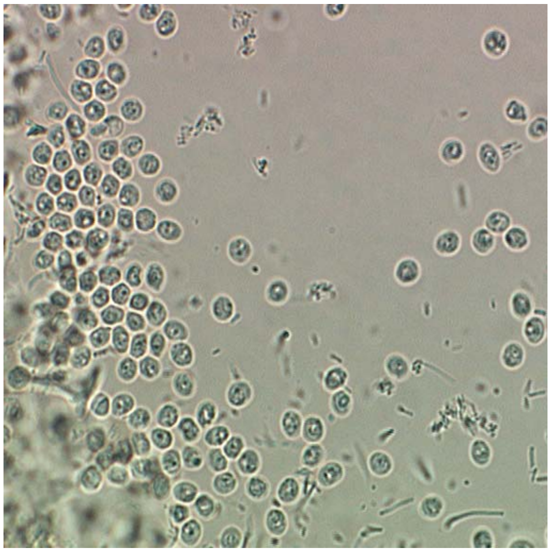

Figure (2.2): Malachite green staining of *Cryptosporidium spp.* oocysts with (100 X magnification) (Hijjawi, N, personal communication).

2.8.3 DNA extraction

Genomic DNA extraction was performed from approximately 150 mg of fecal samples or from the mixture of 1 ml concentrated water with the QIAamp DNA stool mini test kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. PCR primers were designed against DNA codes for DNA J-like protein of *Cryptosporidium spp* [65]. A set of primers were used; sense primer [5'-CCGAGTAAAGATCAAAAATTTACGAA-3'] and anti-sense primer [5'-TAGCTTTCCATATGCCTGATTGAGTA-3']. A 420 base pair segment was amplified using a 100 µl. PCR reactions were carried out in 30 µl volumes containing 1 x Tag polymerase buffer (PeQlab, Fareham, UK), 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, 150 nM of each primer and 200 ng genomic DNA. The PCR condition consisted of 94 ºC for 5 min followed by 30 cycles of following program; 94 ºC for 30 s, 50 ºC for 1 min, 72 ºC for 1 min and an extension reaction at 72 ºC for 5 min. PCR products were subjected to electrophoresis in 1.5 % (w/v) Agarose-TAE (40mM Tris-acetate, 1mM EDTA, pH 8.3) gel stained with Ethidium bromide.

Signed consent was obtained from the parents or guardians of the selected study children before enrollment in the study, and they were informed of the purpose and objectives of the study.

Alongside of sample collection a questionnaire covering demographic information, health status, children's lifestyle and other relevant information was filled up by the researcher herself by interviewing children parents.

The questionnaire which was designed by the author has been tested by giving it to 10 parents of children aged ≤ 5 years old. The questionnaire has been modified taking parent’s notes in consideration.

2.9 Statistical analysis

Statistical analysis was performed by using Statistical Package of Social Sciences (SPSS) version 13. Cross tabulation and Chi square test (X^2) were used to detect significant differences between the various groups. Values were considered to be statistically significant when the p-value obtained was less than 0.05.

Chapter Three
Results

In the present study, the mean age of subjects was 2.4 years old and females were 46.8% (234) of the sample.

3.1 Demographic data

Table 3.1 shows the age distribution of children at the time of the study. The highest percentage was 27.8% (139/500) for children aged 0-1 years old.

Table (3.1): Age distribution of children selected from districts of North West Bank

Age (year)	Frequency	Percentage %
0-1	139	27.8
> 1 -2	128	25.6
> 2-3	78	15.6
> 3-4	75	15.0
> 4-5	80	16.0
Total	500	100.0

Table 3.2 represents the distribution of samples by different regions. The highest percentage was taken from cities 58.2% (291/500), followed by villages 32.6% (163/500) and then refugee camps 9.2% (46/500).

Table (3.2): Samples distribution by region.

District / Region	Nablus	Tulkarm	Qalqilia	Tubas	Total	Percentage (%)
City	140	80	45	26	291	58.2
Villages	95	27	31	10	163	32.6
Refugee camps	27	14	O*	5	46	9.2
Total	262 (52.4%)	121 (24.2%)	76 (15.2%)	41 (8.2%)	500 (100%)	100

*No refugee camps in Qalqiliah district.

Table 3.3 summarizes the prevalence of infection by intestinal parasites among children. Only *Amoeba*, *G. lamblia* in addition to the

Cryptosporidium have been detected in stool samples. The prevalence of *Cryptosporidium* is 13.6%. The distribution is nearly equal among all ages of the participants.

Table (3.3): Prevalence of intestinal parasites among participants

Parasite / Age	Nil	*Entamoeba spp.*	*Giardia lamblia*	*Cryptosporiduim spp.*	Total	*P-Value*
> 0 – 1	94	19 (13.6%)	10 (7.1%)	16 (11.5%)	139	0.365
> 1 – 2	90	12 (9.3%)	7 (5.4%)	19 (14.8%)	128	
> 2 – 3	55	12 (15.3%)	3 (3.8%)	8 (10.2%)	78	
> 3 – 4	55	6 (8.0%)	1 (1.3%)	13 (17.3%)	75	
> 4 – 5	61	6 (7.5%)	1 (1.2%)	12 (15%)	80	
Total	355	55 (11%)	22 (4.4%)	68 (13.6%)	500	

Table 3.4 represents the father's and mother's education and mother's profession. The percentage of educated fathers 66.0% (330/500) and educated mothers 67.2% (336/500) were higher than the percentage of illiterate fathers 34.0% (170/500) and illiterate mothers 32.8% (164/500), while the percentage of housewives mothers 75.8% (379/500) was found to be higher than worker ones (24.2%, 121/500).

Table (3.4): Samples distribution by father's education as well as mother's education and profession

Father Education		
	Frequency	Percentage (%)
Illiterate*	170	34.0
Educated†	330	66
Total	500	100
Mother Education		
Illiterate	164	32.8
Educated	336	67.2
Total	500	100
Mother Profession		
House wife	379	75.8
Worker	121	24.2
Total	500	100

* Less than Tawjihi
† With Tawjihi or University degree

3.2 Data analysis for *Cryptosporidium*

The prevalence of *Cryptosporidium* in the different districts by region has been determined (figure 3.1, table 3.5). The rate was not found to be significantly different between the three regions ($p>0.05$). However, the prevalence rate of the *Cryptosporidium spp.* was found to be higher in refugee camps (21.7%, 10/46) than villages (14.1%, 23/163) and cities (12.0%, 35/291).

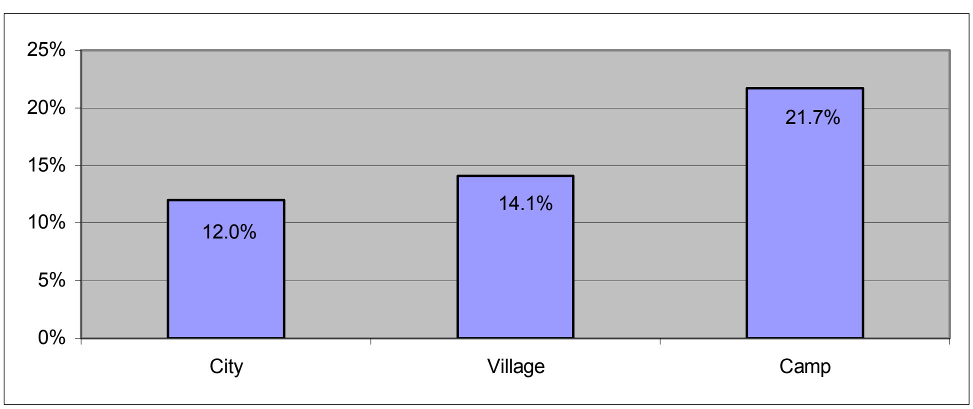

Figure (3.1): Prevalence of *Cryptosporidium* in the different districts by region.

Table (3.5): Association of *Cryptosporidium* infection with various variables.

Variables	Number		Percent%		P-Value
	+ve	-ve	+ve	-ve	
Place					
City	35	256	12.0	88.0	> 0.05
Village	23	140	14.1	85.9	
Camp	10	36	21.7	78.3	
Gender					
Male	27	239	10.2	89.8	0.023
Female	41	193	17.5	82.5	
Father education					
illiterate	26	144	15.2	84.8	> 0.05
Educated	42	288	12.7	87.3	
Mother education					
illiterate	30	134	18.3	81.7	> 0.05
Educated	38	298	11.3	88.7	
Mother profession					
House wife	58	321	15.3	84.7	> 0.05
Worker	10	111	8.3	91.7	

Infection rate with *Cryptosporidium spp.* was found to be significantly different ($p<0.05$) between males and females. The prevalence rate was however higher in females (17.5%, 41/234) than in males (10.2%, 27/266), (Figure 3.2).

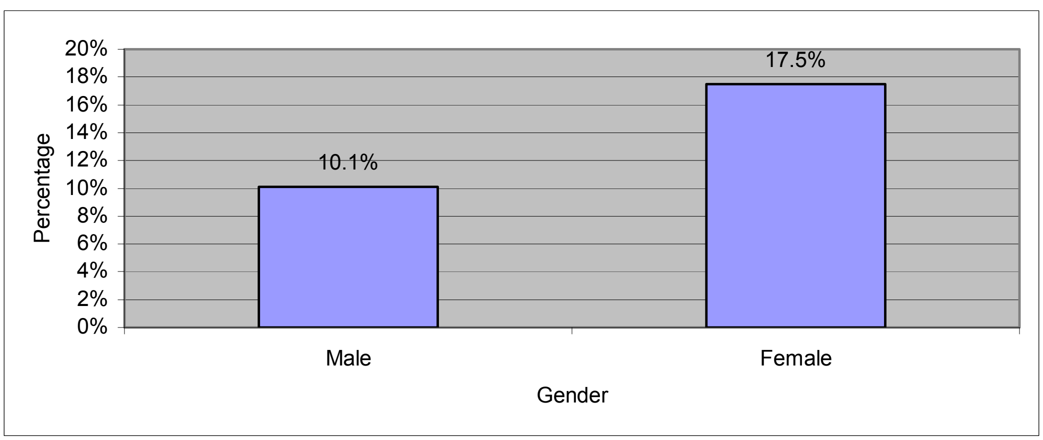

Figure (3.2): Prevalence of *Cryptosporidium* in the different districts by gender

Figure 3.3 shows clearly the effect of father's education on children infection by *Cryptosporidium*. The prevalence rate of the parasite was higher in children for illiterate fathers (15.2%, 26/170) than in children for educated fathers (12.7%, 42/330). But the difference was found to be non-significant (p>0.05).

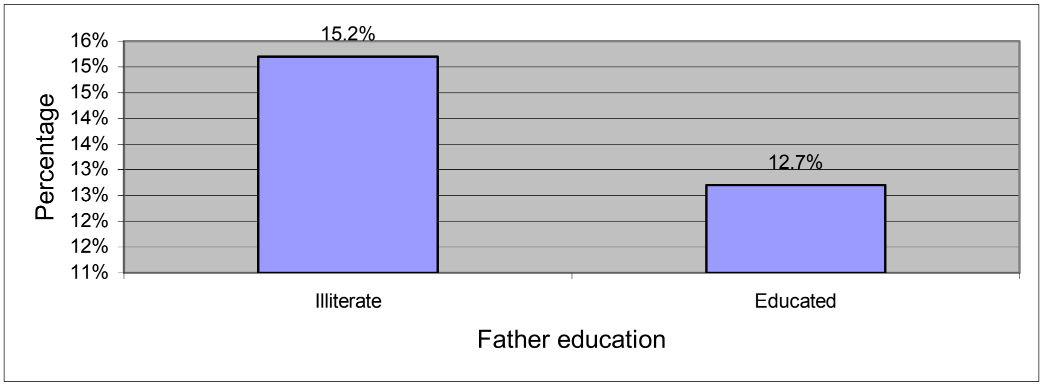

Figure (3.3): Prevalence of *Cryptosporidium* in the different districts by father's education

Similarly, there was no significant difference (p>0.05) between illiterate and educated mothers, while the prevalence rate of the parasite was higher in children for illiterate mothers (18.3%, 30/164) than in those for educated mothers (11.3%, 38/336) (Figure 3.4).

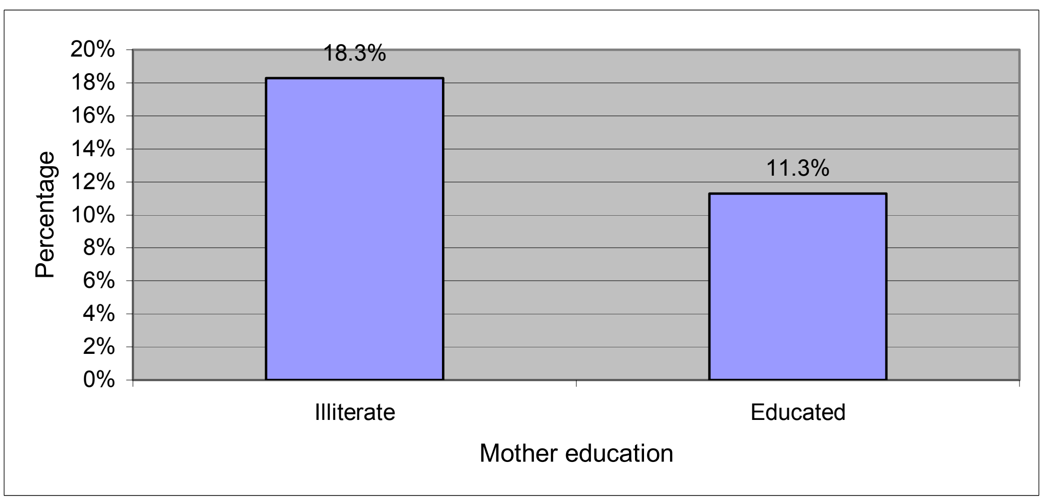

Figure (3.4): Prevalence of *Cryptosporidium* in the different districts by mother's education

Moreover, the prevalence rate of parasite was found to be higher in children for housewives mothers (15.3%, 58/379) than in those for workers mothers (8.3%, 10/121), but there was no significant difference between the two groups ($p>0.05$) (Fig 3.5).

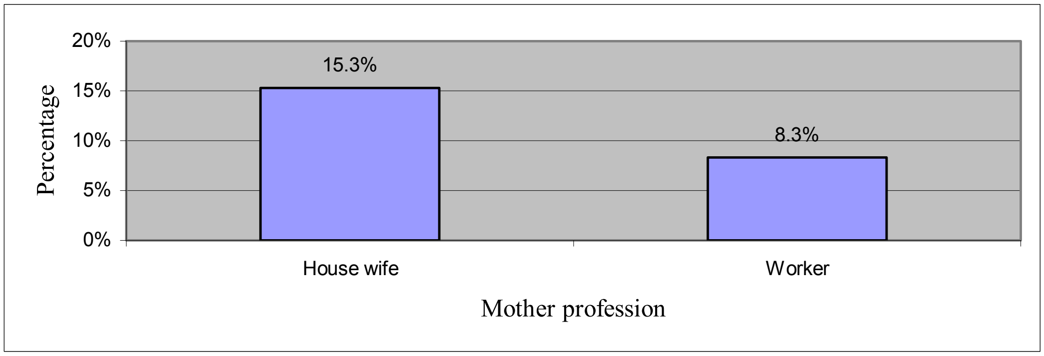

Figure (3.5): Prevalence of *Cryptosporidium* in the different districts by mother's profession

3.3 Prevalence of *Cryptosporidium* in each district by regions

Figure 3.6 demonstrates the rate of infection by *Cryptosporidium* among participants from different regions in North West Bank. The rate in Tubas city was the lowest compared to other cities. However, data analysis showed no significant difference between these cities on the prevalence rate ($p>0.05$).

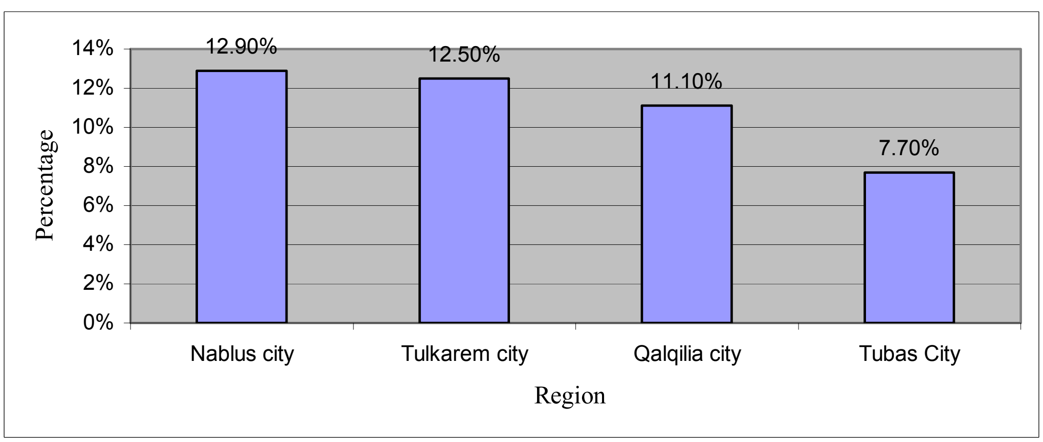

Figure (3.6): Prevalence of *Cryptosporidium* in each district by cities

In figure 3.7, the prevalence rate of the parasite showed no significant difference ($p>0.05$) among the different villages of each district. But it was clearly higher in Qalqilia villages compared to others.

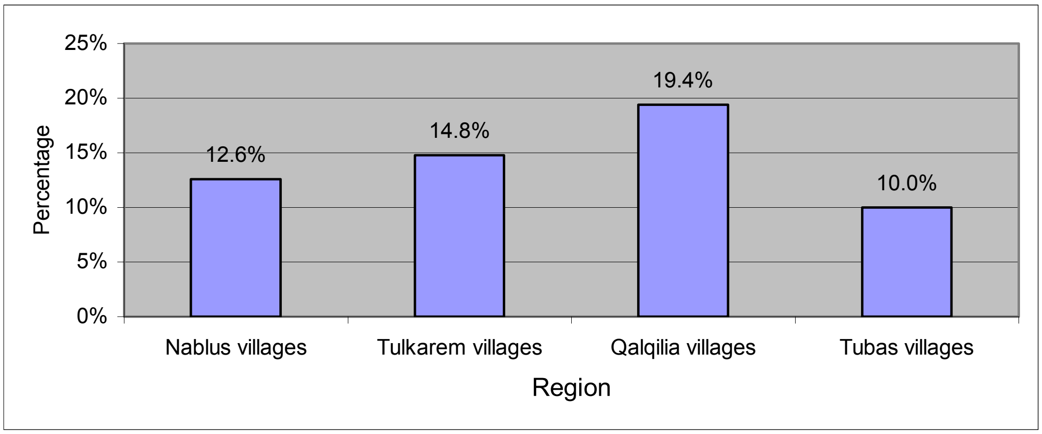

Figure (3.7): Prevalence of *Cryptosporidium* in each district by villages

Analysis of infection by *Cryptosporidium* in children from refugee camps showed higher prevalence in Tulkarm refugee camp 35.7% (5/14), (Fig. 3.8). It is worth mentioning that no infection has been detected in Alfar'a camp in Tubas district.

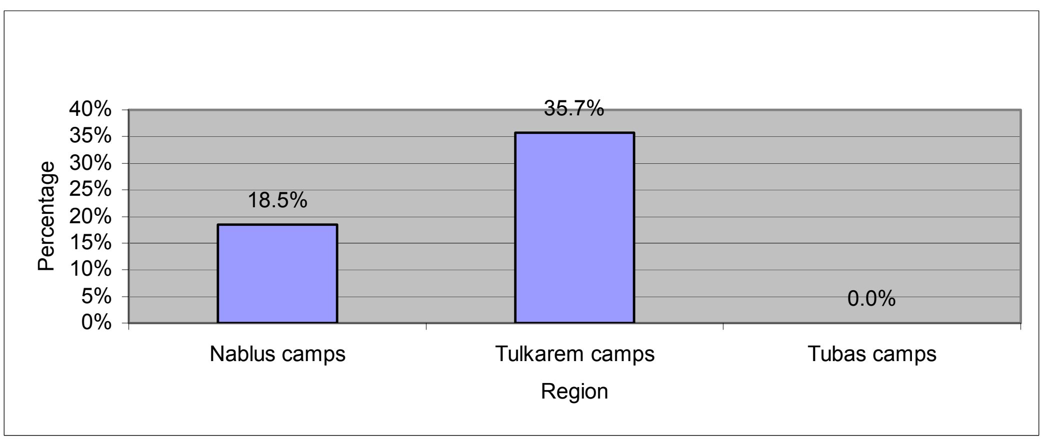

Figure (3.8): Prevalence of *Cryptosporidium* in each district by refugee camps

Table 3.6 summarizes the association of *Cryptosporidium* with environmental risk factors and other variables. Statistical analysis showed no significant relationship between these variables listed in table 3.6 and parasite infection ($p>0.05$).

Table (3.6): Relationship of cryptosporidiosis with a number of environmental risk factors.

Characteristic	Number +ve	Number -ve	Percent% +ve	Percent% -ve	P-Value
No. of children					> 0.05
≤ 3	37	277	11.8	88.2	
> 3	31	155	16.7	83.3	
Washing hands before feeding					> 0.05
yes	7	81	8.60	91.4	
no	61	351	14.8	85.2	
Washing hands after feeding					> 0.05
yes	4	24	14.3	85.7	
no	64	408	13.6	86.4	
Milk (pasteurized or boiled)					> 0.05
yes	9	35	20.5	79.5	
no	58	392	21.9	78.1	
Washing fruits and vegetables					> 0.05
yes	35	184	16.0	84.0	
no	31	236	11.6	88.4	
Hands washing after bath					> 0.05
yes	67	419	13.7	86.3	
no	1	12	7.70	92.3	
Hands washing after diaper change					> 0.05
yes	47	291	13.9	86.1	
no	1	10	9.10	90.9	
Child's hands washing after bath					> 0.05
yes	27	151	15.2	84.8	
no	10	46	17.9	82.1	
Child infection with a parasite					> 0.05
yes	58	347	14.3	85.7	
no	9	84	9.70	90.3	
Child taking medicines					> 0.05
yes	61	405	13.1	86.9	
no	6	19	24.0	76.0	
Family infection with a parasite					> 0.05
yes	49	343	12.5	87.5	
no	17	87	16.3	83.7	

Characteristic	Number		Percent%		P-Value
	+ve	-ve	+ve	-ve	
Having cows and sheep at home					
yes	61	385	15.6	84.6	
no	6	45	11.8	88.2	
Living near cows and sheep					> 0.05
yes	51	281	15.4	84.6	
no	17	150	10.2	89.8	
Child playing with pets					> 0.05
yes	52	347	13.0	87.0	
no	15	84	15.2	84.8	
Child washing hands after playing with pets					> 0.05
yes	12	50	19.4	80.6	
no	11	57	16.2	83.8	
Water resource at home					> 0.05
Tap	48	349	12.1	87.9	
Filtered	6	13	31.6	68.4	
Well	13	66	16.5	83.5	
Mineral	1	3	25.0	75.0	
Water resource at kindergarten					> 0.05
Tap	37	255	12.1	87.9	
Filtered	0	1	0.00	100.0	
Well	1	12	7.70	92.3	
Mineral	1	0	100.0	0.00	

3.4 Data analysis for intestinal parasites

Table 3.7 shows the prevalence of *Cryptosporidium spp.*, *Amoeba* and *G. lamblia* in each district by regions.

Table (3.7): Prevalence of intestinal parasites in each district by regions

District	Region	Nil		*Entamoeba spp.*		*Giardia lamblia*		*Cryptospordium spp.*		Total	
		Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%
Nablus	City	103	73.6	12	8.6	7	5.0	18	12.9	140	100
	Villages	66	69.5	15	15.8	2	2.1	12	12.6	95	100
	Refugee camps	18	66.7	2	7.4	2	7.4	5	18.5	27	100
Tulkarm	City	60	75.0	7	8.8	3	3.8	10	12.5	80	100
	Villages	19	70.4	3	11.1	1	3.7	4	14.8	27	100
	Camp	4	28.6	2	14.3	3	21.4	5	35.7	14	100
Qalqilia	City	34	75.6	5	11.1	1	2.2	5	11.1	45	100
	Villages	20	64.5	4	12.9	1	3.2	6	19.4	31	100
Tubas	City	20	76.9	3	11.5	1	3.8	2	7.7	26	100
	Villages	7	70.0	1	10.0	1	10.0	1	10.0	10	100
	Refugee camps	4	80.0	1	20.0	0	0	0	0	5	100
	Total	355	71.0	55	11.0	22	4.4	68	13.6	500	100

The relationship of parasitic infection with various variables was illustrated in table 3.8. A significant difference ($p<0.05$) on the prevalence rate of parasites infection between cities, villages and refugee camps was demonstrated. The prevalence rate of the various parasites was found to be lower in cities (25.4%, 74/291) than in villages (31.3%, 51/163) with the highest prevalence rate in refugee camps (43.5%, 20/46). While the rate of these parasites appeared to be higher in females (34.2%, 80/234) than in males (24.4%, 65/266) with a significant difference ($p<0.05$).

Table (3.8): Relationship of parasitic infection with various variables.

Variables	Number		Percent%		P-Value
	+ve	-ve	+ve	-ve	
Place					
Camp	20	26	43.5	56.7	0.037
Village	51	112	31.3	68.7	
City	74	217	25.4	74.6	
Gender					
Male	65	201	24.4	75.6	0.021
Female	80	154	34.2	65.8	
Father education					
illiterate	53	117	31.2	68.8	> 0.05
Educated	92	238	27.9	72.1	
Mother education					
illiterate	53	111	32.3	67.8	> 0.05
Educated	92	244	27.4	72.6	
Mother profession					
House wife	116	263	30.6	69.4	> 0.05
Worker	29	92	24.0	76.0	

Table 3.9 represents the relationship of intestinal parasites with environmental risk factors and other variables. The results showed that there was a significant difference ($p<0.05$) on the prevalence of parasites infection among children whose families had history of parasitic infection (38.5%, 40/104) and those children whose families did not (26.3%, 103/392).

Table (3.9): Relationship of parasitic infection with a number of environmental risk factors.

Characteristic	Number		Percent%		P-Value
	+ve	-ve	+ve	-ve	
No. of children					
≤ 3	85	229	27.1	72.9	> 0.05
> 3	60	126	32.3	67.7	
Washing hands before feeding					
yes	24	64	27.3	72.7	> 0.05
no	121	291	29.4	70.6	
Washing hands after feeding					
yes	14	14	50.0	50.0	0.021
no	131	341	27.8	72.2	
Milk (pasteurized or boiled)					
yes	15	29	34.1	65.9	> 0.05
no	126	324	28.0	72.0	
Washing fruits and vegetables					
yes	63	156	28.8	71.2	> 0.05
no	78	189	29.2	70.8	
Hands washing after bath					
yes	142	344	29.2	70.8	> 0.05
no	3	10	23.1	76.9	
Hands washing after diaper change					
yes	100	238	29.6	70.4	> 0.05
no	6	5	54.5	45.5	
Child's hands washing after bath					
yes	55	123	30.9	69.1	> 0.05
no	17	39	30.4	69.6	
Child infection with a parasite					
yes	122	283	30.1	69.9	> 0.05
no	22	71	23.7	76.3	
Child taking medicines					
yes	11	14	44.0	56.0	> 0.05
no	128	338	27.5	72.5	
Family infection with a parasite					
yes	40	64	38.5	61.5	0.020
no	103	289	26.3	73.7	
Having cows and sheep at home					
yes	13	38	25.5	74.5	> 0.05
no	131	315	29.4	70.6	

Characteristic	Number		Percent%		P-Value
	+ve	-ve	+ve	-ve	
Living near cows and sheep					> 0.05
yes	42	125	25.1	74.9	
no	103	229	31.0	69.0	
Child playing with pets					> 0.05
yes	32	67	32.3	67.7	
no	112	287	28.1	71.9	
Child washing hands after playing with pets					> 0.05
yes	22	46	32.4	67.6	
no	24	38	38.7	61.3	
Water resource at home					> 0.05
Tap	107	290	27.0	73.0	
Filtered	8	11	42.1	57.9	
Well	26	53	32.9	67.1	
Mineral	4	0	100.0	0	
Water resource at kindergarten					> 0.05
Tap	69	223	23.6	76.4	
Filtered	1	0	100.0	0	
Well	2	11	15.4	84.6	
Mineral	1	0	100.0	0	

Chapter Four
Discussion

Cryptosporidiosis is a common gastrointestinal disease, and it has been recognized worldwide as a common cause of diarrhea in otherwise healthy children. The disease is widespread in many developed and developing countries [66]. According to the World Health Organization, more than 33% of global deaths are due to parasitic diseases [67]. Intestinal parasitic infections are among the most common infections in the world responsible for mortality and morbidity [67]. In terms of pathogenic importance, *G. lamblia, Cryptosporidium parvum* and *E. histolytica* have been shown to be responsible for severe diarrhoeal episodes especially in immunocompromised and younger children (1-5) years old [45].

The current study explores the prevalence of *Cryptosporidium* and other intestinal parasites among children ≤5 years old in four districts of the North West Bank- Palestine. The results demonstrate clearly a high prevalence rate of *Cryptosporidium* (13.6%) among children of less than 5 years old. The figure is similar to the prevalence of the disease reported in other studies conducted in West Bank and Gaza Strip (14.6%) [60, 61, 68]. For example, Abu-Alrub [61] studied the prevalence of cryptosporidiosis in children with diarrhea in West Bank. His results showed that 14.4% of children aged >0-5 years old had *Cryptosporidium spp.* in their stool samples.

Similarly, the prevalence of *Cryptosporidium* was done on children hospitalized with diarrhea and was found to be 13.5% [60]. It is worth mentioning that although the prevalence rates of both studies [60, 61] were close to the results of this recent study but they were done on children with clinical symptoms or children > 5 years. However, our study has been performed on children ≤5 years old that has been selected randomly. Our findings thus is the first of its type to report the prevalence of *Cryptosporidium* in children aged >0-5 years old.

The prevalence of the disease has been similar in Egypt (17.0%)[55], but different from reports from other countries such as the study conducted in northern Jordan by examining stool samples from 265 children admitted to the pediatric ward at Princess Rahma Hospital, where the prevalence rate of *Cryptosporidium* among the >0-5 years age category was (1.5%) [69]. And Saudi Arabia where the prevalence rate of *Cryptosporidium* was (37.0%) [54].

The results showed that Palestinian children aged ≤5 years old have also amebiasis and giardiasis. There was no other parasites found in the stool of the participants. The prevalence rates of amebiasis and giardiasis were found to be 11.0% and 4.4%, respectively. Similar figures have been reported from other studies carried out on children with diarrhea and of similar age from West Bank (7.2%, 3.6% respectively) [60].

Higher level of cryptosporidiosis in refugee camps than villages and cities without a significant difference is in agreement with the study conducted in the West Bank in 2004 [61]. Cryptosporidiosis is considered as a waterborne disease and this may explain why there was no significant difference between infection and other environmental factors such as crowding condition, poor sanitary and hygienic conditions …etc. However, the results showed a significant difference ($p<0.05$) between infection with

Giardia or *Amoeba*, since both parasites are transmitted through food and water, i.e. fecal-oral route.

Surprisingly, the result showed a significant difference between males and females ($p<0.05$). Females had higher prevalence rate than males. This difference may be explained to the differences in the immune response by both sexes. The data is in agreement with results from other study conducted in the West Bank by Abu-Alrub [61], and also that conducted by Adnan Alhindi in Gaza strip, where (20.3%) of cases were girls, while (12.2%) were boys with a significant difference in the distribution of cases between boys and girls [58].

This study demonstrates clearly a high prevalence rate of *Cryptosporidium*. The tested age group has been reported to be more susceptible to infection, although no significant difference in various age groups (0-1, 1-2 …etc.) was found. Areeshi et al. however reported that most cases of cryptosporidiosis occurred among children less than 7 years of age while those aged 0-2 years old are the most susceptible group [54]. Additionally, the study conducted by Adnan Alhindi [58]; showed that the age group with the highest rate of positivity for cryptosporidiosis was 1-4 years, and there was no significant difference between various age groups, while Abu-Alrub [61] reported that children aged >0-5 years old was the higher risk group for *Cryptosporidium* infection.

Recommendations

- Raise the health awareness about cryptosporidiosis among the population through health education, seminars and leaflets.
- Introduce diagnosis based on molecular technique.
- Genotyping the *Cryptosporidium spp.* in the country which helps in prevention and control of the disease.
- Conduct research on water supply in the country to explore the infection especially *Cryptosporidium* is a waterborne disease.
- Install filters on water sources using < 4 µm filters.
- Routine examination of all diarrheal fecal samples for the presence of *Cryptosporidium*.
- Conduct the same study on larger sample size, to ensure taking the representative number from each country.

References

1. Albonico, M., Crompton, D.W.T., Savioli, L. **Control strategies for human intestinal nematode infections. Adv. Parasitol**. 1999; 42, 277-341.

2. Solomons, N.W.. **Pathways to the impairment of human nutritional status by gastrointestinal parasites**. Paraitology. 1993; 107, S19-S35.

3. Stephenson L.S., Latham M.C. and Ottesen E.A.. **Malnutrition and parasitic helminth infections**. *Parasitology,* 2000; 121:S23-S38.

4. Walsh, J. A. **Problems in recognition and diagnosis of amebiasis: estimation of the global magnitude of morbidity and mortality**. *Rev Infect. Dis*. 1986; 8:228 238.

5. Adams, R.D. **The biology of *Giardia spp*. *Microbiol***. *Rev*. 1991; 55:706- 732.

6. Flanagan P.A. *Giardia* - **diagnosis, clinical course and epidemiology**. *A review Epidemiol. Infect.* 1992; 102:1 – 22.

7. Chen, X. M., Keithly, J. S., Paya, C. V., and Larusso, N. F. **Cryptosporidiosis**. N. Engl. J. Med. 2002; 346: 1723-1732.

8. Guerrant R.L. Cryptosporidiosis: **an emerging, highly infectious threat. Emerg Infect Dis**. 1997; 3: 51-7.

9. Kosek M, Alcantara C, Lima AA, Guerrant RL. **Cryptosporidiosis: an update**. Lancet Infect Dis. 2001; 1: 2629.

10. Xiao L; Fayer R; Ryan U; Upton SJ. **Cryptosporidium taxonomy**. Clin Microbial Rev Jan 2004; 17(1): 72-97.

11. Peng MM; Xiao L; Freeman AR; Arrowood MJ; Escalante AA. **Genetic polymorphism among cryptosporidium parvum isolates: evidence of two distinct human transmission cycles**. Emerg Infect Dis 1997 Oct-Dec; 3 (4): 567-73.

12. Morgan- Ryan UM; Fall A; Ward LA; Hijjawi N; Sulaiman I; Frayer R; Thompson RC; Olson M; Lal A; Xiao L**. Cryptosporidium hominis n. sp**. (Apicomplexa: cryptosporidiidae) from Homo sapiens. J Eukaryot Microbiol 2002 Nov-Dec; 49(6): 433-40.

13. Clark DP**. New insights into human cryptosporidium**. Clin Microbiol Rev 1999 Oct; 12(4): 554-63.

14. Tanriverdi S; Markovics A; Arslan MO; Itik A; Shkap V; Widmer G. **Emergence of distinct genotypes of cryptosporidium parvum in structured host populations. Appl Environ Microbiol**. 2006 Apr; 72(4): 2507-13.

15. Mannheimer, SB, Soave,R. **Protozoal infections in patients with AIDS**. Cryptosporidiosis, isosporiasis, cyclosporiasis, and microsporidiosis. Infect Dis Clin North Am 1994; 8:483.Blan shard C; et,al. Cryptosporidiosis in HIV-seropositive patients. Q J Med 1992 Nov-Dec; 85(307-308): 813-23.

16. Blan shard C; et,al. **Cryptosporidiosis in HIV-seropositive patients**. Q J Med 1992 Nov-Dec; 85(307-308): 813-23.

17. Roberston, L. J., Campbell, A. T., and Smith, H. V. **Survival of Cryptosporidium parvum oocysts under various environmental pressures**. Applied and Environmental Microbiology 1992; 58(11).

18. Juranek, D. D. **Cryptosporidiosis: Sources of infection and guidelines for prevention.** Clin. Inf. Dis. 1995; 21: 57-61.

19. Carpenter, C., Fayer, R., Trout, J., and Beach, M.J. **Chlorine disinfection of recreational water for Cryptosporidium parvum. Emerg**. Inf. Dis. 1999.

20. Hart CA Cryptosporidiosis. In: Gills HM, editor**. Protozoal diseases. London**; Arnold; 1999. p. 592-606.

21. Chappell, CL, Okhuysen, PC, Sterling, CR, DuPont, HL. Cryptosporidium parvum: **Intensity of infection and oocyst excretion patterns in healthy volunteers**. J Infect Dis 1996; 173:232.

22. Meinhardt PL, Casemore DP, Miller KB. **Epidemiologic aspects of human cryptosporidiosis and the role of waterborne transmission**. Epidemiol Rev 1996;18:118-36.

23. Rose, J. **Environmental ecology of *Cryptosporidium* and public health implications**. *Annu. Rev. Public Health*. 1997; 18: 135-161.

24. Kuhls, TL, Mosier, DA, Crawford, DL, Griffis, J. **Seroprevalence of cryptosporidial antibodies during infancy,** childhood, and adolescence. Clin Infect Dis 1994; 18:731.

25. DuPont HL, Chappell CL, Sterling CR, Okhuysen PC, Rose JB, Jakubowski W. **The infectivity of Cryptosporidium parvum in healthy volunteers**. N Engl J Med 1995; 332:855-9.

26. Fayer R. and Ungar, B.L.P. ***Cryptosporidium spp.* and cryptosporidiosis. *Microbiological Reviews***. 1986; 50:458 483.

27. Keithly JS, Zhu G, Upton SJ, Woods KM, Martinez MP, Yarlett N. **Polyamine biosynthesis in Cryptosporidium parvum and its implications for chemotherapy.** Mol Biochem Parasitol 1997;88:35-42.

28. Cordell RL and Addiss DG. **Cryptosporidiosis in child care settings: a review of the literature and recommendations for prevention and control.** Pediatr Infect Dis J 1994; 13: 310-317.

29. Jelinek, T, Lotze, M, Eichenlaub, S, et al. **Prevalence of infection with Cryptosporidium parvum and Cyclospora cayetanensis among international travellers.** Gut 1997; 41:801.

30. Taylor DN, Connor BA, Shlim DR. **Chronic diarrhea in the returned traveler**. Med Clin North Am. 1999; 83: 1033-52.

31. Petersen C. **Cryptosporidium and the food supply**. Lancet 1995 May 6; 345(8958): 1128-9.

32. Cama, VA, Ross, JM, Crawford, S, et al**. Differences in clinical manifestations among Cryptosporidium species and subtypes in HIV-infected persons.** J Infect Dis 2007; 196:684.

33. Butler, B. J., Mayfield, C. I. **Cryptosporidium spp. A review of the organism, the disease and the implications for managing water sources.** Waterloo Center for Ground Water Research. Waterloo, Ontario, Canada. 1996.

34. Centers for Disease Control and Prevention. **Diagnostic procedures for stool specimens**. 2003.

35. Curtis, V. and Cairncross, S. **Effect of washing hands with soap on diarhoea risk in the community**: a systematic review. *Lancet Inf. Dis.* 2003; 3:275 81.

36. Rossignol, JF, Kabil, SM, El-Gohary, Y, Younis, AM. **Effect of nitazoxanide in diarrhea and enteritis caused by cryptosporidium species**. Clin Gastroenterol Hepatol 2006; 4:320.

37. Farthing. M. J. **Clinical aspects of human cryptosporidiosis. Contrib. Mirobiol**. 2000; 6: 50-74.

38. Bern C, Martines J, Zoysa I, Glass RI. **The magnitude of the global problem of diarhoeal disease: a ten-year update**. Bulletin of the World Health Organization 70: 705-714, 1992.

39. Chen XM; Kleithy JS; PAYA CV; LaRusso NF. **Cryptosporidiosis: recent advances and implications for public health**. NEJM May 2002; 346 (2): 1723-31.

40. Tyzzer EE: **A sporozoan found in the peptide glands of the common mouse**. Proc Soc Exp Biol Med 1907;5:12-13.

41. Slavin D: **Cryptosporidium meleagridis sp.** nov. J Pathol Ther 1955;*65:*262-266.

42. Panciera RJ, Thomassen RW, Garner FM: **Cryptosporidiosis in a calf**. Vet Pathol 1971; 8*:*479-484

43. Meisel, J.L., Perera, D.R., Meligro, C. and Rubin, C.E.. **Overwhelming watery diarrhea associated with a *Cryptosporidium* in an immunosuppressed patients.** ***Gastroenterology***. 1976; 70:1156-1160.

44. James, S. J. (ATN) **treatment leads on cryptosporisiosis**: preliminary report on opportunistic infection. *AIDS Treatment News* No. 049. 1988

45. Current WL, Garcia LS. **Cryptosporidiosis**. Clin Microbiol Rev 1991; 4:325-58.

46. Hellard, M., Hocking, J., Willis, J., Dore, J., Fairly, G. **Risk factors leading to Cryptosporidium infection in men who have sex with men. Sex**. Transm. Infec. 2003; 79: 412-414.

47. Wadsworth Center for Laboratories and Research : **Cryptosporidiosis**. Nov 2006.

48. Mackenzie WR; Schell WL; Davis JP. **Massive outbreak of waterborne cryptosporidium infection in Milwaukee,Wisconsin: recurrence of illness and risk of secondary transmission**. Clin Infect Dis 1995 Jul; 21(1): 57-62.

49. Mackenzie WR, Hoxie NJ, and Proctor ME. Massive **outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply**. N. Engl. J. Med. 1994; 331: 161-167.

50. Sulaiman IM, Hira PR , Zhou L, Al-Ali FM, Al-Shelahi FA, Shweiki HM, et al. **Unique endemicityof cryptosporidiosis in children in Kuwait.** J ClinMicrobiol 2005; 43:2805-9.

51. Reinthaler FF, Mascher F, Sixl W, Enayat U, Marth E. **Cryptosporidiosis in children in Idukki District in southern India**. J DiarrhealDis Res 1989; 7:89-91.

52. **Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food-** 10 states, United States, 2005. MMWR Morb Mortal Wkly Rep.2006 Apr 14; 55(14): 392-5.

53. Cryptosporidium and Water: **A Public Health Handbook. Atlanta, Georgia: Working Group on Waterborne Cryptosporidiosis**.1997

54. Mohammed Y. Areeshi Nicholas J. Beeching C. **Anthony Hart. Cryptosporidiosis in Saudi Arabia and neighboring countries**. Ann Saudi Med 2007; 27(5): 325-332.

55. Ibrahim Adib Abdel-Messih, Thomas F. Wierzba, Remon Abu-Elyazeed, Abdel Fatah Ibrahim, Salwa F. Ahmed, Karim Kamal, John Sanders, and Robert Frenck. **Diarrhea Associated with Cryptosporidium parvum among Young Children of the Nile River Delta in Egypt.** Journal of Tropical Pediatrics Advance Access published online on April 14, 2007.

56. E.S. Mahgoub, A. Almahbashi and B. Abdulatif. **Cryptosporidiosis in children in a north Jordanian paediatric hospital**. Health Journal July / September 2004; 4/5(10): 494 – 501.

57. Drora Fraser, Ron Dagan, Lechaim Naggan, Velvl Greene, Joseph El-On, Yunis Abu-Rbiah AND Richard J. Deckelbaum. **Natural History of Giardia lamblia and Cryptosporidium Infections in a Cohort of Israeli Bedouin Infants**: A Study of a Population in Transition. Am. J. Trop. Med. Hyg 1997; 57(5): 544-549.

58. Adnan Ibrahim AL-HINDI, Abdelraouf A. ELMANAMA, Kamal Jad Allah ELNABRIS. **Cryptosporidiosis Among Children Attending Al-Nasser Pediatric Hospital, Gaza, Palestine**. Turk J Med Sci 2007; 37 (6): 367-372.

59. Sallon S, el Showwa R, el Masri M, Khalil M, Blundell N, Hart CA. **Cryptosporidiosis in children in Gaza.** *Ann Trop Paediatr* 1991;11(3):277-81.

60. Sallon, S., Deckelbaum, R. J., Schimd, H., Harlp, S., Baras, M., and Spira, D. T. **Cryptosporidium, Malnutrition, and Chronic Diarrhea in Children.** American Journal of Diseases of Children. 1988; 142: 312-315.

61. Abu-Alrub SM. **Prevalence of Cryptosporidium in Children with Diarrhea in the West Bank**. Birzeit University. 2006.

62. Palestinian Central Bureau of Statistics, 2009. ***Census Final Results – Population Report***. Ramallah - Palestine.

63. Carl M. Johnson. **A rapid technique for Iron Hematoxylin Staining requiring no microscopic control of decolorization**. *Am. J. Trop. Med.* 1935; 551-553.

64. Elliot A., Morgan M. U., and Thompson R. C. **Improved staining method for detecting *Cryptosporidium oocysts* in stools using malachite green.** *The Journal of General and Applied Microbiology*. 1999; 45:139-142.

65. Deng MQ, Peterson RP, Cliver DO. **First findings of *Cryptosporidium* and *Giardia* in California sea lions (Zalophus californianus).** J Parasitol. 2000; 86(3): 490-4.

66. Chalmers R. M., Casemore D. P. **Epidemiology and Strain Variation of *Cryptosporidium***. *The Pathogenic Enteric Protozoa: Giardia, Entamoeba, Cryptosporidium and Cyclospora.* 2003; 8: 27-42.

67. World Health Organization. **Basic laboratory methods in medical parasitology**, Geneva: 1991; 25-26.

68. Sallon, S., el-Shawwa, R., Khalil, M., Ginsburg, G., el-Tayib, J., el-Eila, J., Green, V., and Hart, C. A**. Diarrhoeal disease in children in Gaza.** *Ann. Trop. Med. Parasitol.* 1994; 88(2): 175-182.

69. Youssef, M., Shurman, A., Bougnoux, M., Rawashdeh, M., Bretagne, S., and Strockbine, N. **Bacterial, viral and parasitic enteric pathogens associated with acute diarrhea in hospitalized children from northern Jordan**. *Immunol. Med. Microbiol.* 2000; 3: 257-263.

Appendices

Appendix A
English questionnaire

An-Najah National University
Public Health – High Studies

The Child Guardian Esquire:

Kindly fill the questionnaire, taking into consideration that this data will be employed only for scientific research; abiding by top confidentiality and privacy. Be informed that there is no true or untrue data, but it is your special data that will help us to conduct the study, aimed and designated to study the **Prevalence of *Cryptosporidium* Species among children ≤ 5 years old in north West-Bank, Palestine/ cross sectional study**. The data is a prerequisite to Master degree.

Complete the data in the following tables:

☐ Kindergarten ☐ Clinic Name of kindergarten or clinic: Serial number:		
Age: Sex:		
Place of residence	City ☐ ☐ Village Refugee camp	
Father	Age:	
	Education:	☐ Less than Tawjeehi ☐ Tawjeehi ☐ Diploma ☐ Bachelor's degree ☐ Master's degree
	Profession:	☐ Laborer ☐Employee ☐Teacher ☐ Free work ☐Driver ☐Other than
Mother	Age:	
	Education:	☐ Less than Tawjeehi ☐ Tawjeehi ☐ Diploma ☐ Bachelor's degree ☐ Master's degree
	Profession:	☐ Laborer ☐Employee ☐ Teacher ☐ Free work ☐Housewife ☐ Other than

Options		Questions
1	How many offspring?	________________________
2	Does the child suffer from immune system weakness? e.g: underwent tissue implant or cancer chemical treatment.	□Yes □ No
3	Do you clean your hands before feeding your child?	□Yes □ No
4	Do you clean your hands after feeding your child?	□Yes □ No
5	Does your child drink pasteurized or boiled milk?	□ Yes □ No
6	Do you clean fruits and vegetables before feeding them to the child?	□Always □ Sometimes □ Rarely
7	What do you clean your hands with after leaving toilet?	□With water only □With water and soap □No cleaning
8	What do you clean your hands with after changing your child diaper?	□With water only □With water and soap □No cleaning
9	If your child goes alone to the toilet, what does he clean his hands with after leaving it?	□With water only □With water and soap □No cleaning
10	Was your child infected by any parasites?	□ Yes □ No
11	If the response is yes, what type?	________________________
12	Does he take any medicine for parasites now?	□ Yes □ No
13	Was any family member infected by parasites? e.g: (*Amoeba*, worms).	□ Yes □ No
14	If the response is yes, what is it?	________________________
15	Do you have sheep or cows at home?	□ Yes □ No

16	Do you reside adjacent to sheep, cows or other animals?	□ Yes □ No
17	Does your child play with some pets like cats and dogs?	□ Yes □ No
18	Does he clean his hands after playing with pets?	□With water only □With water and soap □No cleaning
19	What is the source of water your child drinks from at home?	□ Tap water □ Filtered water □ Water from the well □ Mineral water □ Another source ____________
20	If he drinks from a well, is it chlorinated water?	□ Yes □ No
21	What is the source of water your child drinks from at kindergarten? (response from the kindergarten)	□ Tap water □ Filtered water □ Water from the well □ Mineral water □ Another source ____________
22	If he drinks from a well, is it chlorinated water?	□ Yes □ No

Thanks for your cooperation

Appendix B
Arabic questionnaire

جامعة النجاح الوطنية
صحة عامة - دراسات عليا

حضرة ولي أمر الطفل:

أرجو التكرم بتعبئة الاستمارة، مع العلم أن هذه المعلومات ستستخدم فقط من أجل البحث العلمي وستعامل بكامل السرية والخصوصية. وأرجو العلم أنه لا يوجد معلومات صحيحة أو خاطئة إنما هي معلوماتكم الخاصة التي ستساعدنا على القيام بالدراسة التي نحن بصددها والتي تهدف إلى دراسة **معدل إنتشار طفيل "الكريبتوسبوريديوم" بين الأطفال الذين تقل اعمارهم أو تساوي خمس سنوات في شمال الضفة الغربية، فلسطين- دراسة مسحية.** والتي ستقدم استكمالاً لمتطلبات الحصول على درجة الماجستير.

أكمل المعلومات في الجداول الآتية:

☐ حضانه ☐ عياده		اسم الحضانه اوالعياده:		الرقم المتسلسل:
العمر:		الجنس:		
مكان السكن	☐مدينة	☐رية	م☐م	
الأب:	العمر:			
	التعليم:	☐أقل من توجيهي ☐بكالوريوس	☐نوجيهي ☐اجستير	☐لوم
	المهنة:	☐عامل ☐سائق	☐ظف ☐ر ذلك	م☐رس عم☐ حر
الأم:	العمر:			
	التعليم:	☐أقل من توجيهي ☐ بكالوريوس	☐نوجيهي ☐اجستير	☐لوم
	المهنة:	☐عامله ☐عمل حر	☐وظفه ☐ة منزل	م☐رسه ☐ ذلك

	الأسئلة	الخيارات
1	كم عدد الابناء؟	________________________
2	هل يعاني الطفل\ة من ضعف بجهاز المناعة، مثلا سبق أن تعرض\ت لزراعة أنسجة أو تلقى\ت علاج كيميائي للسرطان ؟	□ نعم □ لا
3	هل تغسلين يديك قبل اطعام طفلك ؟	□ نعم □ لا
4	هل تغسلين يديك بعد اطعام طفلك ؟	□ نعم □ لا
5	إذا كان طفلك يشرب الحليب، فهل الحليب الذي يشربه مبستر أو مغلي؟	□ نعم □ لا
6	هل تغسلين الفواكه والخضروات قبل اطعامها للطفل؟	□ دائماً □ أحياناً □ نادراً
7	بماذا تغسلين يديك بعد خروجك من الحمام؟	□ بالماء وحده □ بالماء والصابون □ لا أغسل
8	بماذا تغسلين يديك بعد تغيير الحفاضه لطفلك؟	□ بالماء وحده □ بالماء والصابون □ لا أغسل
9	إذا كان طفلك يذهب للحمام لوحده, بماذا يغسل يديه بعد خروجه من الحمام؟	□ بالماء وحده □ بالماء والصابون □ لا يغسل
10	هل سبق وأن أصيب طفلك بأحد الطفيليات؟	□ نعم □ لا
11	إذا كان الجواب نعم ما هي؟	________________________
12	هل يأخد أي أدوية للطفيليات حاليا؟	□ نعم □ لا
13	هل سبق أن اصيب أحد أفراد العائلة بالطفيليات مثل الأميبا والديدان ؟	□ نعم □ لا
14	إذا كان الجواب نعم ما هي؟	________________________
15	هل لديكم أغنام أو أبقار بالبيت ؟	□ نعم □ لا
16	هل تسكن قريبا من أغنام أو أبقار أو حيوانات أخرى؟	□ نعم □ لا
17	هل يلعب طفلك مع بعض الحيوانات الأليفة كالقطط والكلاب؟	□ نعم □ لا
18	هل يغسل يديه بعد اللعب مع الحيوانات؟	□ بالماء وحده □ بالماء والصابون □ لا يغسل

19	ما هو مصدر ماء الشرب الذي يشرب منه الطفل في البيت ؟	□ ماء حنفية □ ماء مفلتر □ ماء من البئر □ مياه معدنية □ مصدر آخر ____________
20	إذا كان يشرب من البئر ، هل تضع مادة كيميائية مثل الكلور في البئر؟	□ نعم □ لا
21	ما هو مصدر ماء الشرب الذي يشرب منه الطفل في الحضانه ؟ (يجاوب من قبل الحضانه)	□ ماء حنفية □ ماء مفلتر □ ماء من البئر □ مياه معدنية □ مصدر آخر
22	إذا كان يشرب من البئر ، هل تضع مادة كيميائية مثل الكلور في البئر؟	□ نعم □ لا

وشكرا لحسن تعاونكم

جامعة النجاح الوطنية
كلية الدراسات العليا

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قدمت هذه الاطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في الصحة العامة من كلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.
2010م

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الملخص

الخلفية: يسبب الكريبتوسبوريديوم مرضا معديا يدعى الكريبتوسبوريديوسيس. يصيب هذا الطفيلي البرزي الجهاز الهضمي. تشكل هذه العدوى قلقاً واهتماماً كبيرين لأوساط الصحة العامة في البلدان النامية والمتقدمة على السواء.

هذا ومن المحتمل أن يهدد المرض حياة الأشخاص الذين يعانون من ضعف في جهاز المناعة. لكن الأطفال الصغار يكونون أكثر عرضة للعدوى من غيرهم. ويصاب الفرد بالعدوى إما من الطعام أو الشراب.

الأهداف: تصبو هذه الدراسة إلى استكشاف انتشار الكريبتوسبوريديوسيس والطفيليات المعوية المعدية الأخرى ذات العلاقة في الأطفال دون سن الخامسة.

الميثودولوجيا وعلم المنهج: أجريت دراسة مقطعية عرضية لجميع العينات البرازية التي أخذت من 500 طفل من مناطق مختلفة في شمال الضفة الغربية، لقد جرى تحليل تلك العينات مجهرياً باستعمال العينات الايجابية مع الكريبتوسبوريديوم للتشخيص الجزئي عن طريق Polymerase Chain Reaction.

النتائج: لقد كان انتشار الكريبتوسبوريديوسيس في أطفال فلسطين دون سن الخامسة هو: 13.6% وأن معدل انتشار الأمبيا والجياردیا كان بنسبة 11% و4.4% على التوالي. وقد تبين أن العدوى كانت أكبر في مخيمات اللاجئين مع عدم وجود اختلاف كبير ($P>0.05$). وقد كشف

تحليل المعطيات عدم وجود ارتباط احصائياً هاماً له علاقة بين عدوى الكريبتوسبوريديوســـيس والعوامل البيئية (غسل اليدين، غسل الفواكه والخضروات، ...الخ)

النتيجة: ينتشر الكريبتوسبوريديوسيس بين أطفال دون سن الخامسة بشكل كبير وعليـــه، فإنـــه يتوجب اتخاذ الاحتياطات اللازمة لمنع ما يوصف بالعدوى الطفيلية.