Islamic University-Gaza
Deanship of Graduate Studies
Faculty of Science
Biological Sciences Master Program

Cryptosporidiosis in Gaza strip

طفيل البوغيات الخفية وتحديد نسبة شيوعه في قطاع غزة

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Biological Sciences

By
Ahmed M. Tabash

Supervisor
Dr. Adnan Al-Hindi

Ph. D Medical Parasitology

May - 2009

بسم الله الرحمن الرحيم

الجامعة الإسلامية – غزة
The Islamic University - Gaza

عمادة الدراسات العليا
هاتف داخلي: 1150

الرقم Ref. ج.س.غ/35/.....
التاريخ Date 30/08/2008.....

نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة عمادة الدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحث/ **أحمد محمود محمد طبش** لنيل درجة الماجستير في **كلية *العلوم*/ قسم العلوم الحياتية/ تحاليل طبية** وموضوعها:

"Cryptosporidiosis in Gaza Strip"

وبعد المناقشة العلنية التي تمت اليوم الأربعاء 02 جماد آخر 1430هـ، الموافق 2009/05/27م الساعة الثامنة والنصف صباحاً، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

د. عدنان الهندي	**مشرفاً ورئيساً**	.....
أ.د. محمد شبير	**مناقشاً داخلياً**	.....
د. عبد الرؤوف المناعمة	**مناقشاً داخلياً**	.....

وبعد المداولة أوصت اللجنة بمنح الباحث درجة الماجستير في **كلية *العلوم*/ قسم العلوم الحياتية/ تحاليل طبية**.

واللجنة إذ تمنحه هذه الدرجة فإنها توصيه بتقوى الله ولزوم طاعته وأن يسخر علمه في خدمة دينه ووطنه.

والله ولي التوفيق ،،،

عميد الدراسات العليا

د. زياد إبراهيم مقداد

P.O. Box 108, Rimal, Gaza, Palestine fax: +970 (8) 286 0800 فاكس tel: +970 (8) 286 0700 تلفون ص.ب. 108 الرمال، غزة، فلسطين
public@iugaza.edu.ps www.iugaza.edu.ps

DEDICATION

إلى والدي الحبيب

إلى الذي أضاء لي الحياة بنوره, إلى من تعجز حروف اللغة

جمعاء, أن تسعفني بكلمات تفيه حقه في الحب والتضحية والبذل والعطاء

إلى والدتي الحبيبة
أدامك الله تاجا على رؤوسنا، وشمعة في بيتنا أطال الله في عمرك،

تعجز كلماتي عن وصفك.

إلى زوجتي الغالية

إلى رفيقة دربي ومهجة فؤادي وقرة عيني، لك الشكر والحب والامتنان

إلى إخواني الأعزاء

إلى رفاق عمري، ومهجة قلبي، وأنسي في حياتي، ومن أتمنى أن نبقى

معا وسويا و إلى عائلتي كافة، ومن وقفوا بجانبي وساندوني، وتمنوا

لي الخير أبدا

إلى ورثة الأنبياء، أساتذ تي الكرام وقدوتي الطيبة من طفولتي ليومي هذا

إلى من نسكن فيها وسكنت فينا، فلسطين الحبيبة و إلى من رووا

بدمائهم الزكية ثراها الغالي

Acknowledgments

يقول النبي صلى الله عليه وسلم «لا يشكر الله من لا يشكر الناس»

أولا أحمد الله سبحانه وتعالى الذي من علي بنعمته أن وفقني لإتمام هذا العمل المتواضع الذي أتمنى من الله العلي القدير أن يوفقني بما فيه الخير لي ولبلدي العزيز.

أتقدم بالشكر الجزيل والعرفان بالجميل إلى جامعتي الغراء وقسمي الأشم، بكافة أسرته كل باسمه ولقبه وبما يحب أن يكنى، واخص بالشكر الدكتور الفاضل:

عدنان إبراهيم الهندي

الذي لم يبخل علي بجهده وعلمه كما أتقدم بالشكر لكل من ساعدني ودعمني طوال حياتي الدراسية، سواء من هم بجانبي، أو أولئك البعيدين عن العين الساكنين في القلب دوما.

Table of Contents

CHAPTER THREE: MATERIALS AND METHODS

CHAPTER FOUR:

List of tables

Page

List of figures

Abstract

The present study aimed to search for the presence of *Cryptosporidium* and determination the prevalence among patients in Gaza strip. Three hundred stool samples were collected from children less than five years old who attended Al-Nasser hospital and European hospital. The study was done in the period from June to August 2007 and January to March 2008. Stool samples were inspected by wet mount saline, concentration techniques by formalin after that acid-fast stain and ELISA. The results of the present study indicated that the prevalence of *Cryptosporidium* was 18% by modified acid-fast stain and (16.7%) by ELISA where this parasite is uneasy to be detected by direct smear microscopy. It was found that 12-24 month age groups are more susceptible to infection by *Cryptosporidium* and significant relationship was found between age, sex and the infection. A strong association between *Cryptosporidium* and abdominal pain, nausea and vomiting were found with statistical significance p= (0.001). Significant association between children who live in camps and a village and cryptosporidiosis with statistical significance p= (0.03). There was a relationship between *Cryptosporidium* infection and the children who live close to open swage or have septic tank, statistical significance (p=.001). Another association between *Cryptosporidium* and children who their mothers are employee or student with statistical significance p= (0.001). It was concluded that cryptosporidiosis still exist among children in Gaza strip and the prevalence of *Cryptosporidium* is high when compared to that in developed countries and staining was very important methods in the detection of such parasite. It is recommended that cryptosporidiosis should be considered and attention should to be given to such neglected and missed diagnosed parasites. Using acid-fast stain methods was found very important. Improvement of the diagnostic techniques used in routine parasitology in the local hospitals.

Key words: *Cryptosporidium,* prevalence, Gaza, Palestine

الملخص

هدفت الدراسة الحالية إلى الكشف عن وجود طفيل البوغيات الخفية وتحديد نسبة شيوعه في قطاع غزة، حيث تم جمع 300 عينة براز المصاحبة للإسهال فقط من أطفال اقل من خمس سنوات ، في فترة زمنية تتراوح ما بين يونيو إلى أغسطس 2007 ومن يناير إلى مارس2008.

ولقد تم التعامل مع عينات البراز بتقنية المسحة المباشرة وطريقة التركيز باستخدام فورما لين ايثر ومن ثم استخدام الصباغة بواسطة طريقة تسيل نلسن المعدلة والاليزا.

ولقد تبين من خلال الدراسة أن نسبة شيوع الإصابة بطفيل البوغيات الخفية (18%) باستخدام طريقة تسيل نلسن المعدلة ونسبة شيوعه (16. 7%) باستخدام الاليزا، حيث لا يمكن تشخيص هذا الطفيل بطريقة المسحة المباشرة وإنما يحتاج إلى الصباغة.

ولقد تبين من النتائج أن الفئات العمرية ما بين 12الى 24 شهرا كانت معرضة للإصابة بهذا الطفيل ووجود علاقة واضحة بين العمر والجنس من جهة والإصابة من جهة أخرى بدلالة إحصائية P< 0.05. وقد وجدت علاقة واضحة ما بين الإصابة بالبوغيات الخفية وبعض الأعراض مثل الم البطن والغثيان والتقيؤ بدلالة إحصائيةP= 0.001 ووجدت علاقة واضحة بين الأطفال الذين يعيشون في المخيمات والقرى والإصابة بالبوغيات الخفية بدلالة إحصائية P= 0.03. وكذلك علاقة واضحة مابين الإصابة بالبوغيات الخفية والأطفال المجاورين للمجاري المفتوحة والذين يصرفون المجاري في براميل بدلالة إحصائية P= 0.001. وكذلك علاقة واضحة مابين الإصابة بالبوغيات الخفية والأطفال الذين تعمل أمهاتهم كموظفات وطالبات بدلالة إحصائية P= 0.001.

توصلت الدراسة الحالية إلى أن طفيل البوغيات الخفية مازال موجودا قي قطاع غزة ونسبة انتشاره عالية عندما نقارنه بالدول المتقدمة ، وانه لا يمكن فحصه دون صباغة.

وقد أوصت الدراسة بالتحسين في استخدام طرق التشخيص حتى لا يحدث فقد في تشخيص الطفيليات الممرضة مثل البوغيات الخفية.

CHAPTER ONE
INTRODUCTION

1- Overview

Cryptosporidium is a protozoan parasite, which is a well, recognized agent of diarrhoeal disease in animals and humans worldwide. Cryptosporidiosis is transmitted by either direct feacal oral route or by ingestion of food or water contaminated with *Cryptosporidium* oocyst. The two most commonly detected parasites in human cryptosporidiosis are *Cryptosporidium parvum* and *Cryptosporidium hominis,* which is primarily associated with human, cows and sheep. Hence, two or more transmission cycles occur; *Cryptosporidium hominis* is spread by anthroponotic cycle in contrast to *C. parvum*, which can be anthroponotic as well as zoonotic **[134]**. Moreover, these two species show geographic differences in their distribution as a cause of human infection; for example, *C. parvum* is reported to be more common in the UK while *C. hominis* is more common in the US **[43]**. *Cryptosporidium* species is an intestinal protozoan parasite that has been recognized as a human pathogen since 1976 **[40]**. While the role of *Cryptosporidium parvum* as a cause of acute diarrhea in developing countries is well documented (Griffiths, 1998). Studies have proved that there are many places in Gaza strip especially in camps where intestinal parasites are endemic. The most recent study for cryptosporidiosis prevalence was carried out Al-Nasser paediatric hospital was (14.9%) of the tested samples by acid-fast staining technique and (16.3%) using ELISA kit among children **[6]**. Another study in Gaza-Palestine, the prevalence was (14.9%) **[122]**. Other studies in West Bank showed (11.6%) prevalence **[2]**. Epidemiological data on the prevalence of *Cryptosporidium* infections seem to be few in most of the developing countries including Palestine. The prevalence of *Cryptosporidium* in children with diarrhoea in the neighboring countries was found to be (8.8%) in Iraq **[86]**, (1.5%) in Irbid, a city in Jordan, **[153]** and (16.6%), (11.6%), (27.9%) in Egypt for the years 1986, 1987 and 1996 respectively **[91]**. The protozoan parasite *Cryptosporidium* is an important cause of gastrointestinal disease that gives rise to a chronic life threatening condition in immunocompromised individuals and to acute gastroenteritis and diarrhea in healthy people **[28]**.

This parasite invades epithelial cells of the intestinal tract and respiratory tree of vertebrate hosts **[132]**. *Cryptosporidium parvum* is infectious for man and virtually all other mammals **[104]**. There are varieties of methods, including microscopy, immunological and molecular techniques, for the detection of *Cryptosporidium* oocysts. Microscopic methods include concentration techniques and staining of fecal smears. There are difficulties in distinguishing *Cryptosporidium* oocysts from other small particles, such as yeasts, moulds, algae, and plant debris by routine fecal examination techniques in fecal and environmental samples **[42]**. Auramine phenol fluorescence (APF) screening followed by modified acid-fast staining (AFS) is a sensitive and specific approach for the identification of *Cryptosporidium* oocysts in stools **[27]**. The modified acid-fast staining technique is useful and the oocysts appear as pink to red, spherical to ovoid, bodies on a blue or purple background. The stained smears are permanent and can be stored for a long time before examination when the samples are in high numbers **[126]**. Studies done in all continents have shown that the protozoal parasite *Cryptosporidium parvum* is a common enteric pathogen that is associated with diarrheal disease and occasional death among young children, especially in developing countries and reported prevalence of *C. parvum* infection among children have varied from less than 1% to more than 30% on a population basis **[21]**. In regions endemic for *C. parvum* where this parasite actively circulates in a community, the risk of symptomatic infection during early childhood is in part a function of a child's exposure to biologic sources of this parasite (e.g., intra-familial and extra-familial infected contacts, infected domestic animals) and exposure to mechanical vectors such as contaminated food, water, and household surfaces **[93]**. Therefore, reducing a child's exposure to these biologic sources and reducing the parasitic load from mechanical vectors could reduce the annual incidence of this disease in susceptible children. In Brazil, up to 17% of cases of childhood diarrhea were associated with *C. parvum* infection **[52]**. In 2003-2004, *Cryptosporidium* spp. were responsible for 55.6% of gastroenteritis outbreaks associated with treated swimming venues (e.g., swimming pools, water parks) in the United States **[37]**. Cryptosporidiosis is endemic in developing countries, such as Mexico, because of poor sanitation and crowded living conditions **[128]**. *Cryptosporidium* is key role in these outbreaks is likely because of its small size, low infectious dose **[35]**, and high tolerance to chlorine **[72]**.

1.1 Objectives:

1.1.1 The General objectives

To study cryptosporidiosis among children less than 5 years old and evaluate the methods of laboratory diagnosis of *Cryptosporidium* used in Gaza.

1.1.2 Specific objectives:

1. To assess of cryptosporidiosis among children less than 5 years.

2. Evaluation of different diagnostic techniques for *Cryptosporidium* oocyst

3. Detection of *Cryptosporidium* by Ziehl-Neelsen (ZN) staining.

4. Detection and confirmation using ELISA for *Cryptosporidium* oocyst antigen in fecal samples.

1.2 Significance:

Diarrheal diseases are considered as one of the health problems among children in Gaza Strip. Also *Cryptosporidium* is one of protozoan parasites existing in Gaza for about two decades. In addition, *Cryptosporidium* is an important cause of diarrhea among children. In addition, immunodeficient patients and respiratory infection patients are susceptible groups for cryptosporidiosis. This study will bring into focus this public health problem. In addition, the results of this research may draw the attention of local physicians to take this parasite into consideration.

CHAPTER TWO

Literature review

2.1 Morphology:

Cryptosporidium oocysts can be observed without sporocysts, with four naked sporozoites, monoxenous and microgametes without flagella" **[79]**. The organisms are only 4 to 6 µm in diameter. Free sporozoites are fusiform and measure 3.5 to 4.2 x 0.53 to 0.6 µm **[112]**. Sporozoites and merozoites of *Cryptosporidium spp*. appear similar to those of other coccidia with organelles typical of the phylum, such as the pellicle, rhoptries, micronemes, electron-dense granules, nucleus, ribosomes, subpellicular microtubules, and apical rings. However, they lack other organelles such as typical polar rings; mitochondria, micropores, and the conoid Posterior to the apical rings, sporozoites and merozoites have a cylindrical collar that appears to be the site of origin for the inner membrane complex and the subpellicular microtubules **[41]**. Unlike other coccidia, the sporozoites are free within the oocysts and not surrounded by sporocysts **[129]**. *Cryptosporidium parvum* appears to contain neither mitochondrion (although they were in other *Cryptosporidium spp*.) nor the plastid commonly found in apicomplexan parasites **[107]**. Apical organelles (i.e., rhoptries, micronemes, and dense granules) are vesicular secretory organelles that are present in zoites. Specifically, rhoptries and micronemes are at the apical region and have been implicated in host cell adhesion and entry **[16]**. Trophozoite is spherical and contains a prominent nucleolus within a single nucleus surrounded by cytoplasm, and a well-developed attachment/feeder organelle. During nuclear division of schizogony, division spindles, nuclear plaques, and centrioles have been observed **[41]**. The trophozoite stage is intracellular beneath the host cell membrane but is extracytoplasmic **[88]**. Immature microgamonts resemble schizonts but contain small, compact nuclei. Microgametes are rod shaped (1.4 x 0.5 um for *C. parvum*), with a flattened anterior end. Macrogamonts of *C. parvum* are approximately 4 to 6 µm. Macrogamonts of *C. parvum* are spherical to ovoid **[41]**. The oocyst wall consists of outer, central and inner layers, at the outer oocyst wall is a carbohydrate-rich glycocalyx, 20 to 30 nm thick, containing traces of a C18 fatty acid **[98]**. The - 5nm thick central layer is composed of complex lipid, possibly responsible for the acid-fast staining of the oocyst wall, and is thought to provide a large proportion of the oocyst's apparent rigidity **[59]**. The inner layer can be further subdivided into outer- inner and inner-inner layers. Reduker *et al.* (1985a) found that the outer-inner layer was of irregular thickness (mean, 10 nm) and the inner-inner layer comprised an outer zone (mean, 11.6 nm) and an inner zone (mean, 25.8 nm). The inner layer is composed of a filamentous glycoprotein **[18]**, susceptible to proteinase K and trypsin, but not pepsin digestion, and may provide much of the rigidity and elasticity present in intact oocyst walls **[59]**. Oocyst

wall functions include protecting sporozoites from various adverse chemical and physical environments, while remaining robust, yet deformable and sensitive to triggers for excystation **[98]**. Many lipid species are present in *C. parvum* oocysts. White et al**.** (1997) found different types of both polar and neutral lipids and a number of different glycolipid species. Not only were differences in the ratios of these different lipid species noted between *C*. *parvum* and *C. muris* oocysts, but also a noticeable variation in the ratios of these lipids occurred in viable and non-viable oocysts, with a decrease in the levels of cholesterol in *C. parvum* oocysts killed by freezing. Therefore, the nature and quantity of lipid species in oocysts can be used to distinguish between live and dead oocysts is an indicator of their importance to the integrity and survival of the oocyst **[98]**. Carbohydrates are at the host- parasite interface and play an important role in the composition, structure and antigenicity of *C*. *parvum* oocyst walls. Studies on the antigenic composition of *C*. *parvum* have highlighted the role of carbohydrate moieties as significant epitopes **[144]**.

2.1.1 Taxonomy and history

The first individual to establish the genus *Cryptosporidium* and to recognize its multispecies nature was Ernest Edward Tyzzer, who described the type species, *C. muris*, from the gastric glands of laboratory mice. *C. parvum* differed from the type species not only by infecting the small intestine rather than the stomach but also because the oocysts were smaller **[151]**. Following the initial discovery of *Cryptosporidium*, over 50 years elapsed during which the parasite was commonly confused with other apicomplexan genera, especially members of the coccidian genus *S*arcocystis*.* Because many *Sarcocystis* spp. have oocysts with thin walls that often rupture, releasing free sporocysts, and because each sporocyst contains four sporozoites like *Cryptosporidium* oocysts, a variety of named and unnamed species were erroneously assigned to the genus **[146]**. Subsequent ultrastructural studies, however, supported earlier light microscopy studies and reaffirmed endogenous stages of *Cryptosporidium* spp. to possess a unique attachment organelle **[143]**. This attachment organelle, rather than the oocysts, is the key feature that currently defines the genus and family **[142]**. However, it has actually been an integral component of the taxonomic definition of the family since at least 1961 **[81]**. Subsequent cross-transmission studies demonstrated that *Cryptosporidium* isolates from different animals could frequently be transmitted from one host species to another, which ended the practice of naming species based on host origin and the synonymization of many of these new *Cryptosporidium* species as *C. parvum.* However, for a brief period, these very limited transmission studies were used as evidence for the monospecific nature of the genus *Cryptosporidium*, resulting in the widespread use of the name *C. parvum* for *Cryptosporidium* parasites from all kinds of mammals, including humans. In recent years, molecular characterizations of *Cryptosporidium* have helped to clarify the confusion in *Cryptosporidium* taxonomy and validate the existence of multiple species in each vertebrate class. As a result, several new species of *Cryptosporidium* have also been named. Thus, *C. andersoni* from cattle, *C. canis* from dogs, *C. hominis* from humans, and *C. molnari* from fish were all established by using multiple parameters that included not only morphology but also developmental biology, host specificity, histopathology, and/or molecular biology **[95]**. The clarification of *Cryptosporidium* taxonomy is also useful for understanding the biology of *Cryptosporidium spp*. assessing the public health significance of *Cryptosporidium spp*. in animals and the environment, characterizing transmission dynamics, and tracking infection and contamination sources **[151]**.

Classification of *Cryptosporidium* [20]

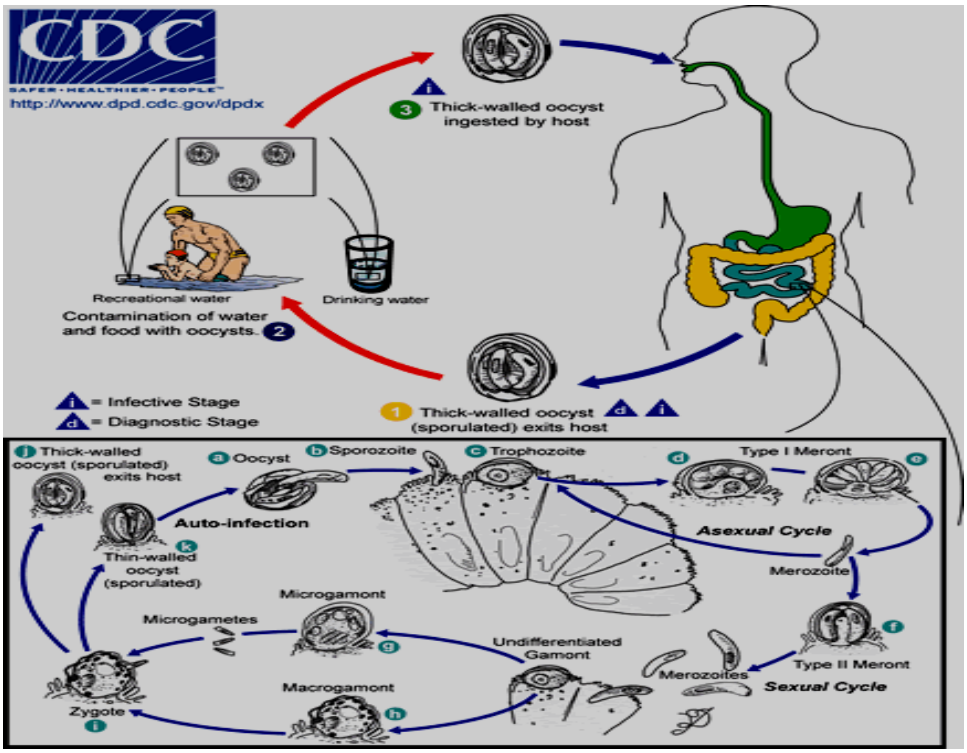
Kingdom	**Protozoa**
Phylum	**Apicomplexa**
Class	**Sporozoea**
Subclass	**Coccidia**
Order	**Eucoccidiida**
Suborder	**Eimeriina**
Family	**Cryptosporidiidae**
Genus	***Cryptosporidium***

2.1.2 Habitat and life cycle

Cryptosporidium is taxonomically classified as a Sporozoa, since its oocyst releases four sporozoites (its motile infectious agents) upon excystation. *C. parvum* infects the epithelial of the intestinal tract (enterocytes) of various mammals. Exposure to the environments of the gastrointestinal tract triggers the poorly understood process of excystation, whereby sporozoites are actively released through the oocyst. Known triggers include temperature (37°C), acidity (- pH 2), slight alkalinity (- pH 7.6) exposure to bile salts and trypsin **[117]**. However, it differs from related parasites such as *Toxoplasma* by its monoxenous life cycle--completing its entire cycle within a single host (Flanigan and Soave, 1993). *Cryptosporidium spp* oocysts are sporulated and infectious at the time of excretion **[31]**. Furthermore, sporulated *Cryptosporidium spp* oocysts can excyst in the intestine before being excreted in the feces. Thus, the infection persists until the immune response of the host clears the parasite. One interesting aspect of the *Cryptosporidium* life cycle is that sexual and asexual reproduction can occur. This life cycle begins by fecal-oral contamination, that is, substances (food or water) that have been in contact with infected feces that contain oocysts. Oocysts contain sporozoite. These ingested oocysts eventually end up in the small intestine where excystation, where sporozoites are released into the lumen of the small intestine **[127]**. Sporozoites excyst from the oocyst and parasitize epithelial cells of the gastrointestinal or respiratory tract **[41]**. This phase of the life cycle is most commonly called schizogonic phase or merogony. Invasion of a host cell by coccidian sporozoites is a dynamic event of considerable interest as the attachment and entry processes involve the sequential secretion of the contents of discrete compartments from within the sporozoite. The released materials are thought to participate in a number of ways, including the penetration event itself and the formation of the vacuolar membrane, which initially surround the intracellular parasite. The machinery mediating this invasion process is collectively housed in the anterior region of the sporozoite and is known as the apical complex **[136]**. The sporozoite then invades the intestinal cell wall to become a trophozoite, the feeding stage of the organism. Soon after becoming a trophozoite, the asexual reproduction of this begins with multiple mitoses (nuclear divisions) before multiple cytokinesis. These new organisms become merozoites, infective organisms that infect other cells within the other body to repeat the cycle **[127]**. Therefore asexual multiplication, called schizogony or merogony, results when the trophozoite nucleus divides **[41]**. Another course of action is to undergo gamegony. The sexual phase of the

Cryptosporidium life cycle in which meronts invade different or same cell types is called gamegony. These meronts differentiate into gamonts by the way of gametogenesis creating male gametocytes or female gametocytes. These gametocytes create gametes, microgamete or macrogametes depending on the sex type of the gametocytes. Microgametes escape the infected cell and locate macrogametes in infected cell types. The merozoites then invade other epithelial cells where they undergo another cycle of type I merogony or develop into type II meronts. The type II meronts form 4 merozoites which do not undergo further merogony but produce sexual reproductive stages (called gamonts) **[104]**. Therefore, only merozoites from type II schizonts initiate sexual multiplication (gametogony) upon infecting new host cells by differentiating into either a microgamont (male) or a macrogamont (female) stage **[41]**. These microgametes will fertilize the macrogametes and form a zygote. After a process of meiosis and genetic recombination, the zygote undergoes mitotic division developing into an oocyst that contains sporozoites during a process known as sporogony. Eventually these oocysts are passed out in feces and then through fecal-oral contamination are ingested by the next subsequent definitive host **[127]**. Some reports suggest that oocysts with thin walls release sporozoites that autoinfect the host, whereas those with thicker walls leave the body to infect other hosts **[41]**. Approximately 20% of the zygotes develop into thin-walled oocysts, which represent auto-infective life cycle forms that can maintain the parasite in the host. This stage and the persistent meronts are believed to be responsible for the life-threatening disease in immunodeficient persons who do not have repeated exposure to environmentally resistant forms **[88]**. Fertilized macrogamonts develop into oocysts that sporulate in situ and contain four sporozoites with either a thin or a thick wall **[41]**.

Fig. 1. Life cycle of cryptosporidiosis [150]



2.1.3 Symptoms

Symptomatic immunocompetent patients usually present with mild to profuse watery diarrhea with an incubation period of 1 to 12 days, with or without mucous, rarely with blood or leukocytes. Frequent and voluminous bowel movements can contribute to rapid weight loss, dehydration, in 92% of symptomatic immunocompetent patients. Other frequently reported symptoms include nausea, vomiting, in 51% of symptomatic immunocompetent patients. Other frequently reported symptoms include abdominal cramping observed in 45% of symptomatic immunocompetent patients, mild fever (less than 39 degrees Celsius) observed in 36% of symptomatic immunocompetent patients, malaise, fatigue, weakness, respiratory problems **[11]**. Cough observed in about 30% **[58]**. Respiratory cryptosporidiosis has been increasingly reported, particularly in immunocompromised individuals. Symptoms included cough, croup, wheezing, hoarseness, and shortness of breath. Chronic cryptosporidial disease is commonest in patients with AIDS or malnutrition. Fulminant intestinal cryptosporidiosis essentially exclusively seen in person with AIDS or chemotherapy-induced immunosuppression, fuliminant cryptosporidiosis is a dramatic cholera-like illness with a very high mortality. Profound hypovolemia and shock may require intensive care unit management of fluid and electrolyte balances **[58]**. AIDS patients with pancreatic cryptosporidial infections have also exhibited bile duct or gall bladder infections. Severe combined immunodeficient infant with cryptosporidial enteritis had cryptosporidial in the pancreatic duct epithelium at autopsy **[11]**.

2.1.3.1 Sequelae

Few studies exist examining the long-term health effects of *Cryptosporidium* infection. Like other diarrheal diseases, C*ryptosporidium* infections may impair growth and development in children **[82].** In addition, some case reports indicate the possibility of cryptosporidiosis starting enteropathic arthropathies such as reactive arthritis, reiter syndrome, and sacroiliitis **[29]**.

2.1.4 Incidence and distribution

Cryptosporidium is parasite that has a ubiquitous geographic distribution and range of vertebrate hosts **[104]**. In 16 reports from Europe, North America, and Australia, 2.1% were positive for *Cryptosporidium* in more than 36.000 patients with diarrhea and in 14 reports from Asia, Africa, and Latin America, 8% were positive **[7]**. The prevalence in developing countries is more than in developed countries **[131]**. The study period from September 1995 to August 19997 in Kuwait collected 3549 stool samples, 509 children had diarrhea. *Cryptosporidium* oocysts were detected in 51 (10%) children with diarrhea; the fecal sample was analyzed by modified safranine methylene blue staining and direct immunofluorescence test **[67]**. *C*ryptosporidiosis in the Philippines is limited. The disease is not routinely diagnosed in the country medical institutions. The researcher collected 53 Filipino cancer patients who were surveyed for cryptosporidiosis using an indirect immunofluorscent antibody test. Fifteen patients (28.3%) were found to be positive for antibodies against *Cryptosporidium*. This study contributes to a better understanding of the incidence of cryptosporidiosis in this country **[115]**. *Cryptosporidium* oocyst observed in sputum sample of a patient with HIV. The samples studied by using DNA markers to determine the species of the parasite. *C. hominis* was identified as the species infecting the patient in respiratory tract; a finding that strengthens evidences regarding these pathogens and their role in human disease. The researcher describes the detection and identification of *C*. *h*ominis in respiratory secretions of a patient with HIV **[90]**. Thirty stool samples were collected from children suffering from diarrhea and were examined using modified acid-fast stain, immunfluorescent test and ELISA; this study was in center of abou-El-Reish children hospital. The researcher proved that immunofluorscence technique qualifies as the test of choice followed by the modified acid-fast stain for the diagnosis of cryptosporidiosis **[12]**. Stool samples from 200 children with diarrhea and from 50 healthy children were examined by modified acid-fast stain, Giemsa stain and direct and indirect immunofluorescence antibody methods, in order to determine cryptosporidiosis prevalence under the age of 12 and to detect the most efficient identifying method for use in Turkey. *Cryptosporidium* oocysts were detected in seven (3.5%) of the cases. This result showed that *Cryptosporidium* could also be a causative agent of diarrhea in children **[4]**. In study to estimate the frequency of *Cryptosporidium* infections in Switzerland, stool samples from patients found to be positive for *Cryptosporidium* by modified ziehl neelson staining and fluorescence microscopy were used for genotyping experiments. With nine of 12 samples DNA extraction and subsequent genotyping

was successful. All *Cryptosporidium* isolates belong the bovine genotype **[46]**. Stool samples collected in Iran include 104 children and adult patients with gastroenteritis referred to the children hospital center and Pasteur institute of Iran. Control samples from healthy individuals (36 children and adult) were also collected. Stool samples were primarily examined by the direct method, then fixed and tested by three assays including acid-fast staining, auramine phenol fluorescence and direct fluorescence using monoclonal antibody. The study revealed that *Cryptosporidium* infected 2.9% of the patients and other parasites observed in different percentages **[97]**. In Canada the researcher found that, *Cryptosporidium spp*. infection occurred at an overall rate of 6.0 per 100.000 population per year although a large out break of *Cryptosporidium spp*. infections occurred in the second half of the summer of 2001. During August and September of 2001, the incidence of cryptosporidiosis was 55.1 per 100.000 per year. Stool samples were primarily examined by immunoassay **[74]**. Colonias along the border a clinic in an urban border community, and clinics. In large urban nonborder area. Serum IgG and IgA anticryptosporidial antibodies were measured by enzyme-linked immunosorbent assay (ELISA). Overall, 70.2% (196/279) of subjects had detectable *C. parvum* antibodies. Prevalence rates were higher (93/105 [89%]) in the colonias and urban border community (53/65 [82%]) compared to the urban nonborder community (50/109 [46%]). Within colonias, independent risk factors for *C*. *parvum* infection included consumption of municipal water instead of bottled water, older age, and lower household income. Children living along the Texas-Mexico border have a higher rate of infection with *C. parvum* compared to children living in a large nonborder urban area. Within colonias, *C*. *parvum* infection was associated with source of water supply, age, and socioeconomic status **[75]**. *Cryptosporidium parvum* in diarrheic children who were hospitalized in Goiânia, capital of Goiás State in Brazil. A crude prevalence of 14.4% (64 of 445) was observed using a direct immunfluorescent assay (DFA), but the true prevalence was 18.7% (83 of 445) when a gold standard of immunomagnetic separation was used in combination with the DFA. Infection was more predominant in children less than 24 months old and males were 2.2 times more at risk for infection when compared with females **[106]**. Stool samples taken from 50 children with malignancy and from 92 healthy children were investigated for intestinal parasites, using the modified formol ethyl acetate concentration method, and native-lugol, trichrome and Kinyoun acid-fast stain methods. Thirty-eight (76.0%) of the 50 patients had lymphoma or leukemia and were considered immunosuppressed. Several different parasites were found in 21 (42.0%) of the 50 patients with

malignancy and in 16 (47.3%) of the 38 patients with immune deficiency compared to in only 16 (17.3%) of the 92 healthy children **[3]**. Prevalence and consequences of cryptosporidiosis in children with and without diarrhea at Mulago Hospital in Kampala, Uganda were studied. Overall, 444 (25.0%) of the 1,779 children with diarrhea had *C*. *parvum* compared with only 57 (8.5%) of the 667 children without diarrhea. The age distribution of children with cryptosporidiosis followed the same age distribution of children with diarrhea in this population (3-36 months). The PCR-RFLP analysis showed that of the 444 children with *C*. *parvum*, 326 (73.7%) were infected with genotype 1 (human), 85 (19.2%) with genotype 2 (zoonotic), and 19 (4.2%) with a mixture of genotypes 1 and 2. Fourteen (3%) isolates could not be classified as either genotype. Five (isolates were classified as *C. meleagridis*. *Cryptosporidium* oocysts were detected by modified acid fast staining of fecal smears and analysis by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) **[139]**.

2.1.5 Pathogenesis

In general, diarrhea develops when intestinal absorption is impaired or secretion is enhanced. Both of these processes are regulated by the intestinal epithelial cells, which are infected by *Cryptosporidium* **[27]**. Upon oocyst excystation, four sporozoites are released which adhere their apical ends to the surface of the intestinal mucosa. Sporozoite-specific lectin adherence factor has been identified as the agent of attachment to the intestinal surface **[71]**. After sporozoite attachment, it has been hypothesized that the epithelial mucosa cells release cytokines that activate resident phagocytes **[55]**. These activated cells release soluble factors that increase intestinal secretion of water and chloride and inhibit absorption. These soluble factors include histamine, serotonin, adenosine, prostaglandins, leukotrienes, and platelet-activating factor, and they act on various substrates, including enteric nerves and on the epithelial cells themselves **[55]**. Consequently, one of two models damages epithelial cells:

1. Cell death is a direct result of parasite invasion, multiplication, and extrusion.

2. Cell damage could occur through T cell-mediated inflammation, producing villus atrophy either model produces distortion of villus architecture and is accompanied by nutrient malabsorption and diarrhea. Experimental evidence supporting this pathogenic hypothesis exists in a pig model system, where decreased intestinal sodium absorption has been correlated with "both decreased villus surface area and inhibition by prostaglandin E2 produced by inflammatory cells" **[55]**. Infection causes accelerated loss of absorptive intestinal epithelial cells and mucosal inflammation that result in nutrient malabsorption, fluid secretion, and debilitating diarrhea **[56]**. Malabsorption and abnormal intestinal permeability (decreased vitamin B_{12} absorption, decreased D-xylose absorption, abnormal lactulose/mannitol permeability test) have been confirmed in people with AIDS and cryptosporidiosis **[130]**. This point deserves emphasis, because diagnostic evaluations, such as endoscopy, may miss the site of infection. In patients with AIDS, other sites within the gastrointestinal tract may be involved, including the stomach, duodenum, and colon, as well as the biliary tract. The histopathological features of cryptosporidiosis include a minimal inflammatory infiltrate and blunting of the villus. More pronounced inflammatory changes, such as disruption of the epithelial cell barrier and more extensive infiltration of the lamina propria with inflammatory cells, are seen in immunodeficient patients **[76]**. The mechanism by which *Cryptosporidium* infection causes diarrhea remains elusive. The diarrhea is

typically noninflammatory and is often profuse. The parasite does elicit a local inflammatory response, and increased production of prostaglandins and several cytokines. These inflammatory mediators may consequently alter solute transportation in the intestinal epithelial cell, leading to osmotic diarrhea. *Cryptosporidium* infection has also been shown to inhibit apoptosis in infected epithelial cells as well as promote it in adjacent epithelial cells in vitro. This could theoretically prolong parasite survival and impair absorption in the intestinal mucosa. The presence of an enterotoxin has also been hypothesized but never conclusively demonstrated. Whatever the mechanisms by which *Cryptosporidium* infection causes disease may be, attachment to and invasion of host cells are crucial primary events in pathogenesis. However, little is known about specific parasite and host molecules involved in these processes **[76]**.

2.2 Epidemiology

Cryptosporidium has emerged as the most frequently recognized cause of recreational water–associated outbreaks of gastroenteritis, particularly in treated (disinfected) venues. The infectious dose is low, and ingestion of as few as 10-30 oocysts can cause infection in healthy persons. *Cryptosporidium* does not multiply outside of the host. The oocyst stage can resist disinfections, including chlorination, and can survive for a prolonged period in the environment, thus facilitating waterborne transmission. Because the oocysts are infectious when shed, the parasites are readily transmitted person-to-person. Some genotypes have animal reservoirs, and, thus, animal contact can be associated with transmission. Host immune response limits the duration and severity of infection **[64]**.

2.2.1 Cryptosporidiosis in United States

Cryptosporidiosis is widespread geographically in the United States. National cryptosporidiosis surveillance data are used to assess the epidemiologic characteristics and disease burden of cryptosporidiosis in the United States. In the U.S. an estimated 60,000 to 301,600 cases of cryptosporidiosis occur per year. In 2002, incidence varied by geographic location from less than one to almost 10 cases per 100,000 persons per year **[62]**. During 2003-2005, the total number of reported cases of cryptosporidiosis increased from 3,505 for 2003 to 3,911 for 2004 and to 8,269 for 2005. All reporting areas submitted reports, with more reports from northern states. Increase in cases reported for 2005 was attributable primarily to the occurrence of a single large recreational water-associated outbreak **[152]**.

2.2.2 Cryptosporidiosis in Europe

Cryptosporidiosis is a notifiable disease at the European Union level, and surveillance data are collected through the European Basic Surveillance Network. The disease distribution in Europe for 2005 included 7,960 cryptosporidiosis cases reported among 16 countries. The crude incidence rate was 1.9 cases per 100,000 populations, although considerable differences in the rates of cryptosporidiosis between countries were observed. A pronounced seasonal peak was observed in the autumn season, with 59% of cases reported between August and November. However, Ireland and Spain experienced a peak in spring and summer, respectively. Routine cryptosporidiosis surveillance in Northwest England over 17 years revealed that the cases predominantly occurred in spring and autumn **[125]**.

2.2.3 Cryptosporidiosis in Southeastern mediterranean

Cryptosporidiosis is endemic in developing and neighboring countries, such as in Fayoum 15%) **[38]** and among young children of the Nile River Delta in Egypt (17%) **[1]**; in Israel (3.4-7.4%) **[92]**; and among children in Jordan (37.3%) **[87]**.

2.2.4 Cryptosporidiosis in Other countries

2.2.4.1 Saudi Arabia

In Saudi Arabia, diarrhoeal disease (DD) is an important cause of morbidity in children but the contribution made to it by coccidian parasites is unknown and have revealed the highest prevalence of *Cryptosporidium* infection among children with diarrhea who were presenting to paediatric out patient clinics was (32%). This indicates that there is particularly high level of exposure to *Cryptosporidium* in this setting **[5]**

2.3 Prevention

Patients with underlying immune system weaknesses are at risk for the more severe complications of *Cryptosporidium* infection. In the absence of effective, specific therapy against infection with this parasite, preventative measures are of great importance among this patient population. Such measures include extensive hand washing, avoiding direct contact with stool from animals or humans, avoiding the accidental ingestion of water used in recreational activities, and taking measures to ensure the safety of the drinking water. It should be noted that the quality of the local drinking water is regionally and seasonally variable. Local public health and municipal water authorities can provide specific information about the safety of the water supply. *Cryptosporidium* species can be removed from drinking water by either boiling for 1 min or by filtering the water through a filter with a pore size of less than one µm. These recommendations are well summarized on the Centers for Disease Control **[76]**. The multiple barrier concepts for prevention of waterborne disease in drinking water include protection of source water from contamination. Protected watersheds generally have lower oocyst levels than sites receiving agricultural, sewage. Limiting these activities in a watershed might help reduce the burden on the water treatment process, but storm events that wash fecal material into receiving streams, animal migration, or epizootic infections may create peaks in the oocyst densities **[120]**.

2.3.1 Efficacy of Common Laboratory Disinfectants on the Infectivity of *Cryptosporidium*

The oocysts are also unaffected by commonly used laboratory disinfectants such as 6% sodium hypochlorite, 70% ethanol, and a variety of commercial preparations used domestically or in animal husbandry **[145]**. Although exposure to 10% Formol, aqueous or gaseous ammonia, or hydrogen peroxide has been reported to greatly reduce or eliminate oocyst infectivity **[9]**. Low concentrations of ammonia (0.007 M) significantly decrease the viability of oocysts after 24 h of exposure, as determined with in vitro assays **[68]**. Also a 4 min. exposure to 6% hydrogen peroxide or a 13 min. exposure to ammonium hydroxide reduces infectivity of *C. parvum* oocysts in cell culture 1,000-fold **[145]**.

2.4 Transmission

The best-documented routes of transmission are waterborne, foodborne, and person-to-person spread. The majority of the documented outbreaks of waterborne infection in the world have been attributed to contaminated drinking water supplies, although contaminated water used for recreational activities has also been implicated. The first reported waterborne outbreak of cryptosporidiosis occurred in 1984 and was attributed to fecal contamination of a public artesian well in Texas. In the spring of 1993, the largest outbreak of waterborne disease of any kind recorded occurred in Milwaukee. More than 400,000 of ~1,600,000 people in the greater Milwaukee area developed cryptosporidiosis after consuming contaminated drinking water. The onset of illness correlated with an increase in the turbidity of the treated water from Lake Michigan. Despite use of water purification methods that were standard at the time **[84]**. Although the source of contamination remains uncertain, recent genotypic analysis of four of the isolates indicates consistency with a human origin **[105]**. Unfortunately, despite the public attention garnered by this massive outbreak, there have been many subsequent outbreaks of *Cryptosporidium* infection attributed to contaminated drinking water in the United States and the United Kingdom. This is in part due to increased surveillance, but it also reflects our inability to rid the public water supplies of this troublesome parasite. Concern about the safety of the public water supply has prompted government authorities to issue standards for the detection of *Cryptosporidium* species. These guidelines have been updated as recently as 2001, and our ability to detect *Cryptosporidium* species in the public water supply has unquestionably improved **[110]**. The current strategy for elimination of *Cryptosporidium* species from the public water supply involves preventing contamination of water sources, physical removal of the organisms, and chemical or physical disinfection. However, these methods are only capable of reducing the number of oocysts, not eliminating the parasite from the water supply. Therefore, it is imperative that patients at risk for *Cryptosporidium* infection contact their local public water authorities for advice about the safety of their water supply. More sophisticated approaches, such as filtration, ultraviolet light irradiation, and ozone treatment, have not been widely applied for various reasons, including financial and health-related concerns **[120]**. Although waterborne routes of transmission have been the most notable, foodborne and person-to-person spread have also been documented. Cryptosporidiosis has been attributed to ingestion of contaminated apple cider, chicken salad, milk, and food prepared by an ill food handler. *Cryptosporidium*

species have also been detected in seawater and have even been found in commercially harvested oysters. Another potential source of infection may be raw vegetables sold in the marketplaces in developing countries. Finally, it should be mentioned that, although cattle are thought to be the most common animal reservoir of *Cryptosporidium* species, other species of animals, including reptiles, birds, and insects, have been shown to harbor the parasite **[42]**. It is not clear what role these other animal hosts have in the transmission of *Cryptosporidium* species.

2.5 Immunity

Exposure to *Cryptosporidium* does not necessarily lead to clinical disease. There is some indication that prior exposure results in protective immunity from cryptosporidiosis, though the duration of this immunity is unknown **[48]**.

2.6 Diagnosis of *Cryptosporidium*

Routine ova and parasite examination of feces is not sufficient for the detection of *Cryptosporidium* oocysts. The organism is the size of yeast, 4 to 6 µm, and is easily mistaken for yeast on routine stool examination **[148]**. Therefore Garcia et al. highly recommended the modified Ziehl-Neelsen carbol fuchsin stain technique for the identification oocysts in feces **[49]**.

2.6.1 Differential staining methods:

Oocysts are excreted in the faeces of human and animal hosts, and they can be preserved in 10% formalin or SAF (sodium acetate-acetic acid-formalin). Preserved stools can be concentrated to increase the yield of oocysts by sedimentation using the formol-ether or formol-ethyl acetate techniques. Current laboratory methods for the diagnosis of cryptosporidial infections generally rely on microscopic examination of fecal samples for the presence of *Cryptosporidium* oocysts. These oocysts are similar in size and shape to other fecal components (especially some yeasts), and therefore staining techniques that differentiate between the two are very desirable to avoid confusion **[104]**. Differential staining methods including safranin methylene blue stain, Kinyoun, Ziehl-Neelsen and carbol fuchsin stain oocysts red and counter stained the background. Baxby et al. (1984), which stains oocysts red, yeasts, and other fecal debris blue, have described a safranin methylene blue stain. Acid-fast stains such as Kinyoun **[83]**, Ziehl-Neelsen **[61]**, and Dimethyl Sulfoxide (DMSO) carbol fuchsin **[108]** stain oocysts red with the background counter stained and are commonly used in many laboratories including a negative malachite green stain, which we have found to be fast and reliable and should improve the microscopic diagnoses of cryptosporidial infections in clinical laboratories. The Ziehl-Neelsen staining method, first used to detect *Cryptosporidium* oocysts in feces in 1981 **[61]**. It is probably the most widely used method because of its simplicity and low cost **[39]**. Differential staining techniques, however, are time consuming and vary in sensitivity and specificity **[94]**. Fluorochrome stains, although sensitive, are complex and oocyst like the one structure in fecal debris often takes up the stain.

Negative staining techniques using nigrosin **[108]**, light green, merbromide **[24]**, which stain background yeasts and bacteria but not oocysts. Negative staining methods are faster but are considered by some to be less sensitive than conventional staining techniques **[20]**. Effective diagnosis of cryptosporidial infections requires diagnostic tools to be fast, cost effective, accurate, and sensitive. Counterstaining methods using carbol fuchsin and safranin are time consuming, frequently stain for yeasts, and lack sensitivity **[94]**. Microscopy provides the advantage of direct visual confirmation of the presence of *Cryptosporidium* oocysts. The malachite green method is practical, safe, and sensitive method of detecting *Cryptosporidium* oocysts in stool samples and was significantly more sensitive than the other three staining methods tested. With the malachite green stain, yeasts were clearly differentiated from oocysts as they took up the stain. Because of the ease with which oocysts can be differentiated from yeasts, less experienced microscopists can still accurately diagnose cryptosporidial infections. Because pathology laboratories can vary widely in their expertise in diagnosing *Cryptosporidium* and because variable laboratory expertise can result in a large variation in results **[25]**. The malachite green staining procedure described here should greatly improve microscopic diagnoses and the detection of cryptosporidial infections. Therefore, by acid-fast stain the oocyst wall will usually stain pink to purple, the background will stain blue or green, depending on the counter stain used. Many of these stains require an experienced microscopist, however, and are labor-intensive **[42]**. The "gold standard" and perhaps most widely used test for the detection of *Cryptosporidium* oocysts in stool remains the modified acid fast or Kinyoun stain. The test should be specifically requested, because it will not be performed as part of a routine examination for ova and parasites. Interpretation of the stained smear requires experience, because other organisms in the stool may stain acid fast **[76]**. Conventionally, diagnosis is made by concentration of stools followed by acid-fast staining (AF) or immunofluorescent staining. The threshold of detection in human stool specimens by these methods may require the presence of 50,000 (immunofluorescent staining) to 500,000 (AF) oocysts per g of stool **[13]**

2.6.2 Auramine phenol staining method

The fixed smear was stained with auramine O (15 min), rinsed tapwater, destained with 3% acid alcohol, restained for background color with .5% potassium permanganate (3min), rinsed with tapwater, dried at room temperature, and observed under fluorescence microscope (all materials from sigma) **[33]**.

2.6.3 Indirect immunofluorescent assays:

Immunologic techniques for the detection of cryptosporidial in stool samples were introduced in 1985 and 1986. Indirect immunofluorescent assays were described for the detection of oocysts employing recovered human serum and oocyst immunized rabbit antiserum. In immunofluorescent assays, employing oocyst-reactive monoclonal antibodies were also introduced. The immunofluorescent methodologies showed significantly increased sensitivities and specificities compared to conventional staining techniques and have found widespread application in research and clinical laboratories, as well as for monitoring oocyst presence in environmental samples. The assays generally work well with fresh or preserved stools (formalin, potassium dichromate), but some fixatives can cause problems (e.g. MIF) (Merthiolate Iodine Formaldehyde). Immunofluorescence assays demonstrating cryptosporidial life-cycle stages (e.g. oocysts) in infected tissues or biopsy specimens have been reported and can be performed using reagents available in commercial diagnostic kits. These immuno histological assays are primarily of research value, given the broad availability of stool-based diagnostic assays and the ready identification of *Cryptosporidium* in tissue sections (cryptosporidia are uniquely found on the lumenal surface of epithelial cells and are apparent in samples stained with hematoxylin and eosin or other routine histology stains **[11]**. (Several immunofluorescent assays and enzyme immunoassay (EIA) kits have become commercially available and show promising sensitivity and specificity. These tests use antibodies against *Cryptosporidium* antigens to detect the parasite in stool specimens. One of these kits, the ColorPAC *Cryptosporidium/Giardia* rapid assay (Becton-Dickinson), was recently recalled because of a cluster of false-positive results **[76]**.

2.6.4 Direct fluorescent antibody

Direct Fluorescent antibody (DFA). The most widely used antigen detection immunoassays for Giardia and *Cryptosporidium* are the direct fluorescent-antibody (DFA) tests, which detect intact organisms, and enzyme immunoassays (EIAs), which detect soluble stool antigen. DFA tests utilize fluorescein-labeled antibodies directed against cell wall antigens of *Giardia* cysts and *Cryptosporidium* oocysts and allow visualization of the intact parasites, providing a definitive diagnosis. The sensitivity and specificity of the most commonly used commercial DFA test, the Merifluor DFA test, have been reported to be 96 to 100% and 99.8 to 100%, respectively, for both *Giardia* and *Cryptosporidium.* This test has a greater sensitivity than traditional examination of permanent smears for *Giardia* and sensitivity equal to or greater than that of traditional examination of permanent smears prepared from concentrated stool specimens for *Cryptosporidium* **[69]**.

2.6.5 Oocyst concentration

The flotation-concentration method by Sheather was found to provide the best results of all selected methods. The merthiolate iodine formaldehyde concentration (MIFC) method was the least specific one. The least suitable method concerning sensitivity and costs was the flotation method with caesium chloride (CsCl) with a specificity of 29%. Only sheather's method detected all samples as positive **[73]**.

2.6.6 Antigen-capture ELISA

The diagnosis of the small (4- to 6-microns) *Cryptosporidium* oocysts is labor intensive and relies on stool concentration, with subsequent staining and microscopy. The primary purpose of this study was to evaluate the clinical utility of an antigen capture enzyme-linked immunosorbent assay (ELISA) in detecting *Cryptosporidium* oocysts in human stools. A total of 591 samples (76 diarrheal, 515 control) obtained from 213 inhabitants of an urban slum in northeastern Brazil were examined by both ELISA and conventional microscopic examination (CME) of formalin-ethyl acetate- concentrated stool samples stained with modified acid-fast and auramine stains. Forty-eight diarrheal stools (63.2%) were positive for *Cryptosporidium* oocysts by CME, with 40 of these positive by ELISA. Thirty-five control stools (6.8%) had *Cryptosporidium* oocysts detected by CME, with 15 of these also positive by ELISA. All of the 480-nondiarrheal stools and all but one of the diarrheal stools negative by CME were negative by ELISA. The test had an overall sensitivity of 66.3% and a specificity of 99.8% (positive predictive value, 98.2%; negative predictive value, 94.8%). In the evaluation of human diarrheal stool samples, the test sensitivity increased to 83.3%, with specificity of 96.4%, and, in analysis of samples from individual patients with diarrhea, the sensitivity was 87.9%, with a specificity of 100%. These results indicate that this stool ELISA is sensitive and specific for the detection of *Cryptosporidium* oocysts in human diarrheal stool specimens but has limited use in epidemiologic studies for the diagnosis of asymptomatic *Cryptosporidium* infection **[101]**.

2.6.7 PCR

PCR-based detection of microbes in clinical samples is attractive due to its extreme sensitivity and specificity. Additionally, the genetic information obtained from the sample may permit nonhuman pathogens to be distinguished from human pathogens. Methods for PCR-based detection of *Cryptosporidium* in clinical samples and drinking water have recently been reviewed **[47]**. Some investigators have found high sensitivity for PCR-based assays (one oocyst) and suggest that these assays are more sensitive than microscopic analysis of acid-fast smears **[95]**. Several factors complicate the PCR-based detection of *C. parvum* in stool. Standard fixation in 10% buffered formalin may reduce the sensitivity of the PCR, particularly if fixation occurs over an extended period. In addition, extended formalin fixation may alter the buoyancy of *C. parvum* oocysts, interfering with standard methods for purification of *C. parvum* oocysts from stool. PCR detection of oocysts from frozen stool is also possible, but the sensitivity may be reduced, probably due to rupture of oocysts during thawing. One method for oocyst purification from stool commonly used in the research laboratory involves density gradient centrifugation of stool **[141]**. While this method provides purified oocysts, ideal for PCR analysis, it is more suitable for a research laboratory than a clinical laboratory and may not be useful for samples containing few oocysts. Numerous substances can inhibit the PCR, including some stool components. Several investigators have developed nucleic acid extraction methods for stool to remove these inhibitors **[154]**.

2.7 Treatment

There have been a large number of studies aimed at developing a satisfactory therapy for cryptosporidiosis, particularly in patients with AIDS. Although several agents have been found to have some No safe and effective therapy for cryptosporidial enteritis has been successfully developed. Since cryptosporidiosis is a self-limiting illness in immunocompetent individuals, general, supportive care is the only treatment for the illness. Oral or intravenous rehydration and replacement of electrolytes may be necessary for particularly voluminous, watery diarrhea. Oral rehydration treatment can include Gatorade, bouillon, or oral rehydration solution, containing glucose, sodium bicarbonate, and potassium **[44]**. Although several agents have been found to have some activity (most notably macrolides such as spiramycin and clarithromycin, the aminoglycoside paromomycin, and ionophores such as Lasalocid and maduramycin), results have been mixed **[58]**. In part because of the failure of other therapeutic approaches, there have been several attempts at passive antibody-based immunotherapy for cryptosporidial infections **[30]**. These have also had limited success. One therapeutic intervention that has a dramatic effect on cryptosporidiosis in AIDS patients is antiretroviral therapy leading to recovery of the CD4 count. In one study of two patients with cryptosporidiosis, both were free from the parasite within 24 weeks after starting antiretroviral therapy **[45]**. This finding was confirmed in another, larger study, where all patients taken antiretroviral agents showed clinical recovery **[85]**. Two patients subsequently relapsed after the therapy, was stopped. The authors noted that resolution of the diarrhea seemed to be related to an increased CD4+ cell count rather than to the viral load. Furthermore, at least one clinic has noted a decrease in problems related to cryptosporidiosis in their AIDS patients since the onset of the widespread use of protease inhibitors **[78]**.

2.7.1 Thrombospondin-related adhesive protein of *Cryptosporidium*

Researchers have discovered the presence of *Cryptosporidium* proteins that may be involved in the adhesion of the protozoan to the intestine. These proteins are called Thrombospondin Related Adhesive Proteins 1 and 2(TRAP-C1 and TRAP-C2). Receptor Binding Motifs (RBM's) located on these proteins may play a role in *Cryptosporidium* intestinal adhesion. If these binding motifs can somehow be disrupted or blocked with monoclonal antibodies, then adhesion and the subsequent infection can be inhibited **[60]**. Colostrum derived bovine immunoglobulin concentrate has been shown to be effective concerning the treatment of diarrhea associated with cryptosporidiosis. Although helping to lessen the severity of the diarrhea, it does not have a role in the eradication of the protozoan **[57]**. Although progress has been made, control of cryptosporidiosis remains problematic due to the absence of approved vaccines or immunotherapies and lack of consistently effective parasite-specific pharmaceuticals **[17]**. Because apical complex and surface molecules of the apicomplexa are involved in attachment, invasion, and intracellular development, these molecules may provide targets for immunological or pharmacological therapy against cryptosporidiosis **[19]**. To this end, we recently reported the production and characterization of a panel of mouse monoclonal antibodies (MAbs) against multiple epitopes of immunoaffinity-purified apical complex and surface antigens of *C. parvum* sporozoites **[114]**. One of the MAbs, designated E2, was central in these studies because of its ability to elicit distinctive morphologic changes in both sporozoites and merozoites, neutralize their infectivity in vitro, and control infection in vivo **[114]**.

CHAPTER **THREE**
Materials and Methods

3.1 Methodology

3.1.1 Ethical considerations and Permissions

Helsinki committee approval and permission from Ministry Of Health (MOH) were obtained. Each child sponsor was given informed consent to get approval for his / her child participation in the study, and the objectives of the study were explained to the child sponsor.

3.1.2 Data collection

3.1.2.1 The study design and population

This is descriptive study a bout cryptosporidiosis in Gaza strip, Palestine

The population included in the present study was patients attending the following locations:

1- AL-Nasser Hospital.

2- European Hospital.

3.1.2.2 Period of the study

The present study has started from June to August, 2007, and January to March, 2008 where single stool sample from each of 200 children attending AL-Nasser and 100 from European hospitals were examined by Ziehl neelsen (ZN) stain and ELISA for the presence of *Cryptosporidium* oocysts.

3.1.2.3 Description of study area

Al Nasser Hospital has four divisions in addition to the blood disease department (leukemia, hemophilia and thalassemia), intensive care unit, immature infants department, reception pharmacy laboratory, and X-ray department. The total number of inpatient beds is 151, with 33 beds for daily care (reception and emergency). The reception department receives 3,500– 4,000 patients monthly. The general department receives 950-1,000 patients monthly, and inpatients normally stay for three days and are then discharged.

European Gaza Hospital is situated in the southern Gaza Governorate of Khanyounis. EGH is considered as one of the biggest investments in the area with total cost around $60 Million. The International management team took the responsibility to commission the Hospital. On 15 October 2000, the Management Authority transferred to local Palestinian staff. In addition to providing excellent care for patients, professional and technical positions. There are continuing education programs and specific training programs. The European Gaza Hospital has four divisions' medical department services, which include accident, and emergency, special surgery, internal

medicine, anesthesia, intensive care unit and operations and paramedical departments' services include laboratory, radiology and occupational health.

3.1.2.4 Sample size

The sample size of the present study was estimated to be 300 samples based on the calculation of the general prevalence of *Cryptosporidium* in the Gaza strip and using sample size calculator.

3.1.2.5 Types of samples distribution

- Two hundred-diarrhea stool samples from children attending AL-Nasser Hospital.
- one hundred-diarrhea stool samples from children patients attending European Hospital.

3.1.2.6 Criteria

Children complaining from diarrhea, their age were less than 5 years.

3.1.2.7 Preservation

A portion of stool samples were fixed and preserved in SAF (Sodium acetate acetic acid formaldehyde) in the same day of collection.

3.1.2.8 Sample collection

All patient sampling was applied and one stool sample was obtained in a dry, fresh stool, a clean, sterile, and well sealed plastic container with a top cover, including a self-spoon to collect the sample, leak proof plastic container. In addition, each container was labeled and the given is matched with names recorded.

3.1.3 Parasitological methods

3.1.3.1 Direct smear microscopy

Direct microscopic examination of stool for intestinal parasites stages was performed using saline.

A drop of saline was placed on a clean slide, stool was mixed with saline, and cover slip placed then the slide was examined under the microscope x10, x40 and x100.

.

3.1.3.2 Concentration technique

The present study employed just one method of concentration, formal ether sedimentation technique to detect parasites and ova.

Formal ether concentration technique

This technique is recommended as the best overall techniques for the concentration of parasites in feces **[149]**.

Procedure

1. Emulsify 1gm of feces in about 10 ml of 10%v/v formal water contained in a cap.
2. By using glass beads, were mixed well by shaking for about 20 seconds.
3. Sieves the emulsified faeces collecting the sieved suspension in beaker.
4. Transfer the suspension in a test tube and was added about 3ml of ether.
5. Stopper the tube and was mixed well for 1 minute.
6. Centrifuge immediately at approx 3,000rpm for 15 minutes.
7. After centrifugation was used a stick to remove the faecal debris from the side of the tube and decants the supernatant layer.
8. The sediment will remain.
9. Washed for three times.
10. Examined microscopically using 10x and 40x to identify the parasite.

3.1.3.3 Modified Acid-Fast Staining technique (Z-N technique)

Stool sample was used from SAF vials.

1. Sediment from concentration method was mixed, and then smear 10 µL on slide within etched circle by use transfer loop (10 µL).
2. Air-dry for at least 1 hour.
3. Fixed in Methanol for 3 to 5 minutes.
4. Stained with carbon fuchsin for 20 minutes.
5. Rinsed with tap-water for 4 minutes.
6. Decolorized with acid alcohol (HCl with 95% alcohol).
7. Rinsed with tap-water for 2 minutes.
8. Counter stain with 3% methylene blue for 30 seconds or malachite green
9. Rinsed with tap water for 2 minutes.
10. Air dry.
11. Examined by standard light microscopy.
12. By Z-N method, *Cryptosporidium sp*. Oocyst stain red against a blue-green background. *Cryptosporidium* is round and approximately four µm in diameter.

3.1.3.4 Enzyme Linked Immunosorbent Assay (ELISA) technique: Stool samples were extracted and processed according to manufacturer's recommendations (International Immuno-Diagnostics, 2003). Absorbance was read at "wavelength 450nm" using StatFax ELISA reader.

1. Break off the required number of wells needed (number of samples plus2 for controls) and place in holder.
2. Added two drops (approximately 100µl) of negative control to well #1 and 2 drops of positive control to well # 2.
3. Added two drop of the stool supernatant to each test well.
4. Incubated for 30 minutes at room temperature, then wash.
5. Added two drops of reagent 1 to each well.
6. Incubated for 5 minutes, then wash.
7. Added two drops of reagent 2 to each well.
8. Incubated for 5 minutes, then wash.
9. Added two drops of chromogen to each well.
10. Incubate for 5 minutes.
11. Added two drops of stop solution to each well. Mix wells by gently tapping the side of the strip holder with index finger.
12. Read results visually or at 450/620-650 nm. Zero reader on air.

3.1.3.5 Questionnaire

Father or mother was interviewed and information obtained were arranged in special form that includes name, age, sex, clinical symptoms, diarrhea, duration of episode and hygienic status, residence and type of sanitation system of study population**.**

3.2 Statistical analysis

Data obtained was entered into computer system and analyzed using SPSS (Statistical Package for Social Studies). Simple distribution of study variables, frequency tables, cross-tabulation, Chi square test, and graphs were carried out.

CHAPTER FOUR

RESULTS

In the present study 300 stool samples were collected from children attending Al-Nasser paediatrics hospital 200 (66.7%) and European hospital 100 (33.3%). Each child submitted one stool sample. Their ages ranged from one month old to 60 month old and distributed as159 males (53%) and 141 female (47%). It was found that 54 of those children were infected with *Cryptosporidium* with a prevalence of (18%) by modified acid-fast stain and the total number of *Cryptosporidium* positive samples was 50 with a prevalence of (16.7%) by stool ELISA in all studied areas (cities, villages, camps).

Table 4.1: Different demographic characters n = (300)

Variable	Number	%
Residency		
City	**150**	**50**
Camp	**94**	**31.3**
Village	**56**	**18.7**
Month		
Jun	**49**	**16.3**
July	**50**	**16.7**
Aug	**51**	**17**
Jan	**50**	**16.7**
Feb	**50**	**16.7**
March	**50**	**16.7**
Sex		
Male	**159**	**53**
Female	**141**	**47**
Hospital		
Al-Nasser	**200**	**66.7**
European	**100**	**33.3**

- Participants in present study were children from city (50%) while in camp and village were (31.3%), (18.7%) respectively. Participants were distributed as (53%) males and (47%) females.

- Al-Nasser paediatrics hospital 200 (66.7%) and European hospital 100 (33.3%) as shown in table 4.1.

Table 4.2: Complaints of children from symptoms n = (300)

Symptoms	Number	%
With dehydration	**185**	**61.7**
Without dehydration	**115**	**38.3**
With abdominal pain	**153**	**51**
Without abdominal pain	**147**	**49**
With fever	**179**	**59.7**
Without fever	**121**	**40.3**
With nausea	**130**	**43.3**
Without nausea	**170**	**56.7**
With vomiting	**56**	**18.7**
Without vomiting	**244**	**81.3**

The most common symptoms reported by children mothers; dehydration 61.7%, fever 59.7%, abdominal pain 51% and nausea 43.3% as shown in table 4.2.

Table 4.3: Environmental health characters

The parameter	Number	%
Sewage system		
Closed sewers	**200**	**66.7**
Open sewers	**65**	**21.7**
Septic tank	**35**	**11.7**
Garbage		
Garbage around home	**136**	**45.3**
Without garbage around home	**164**	**54.7**
Agriculture areas	**65**	**21.7**
Irrigation		
Irrigation of land with pool	**50**	**16.7**
Irrigation of land with rain	**15**	**5**

Families who have open sewers (21.7%) and presence of garbage around home (45.3%) as shown in table 4.3.

Table 4.4: Some parameters; drinking water type, feeding type, vegetables and cheese consumption

The parameter	Number	%
Source of drinking water		
-Filtered	**177**	**59**
-Tap water	**123**	**41**
Feeding type for child		
- Exclusive Breast feeding	**149**	**49.7**
- Artificial	**126**	**42**
- Both	**25**	**8.3**
Vegetables eating	**171**	**57**
Cheese eating	**111**	**37**

It was found that high percentage of children families used tap water for drinking (41%), use exclusive breast feeding (49.7%), vegetables eating (57%) and cheese eating (37%) as shown in table 4.4.

Table 4.5: The detected types of intestinal parasites as reported by father/ mother of child through the questionnaire

Type of parasite	The detected parasites as shown from questionnaire	
	Number	%
Entamoeba histolytica	**15**	**5**
Giardia lamblia	**17**	**5.7**
Ascaris lumbricoides	**4**	**1.3**
Enterobius vermicularis	**3**	**1**
Trichuris trichiura	**1**	**.3**
Hymenolypes nana	**1**	**.3**

The prevalence of *E. histolytica and Giardia lamblia* were similar & close from results were (5%) and *(*5.7*%)* respectively as shown in table 4.5.

Table 4.6: Some demographic parameters; occupation and education level

The parameter	Number	%
Father occupation		
- Employee	**121**	**40.3**
- Laborer	**139**	**46.3**
- Unemployed	**36**	**12**
Father education		
- primary	**59**	**19.7**
- secondary	**94**	**31.3**
- university	**147**	**49**
Mother occupation		
- Employee	**108**	**36**
- House wife	**140**	**46.7**
- Student	**52**	**17.3**
Mother education		
- Primary	**115**	**38.3**
- Secondary	**132**	**44**
- University	**53**	**17.7**

It was found that percentage children of father employed close and similar with children of father labor (40.3%), (46.3%) respectively as shown in table 4.6.

It was found that (54) of children were infected with *Cryptosporidium* a prevalence of (18%) as shown in Fig2. (Appendix 4)

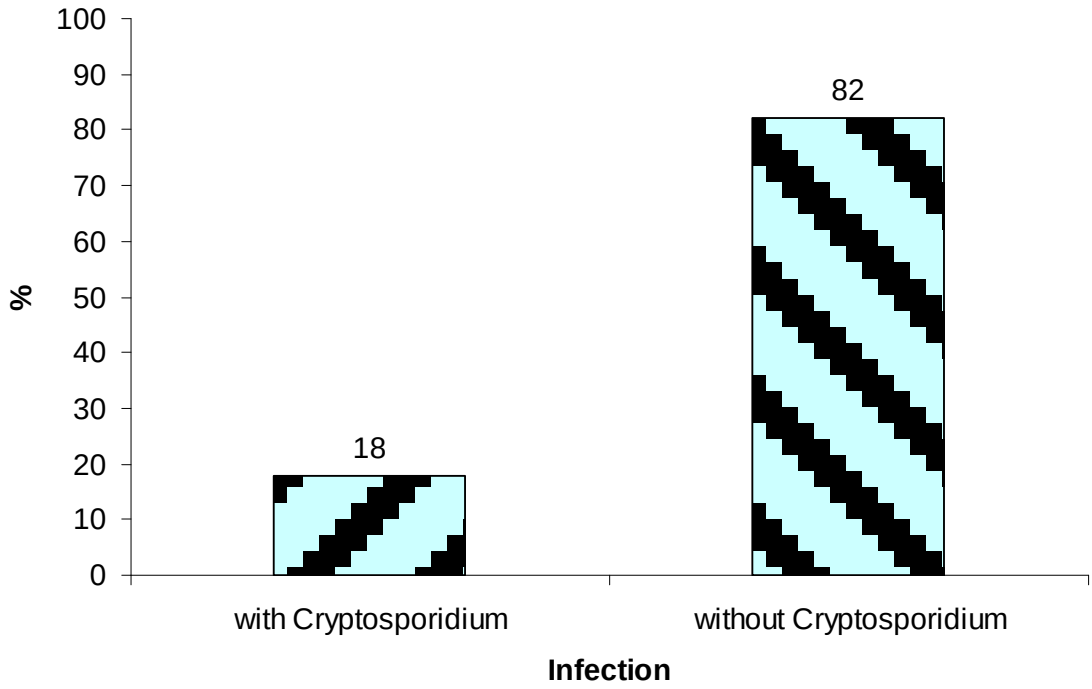


Fig. 2. Distribution of cryptosporidiosis according to modified acid-fast stain

The prevalence among males was (9.4%) and (27.7%) among females as shown in Fig 3. **(χ^2 = 16.8, df = 1, P = 0.001).** (Appendix 5)

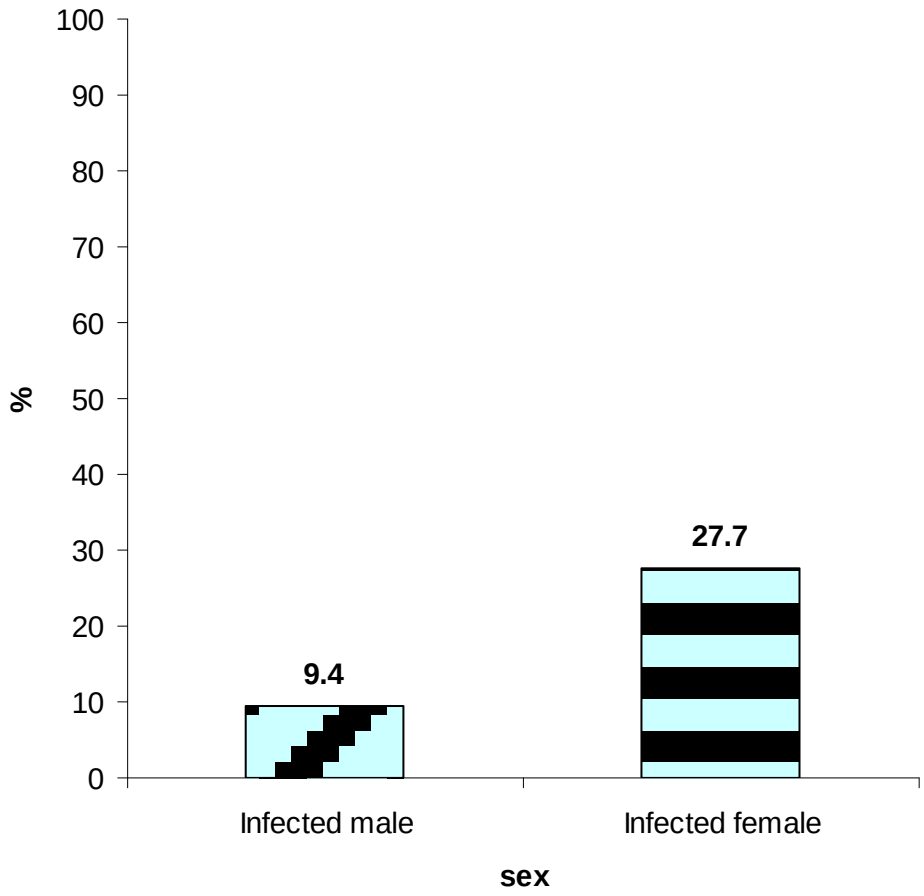


Fig. 3. Distribution of cryptosporidiosis according to sex

Table 4.7: Distribution of *Cryptosporidium* according to residency (n=300)

Residency	Positive for *Cryptosporidium* n=(54)		Negative for *Cryptosporidium* n=(246)		P-value
	No.	%	No.	%	
City n=(150)	18	(12)	132	(88)	0.03
Camp n=(94)	22	(23.4)	72	(76.6)	
Village n=(56)	14	(25)	42	(75)	

(χ^2 = 7.4, df = 2, P =0.03)

Prevalence of *Cryptosporidium* among children according to residency as shown in table 4.7 where the village had the highest prevalence.

Table 4.8: Distribution of *Cryptosporidium* according to age (n=300)

Age	Positive for *Cryptosporidium* n=(54)		Negative for *Cryptosporidium* n=(246)		P-value
	No.	%	No.	%	
<12 month old n=(156)	22	(14.1)	134	(85.9)	0.03
12-24 month old n=(127)	31	(24.4)	96	(75.6)	
25-36 month old n=(17)	1	(5.9)	16	(94.1)	

(χ^2 = 6.83, df = 2, P =0.03)

Children examined in the study ranged in age from less than 12 month to 36 months**.** The most children examined were <12 month of old; 14.1% (22 of 156) had and 24.4% (31 of 127) of those 12–24 month of old were infected. This group had the highest of infection. *Cryptosporidium* prevalence of 5.9% (1 of 17) was observed in children 25–36 month of old. This group had the lowest of infection as shown in table 4.8.

A higher rate of *Cryptosporidium* was recorded in august (24.4 %) while were similar & close from results in January, February, March were (20.8%), (20.4%) and (20%) respectively and lower peak was observed in June, July with infections rates of 9.4% and 14 % as shown in Fig 4. **(χ^2 = 5.04, df = 5, P=0.411).** (Appendix 6)

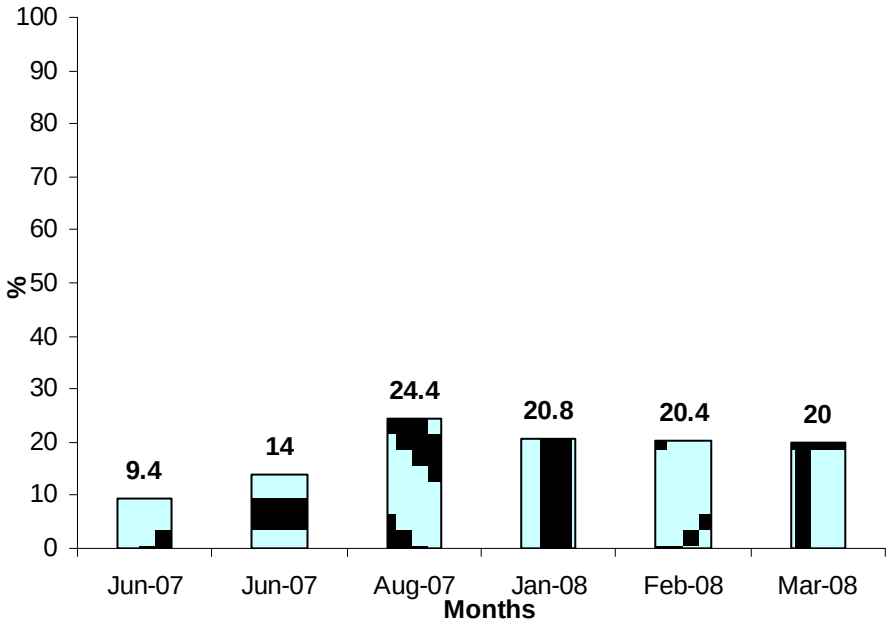


Fig. 4 Distribution of stool sample with *Cryptosporidium* infection

Table 4.9: Distribution of *Cryptosporidium* with the reported symptoms

Symptom	Positive for *Cryptosporidium*		Negative for *Cryptosporidium*		P Value
	No.	%	No.	%	
Had dehydration n=(89)	23	(25.8)	66	(74.2)	
Had no dehydration n=(211)	31	(14.7)	180	(85.3)	.0220
Had fever n=(116)	19	(16.4)	97	(83.6)	
Had no fever n=(184)	35	(19)	149	(81)	0.6
Had nausea n=(130)	45	(34.6)	85	(65.4)	
Had no nausea n=(170)	9	(5.3)	161	(94.7)	.0010
Had vomiting n=(56)	36	(64.3)	20	(35.7)	
Had no vomiting n=(244)	18	(7.4)	226	(92.6)	0.001
Had abdominal pain n=(108)	44	(40.7)	64	(59.3)	.0010
Had no abdominal pain n= (192)	10	(5.2)	182	(94.8)	

P< 0.05 is significant

P> 0.05 not significant

It was found that 40.7% of children infected with *Cryptosporidium* had abdominal pain and 34.6% had nausea, 64.3% had vomiting, 25.8% had dehydration. However, symptoms of abdominal pain, nausea, vomiting and dehydration were significantly higher in children (p<.05). While 16.4% of children infected with *Cryptosporidium* had fever (p> 0.05) as shown in table 4.9.

The distribution of *Cryptosporidium* in the case of opened sewage was more than from closed sewage and Homes close to of garbage had high prevalence with *Cryptosporidium* infection (19.2%) compared to homes away from garbage (16%) as shown in Fig.5. **P< 0.05 is significant, P> 0.05 not significant.** Appendix (7)

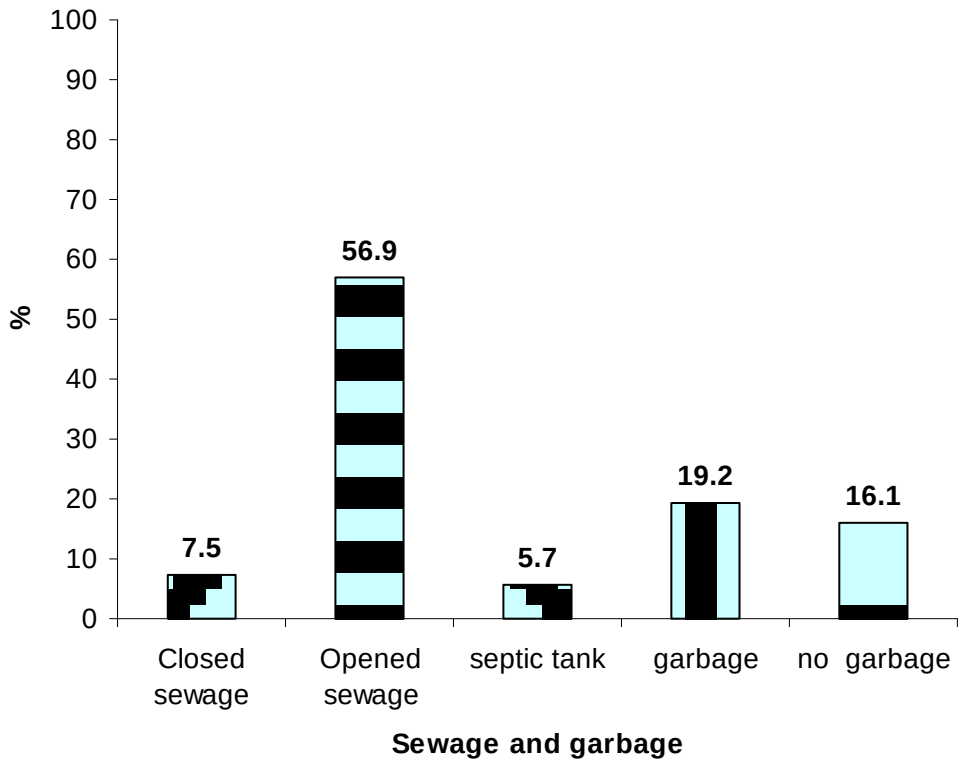


Fig. 5 Distribution of cryptosporidiosis cases according to sewage and garbage

Table 4.10: Distribution of *Cryptosporidium* according to drinking water

Source of drinking water	With *Cryptosporidium*		Without *Cryptosporidium*		P Value
	No.	%	No.	%	
Filtered n=177	4	(2.3)	173	(97.7)	0.001
Tap water n=123	50	(40.7)	73	(59.3)	

(χ^2 =72.5, df =1, P < .05)

The highest prevalence was in children who drank tap water with infection rate of 40.7% and the lowest in those who drank filtered water (2.3%) as shown in table 4.10.

The highest prevalence with *Cryptosporidium* (28%) was in irrigation of lands with pool compared with irrigation of lands without pool (16%) as shown in Fig.6. **(χ^2 =4.1, df =1, P = (0.044).** Appendix (8)

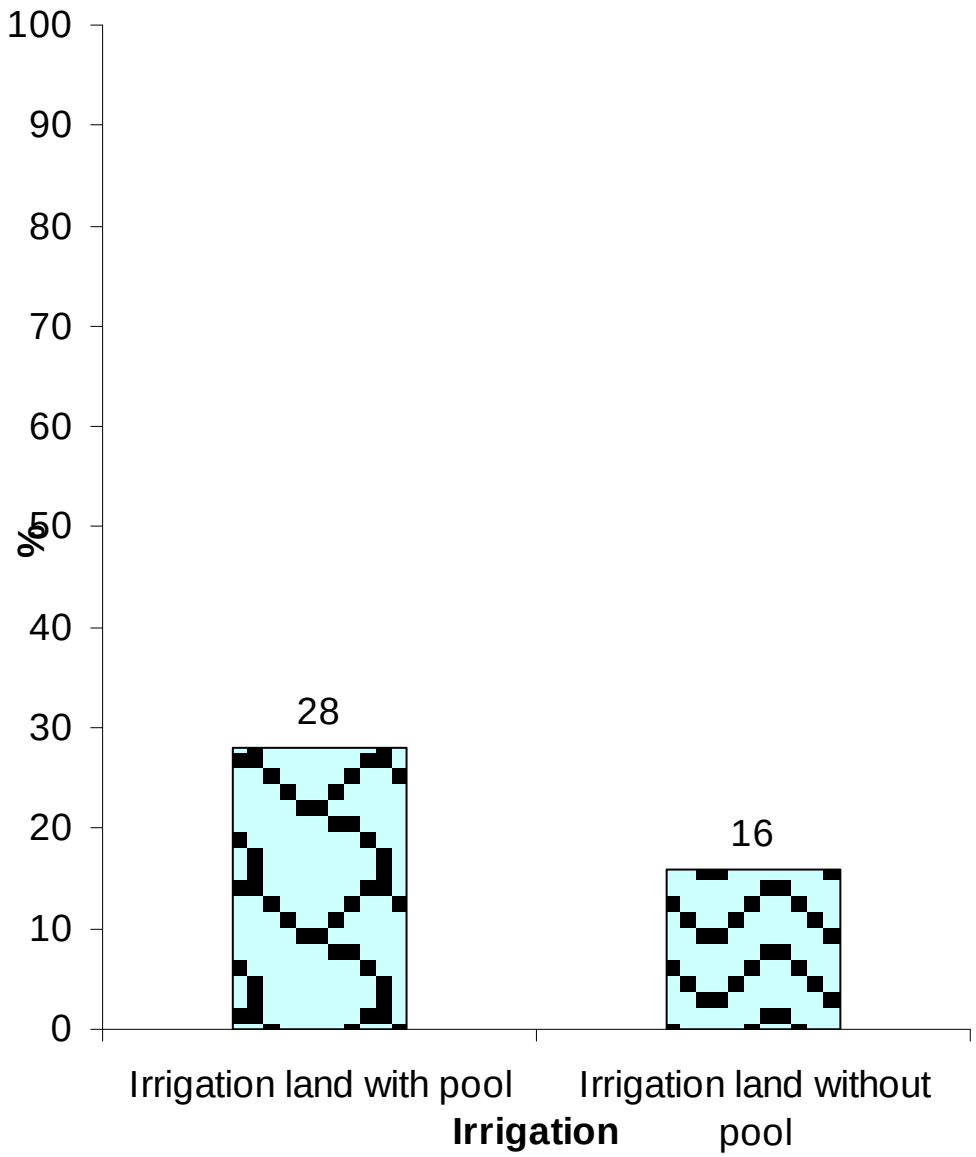


Fig.6. Distribution of *Cryptosporidium* according to Irrigation of land

Prevalence of infection with *Cryptosporidium* was high in children living households that had breeding animals (22.8%) compared to those not breeding animals (15.1%) but no statistically significant association was found between animal contact and acquisition of *Cryptosporidium* in these children as shown in Fig.7. **(χ^2 =2.9, df =1, P > 0.05).** Appendix (9)

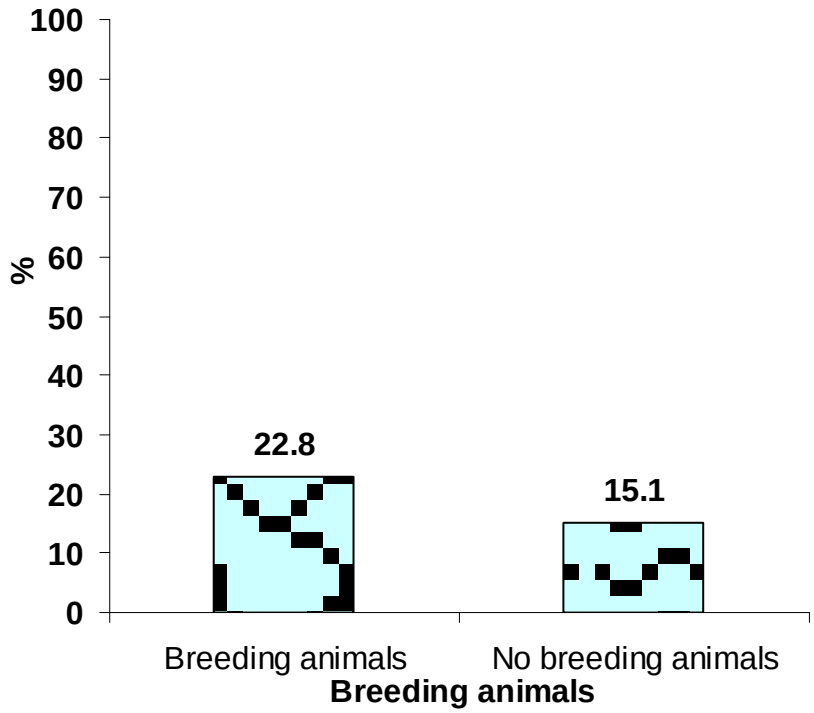


Fig.7. Distribution of *Cryptosporidium* according to breeding animals

Table 4.11: Distribution of *Cryptosporidium* according to vegetables and cheese consumption

Consumed food	With *Cryptosporidium*		Without *Cryptosporidium*		P Value
	No.	%	No.	%	
Had vegetables	28	(16.4)	143	(83.6)	
Had no vegetables	26	(20.2)	103	(79.8)	0.4
Had cheese	18	(16.2)	93	(83.8)	
Had no cheese	36	(19)	153	(81)	0.5

Children eating vegetables infected with *Cryptosporidium* was (16.4%) and eating cheese (16.2%) as shown in table 4.11.

Table 4.12: Distribution of *Cryptosporidium* according to feeding type for the child

(feeding type for the child)	With *Cryptosporidium*		Without *Cryptosporidium*		P Value
	No.	%	No.	%	
Breast feeding n=139	25	(18)	114	(82)	
Artificial n=133	24	(18)	109	(82)	>0.05
Both n= 28	5	(17.9)	23	(82.1)	

(χ^2 = 0.001, df =2, P > .05)

Percentage of children infected with *Cryptosporidium* based on breast feeding and artificial was similar (18%) as shown in table 4.12.

Table 4.13: Distribution of *Cryptosporidium* according to father occupation and education

Father occupation	With *Cryptosporidium*		Without *Cryptosporidium*		P Value
	No.	%	No.	%	
Father occupation					
Employee n=(121)	**30**	**(24.8)**	**91**	**(75.2)**	
Laborer n=(139)	**20**	**(14.4)**	**119**	**(85.6)**	
Un-employment n=(36)	**4**	**(11.1)**	**32**	**(88.9)**	**0.07**
Father education					
Primary n=59	**10**	**16.9**	**49**	**83.1**	
Secondary n=94	**11**	**11.7**	**83**	**88.3**	**0.1**
University n=147	**33**	**22.4**	**114**	**77.6**	

(χ^2 =7.04, df =3, P = 0.07) Father occupation

(χ^2 =4.5, df =2, P = 0.1) Father education

In children who have employee father high prevalence (24.8%) compared with laborer (14.4%) as shown in table 4.13.

Table 4.14: Distribution of *Cryptosporidium* according to mother occupation

Variable	With *C*ryptosporidium		Without *C*ryptosporidium		P Value
	No.	%	No.	%	
Mother occupation					
Employee n=(108)	**31**	**(28.7)**	**77**	**(71.3)**	
House wife n=(140)	**10**	**(7.1)**	**130**	**(92.9)**	**0.001**
Student n=(52)	**13**	**(25)**	**39**	**(75)**	
Mother education					
Primary n=115	**19**	**(16.5)**	**96**	**(83.5)**	
Secondary n=132	**26**	**(19.7)**	**106**	**(80.3)**	**0.8**
University n=53	**9**	**(17)**	**44**	**(83)**	

(χ^2 =21.3, df =2, P = 0.001) Mother occupation

(χ^2 =0.5, df =2, P = 0.8) Mother education

Children who have employed mother and student mother have high prevalence (28.7%), (25%) respectively compared with house wives (7.1%) as shown in table 4.14.

Table 4.15: Co- infection related to *Cryptosporidium*.

Infection of *Cryptosporidium*	Infection of *Giardia lamblia*	Infection of *Cryptosporidium*	Infection of *Entamoeba histolytica*
24	**+**	**106**	**+**
60	**+**	**184**	**+**
93	**+**		
115	**+**		
120	**+**		
135	**+**		
155	**+**		
174	**+**		
193	**+**		
211	**+**		
228	**+**		
238	**+**		
261	**+**		
289	**+**		
298	**+**		
	15/54 (27.8%)		**2/54 (3.7%)**

Fifteen samples of *Giardia lamblia* were associated with *Cryptosporidium* out of 54 (27.3%) and *Entamoeba histolytica* (3.7%) as shown in table 4.15.

Table 4.16: Comparison positive stool ELISA and microscopic examination of *Cryptosporidium* oocysts

Cryptosporidium	NO	%
Positive for *Cryptosporidium* by Microscopy	**54**	**18**
Positive for *Cryptosporidium* by ELISA	**50**	**16.7**

The samples were positive for *Cryptosporidium* oocyst by modified acid-fast stain was 18% and by ELISA 16.7% as shown in table 4.16.

Table 4.17: Correlation between stool ELISA and microscopic examination of *Cryptosporidium* oocysts

Infection of *Cryptosporidium*	Microscopic	ELISA
15	**+**	**-**
18	**-**	**+**
289	**+**	**-**
294	**+**	**-**
298	**+**	**-**

Four of the fifty-four samples that were positive for *Cryptosporidium* by microscopy were negative by stool ELISA. As shown table, 4.17 identified one positive stool sample.

CHAPTER FIVE

DISCUSSION

5.1.1 Geographical distribution

Cryptosporidiosis in humans is predominantly a diarrheal disease, occurring in all age groups, with the organisms growing in an intracellular but extracytoplasmic location in the enterocytes of the gastrointestinal tract. The disease is more protracted, severe, and affects extraintestinal sites in people with innate or acquired deficiencies in immunity **[66]**. According the previous literature few studies have been carried out regarding cryptosporidiosis in Gaza strip and West Bank. It was clear that the prevalence still close to each other in Gaza strip. The techniques applied for *Cryptosporidium* oocyst isolation were acid fast and antigen detection using ELISA. Findings showed that patients with *Cryptosporidium* infection frequently remain undiagnosed due to traditional diagnosis of intestinal parasites using direct smear microscopy which is practiced method in Gaza strip. The present study focused on the occurrence of *Cryptosporidium* among diarrheal children under five years old. This study is considered surveillance study because such a study provides the foundation for understanding the epidemiology of cryptosporidiosis. The present study found that the prevalence rate of *Cryptosporidium* in children with diarrhoea in Gaza strip was slightly high (18%). Our study was similar and close to results reported among children in Gaza (19%) **[121]** but different from that others (14.9%) **[122]**. *Cryptosporidium* oocysts were detected in 62 (14.9%) of the tested samples by acid-fast staining technique and in 68 (16.3%) using ELISA kit **[6]**. The prevalence of *Cryptosporidium* in children with diarrhoea in the West Bank was lower than our study (11.6%) **[2]**. Our results are consistent with those obtained in South Africa with a prevalence rate of (18%) **[123]** and higher rates of infection were reported in Kenya (25%) **[50]** and 26% in Mexican **[101]**. Much lower than that obtained in this study for children under five years old with gastroenteritis in Irbid, Jordan, the prevalence rate for *Cryptosporidium* was 1.5% **[153]**. This difference could be attributed to possible differences in population. The high prevalence rate of *Cryptosporidium* in Gaza strip may be attributed to wastewater disposal methods and possible contaminating of drinking water. In the present study, the detection of 54 cases infected with *Cryptosporidium* was proved using modified acid fat stain but no case was detected by direct smear technique.

Also during the search in the local literature and the annual reports of ministry of health no mention of such parasite. This may be due to the lack of knowledge about this protozoan and the poor laboratory techniques, which are limited to direct smear in the local medical laboratories in hospitals and private laboratories in Gaza.

5.1.2 Demographics:

5.1.2.1 The relationship between *Cryptosporidium* and gender

Studying the gender and cryptosporidiosis infection is a debate issue. It was shown that total positive rate oocysts was (18%) distributed as 9.4% in males and (27.7%) in females where this difference was a statistical significant p = (0.001). The higher number of cases in female children may reflect the increased exposure to sources of contamination by female or another probability they are often fed less, given less nutritious food, provided with less health care. The present findings were consistent with previous study in Gaza **[6]**. In contrast, there was no significant difference observed by sex as reported by **[50]**.

5.1.2.2 Area of residence related to *Cryptosporidium* infection

These data indicate that the prevalence of infection with *Cryptosporidium* is very high among children living in villages (25%) and camps (23.4%) and less than the prevalence observed in city (12%). So the majority of infected children in this study came from the camps and villages thus it was shown that the elevated results in camps and villages areas might be due to deficiency of sanitary facilities and diffused faecal contamination was commonly seen. They live in overcrowded rooms and belong to low socioeconomic classes with poor hygiene, thus direct person to person transmission probably played an important role in cryptosporidiosis epidemiology in children. In a similar study conducted in West Bank, it showed that prevalence was higher in refugee camps and in rural areas as compared to in urban areas **[2]**.

5.1.2.3 The relationship between *Cryptosporidium* and age

Cryptosporidium is a leading cause of persistent diarrhoea in children in developing countries **[34]**. Cryptosporidiosis cases are distributed across all age groups, but in the most studies of incidence or prevalence, the 1 to 2 year-old population seems to be the most age affected. Because cryptosporidiosis has low incidence in infants under one year reflects reduced exposure to oocysts as a result of breast feeding or bottle feeding as well as protection from the limited oral exploration of the world in the period prior to crawling or walking. There will also be a degree of immunity derived from maternal antibodies against *Cryptosporidium* in the mother's breast milk **[66]**. In the present study, cryptosporidiosis was high among children 12–24 months of age as children in this age are the most susceptible to disease. This group consistent with the weaning period where the children are exposed to risks such as contamination of environment, food, water and lack of self-awareness, personal hygiene and cleanliness at this critical age. In addition, crawling sometimes begins at this age and the risk of ingesting contaminated materials is high, and applicable especially in unhygienic environments. This is in contrast to reports on cryptosporidiosis in children in Egypt where infection was most common among children less than 12 months of age **[1]**. Our study is agreement with previous reports showing that a study on cryptosporidiosis in children in Gaza showed the highest levels of infection among children who are 1–2 years of age and a prevalence of 14.9% **[122]**. Another study on cryptosporidiosis in children in Gaza showed the highest levels of infection among children 1–4 years of age and a prevalence of 14.9 % **[6]**. It is not clear why there are differences in susceptibility by age of children but there are some possibilities that they may be due to the prevailing *Cryptosporidium* species endemic in specific areas.

5.1.3 Seasonality

5.1.3.1 The relationship between *Cryptosporidium* and seasonal variation

The seasonality of *Cryptosporidium* varied depending on the geographic locations of the studies, but it was generally most prevalent in the rainy season **[10]**. In the present study no clear trend to the relationship between occurrence of *Cryptosporidium* and seasonal variation but the months from January to March showed similar prevalence but the high peak of prevalence was noticed in August (24.4%). It was reported that *Cryptosporidium* infections were more common in the late summer in Canada **[74]** and this was similar to our findings. In a previous study, a high prevalence of cryptosporidiosis in the hot and dry periods was also reported in Gaza with a significant decrease at the onset of the cooler, wet season **[121]**. This may be attributed to water shortages that are common in these regions during the dry season, which result in poor hygiene. In contrast, study in Kuwait where rainfall is scanty the highest prevalence was found in cool months of November to April **[133]**. Other studies showed that the seasonal increases in cases are linked to increased rainfall **[99]**. It is assumed that this reflects contamination of drinking water sources from sewage and animal waste. Our environment is contaminated where economic conditions are very difficult, removal of garbage and sewage treatment faced many obstacles.

5.1.4 Symptoms in relation to cryptosporidiosis

Rates of clinical symptoms in our study were higher than rates reported in other studies and this could be attributed to differences in study designs. 56 patients had vomiting of whom 64.3% had cryptosporidiosis there was significant association between cryptosporidiosis and vomiting. Our study is in agreement with previous reports showing that the strong association of cryptosporidiosis with vomiting and has been observed in other studies in Egypt **[1]** and in Tanzania **[22]**. A total of 108 patients had abdominal pain of whom 40.7% had cryptosporidiosis there was significant association between cryptosporidiosis and abdominal pain due to direct exposure to the infectious agent or lack of immunity to *Cryptosporidium* and or other infectious agents capable of causing abdominal pain was not identified but could have play role in the symptoms displayed by the children carrying *Cryptosporidium*. Our study is in agreement with previous reports that correlate *Cryptosporidium* infection with abdominal pain in Indians **[15]**. Findings contrast with previous studies where observations of symptoms varied **[63]** result of differences in environmental risk factors or different genetic types of *Cryptosporidium*. Our study is in agreement with previous reports showing that cryptosporidiosis is significantly associated with abdominal pain **[138]**. Dehydration is the most common problem people develop after being infected with cryptosporidiosis. In the present study 89 children had dehydration of whom 25.8% had cryptosporidiosis due to severe diarrhea and there was significant association between cryptosporidiosis and dehydration (p= 0.022).

5.1.5 Cryptosporidiosis and risk factors

Cryptosporidium was increasing with the presence of garbage around homes (19.2%) but no statistical significant difference was found (p=.5). It was observed that, prevalence of *Cryptosporidium* in the case of opened sewage (56.9%) was more than from closed sewage (7.5%). However there was significant association between cryptosporidiosis and sewage due to the type of sewer (p= 0.001), flooding of sewers is periodical matter in Gaza. In addition, this put the risk for transmission and contaminated water, environment, vegetables. Thus, children with weakened immune systems are the most vulnerable to the pathogens found in sewage. Previous study showed that the strong association of *Cryptosporidium* with environments was conducive to human fecal contamination (p = 0.001) **[23]**. Finding of this study is that drinking of tap water without further treatment or processing was associated with cryptosporidiosis when compared to drinking filter water (p= .001). The children who drank tap water the infection rate of *Cryptosporidium* was 40.7% compared to who drank filter water 2.3%. There was significant difference between them due to the lower quality of water and faulty sewage lines. Another possibility due to the resistance of *Cryptosporidium* oocysts to chemical disinfection while filter water was the primary mean to remove oocysts from water sources. Thick-walled *Cryptosporidium* oocysts (3 to 6 µm in diameter) are stable in the environment and have been found to remain viable in water for up to 140 days **[111]**. In similar reports in the US, the potential for drinking water to be associated with cryptosporidiosis among immunocompetent persons is relevant since most major known outbreaks of cryptosporidiosis involved transmission through contaminated water **[54]**. Previous evidence has suggested an association between drinking of unfiltered water from Loch Lomond, Scotland, and cryptosporidiosis **[8]**. In contrast to reports on children in Australian study also found that cryptosporidiosis no association with drinking publicly supplied water **[116]**. In the present study, it was observed that the highest prevalence with *Cryptosporidium* (28%) was in irrigation of lands with pool compared to irrigation of lands without pool (16%). Regarding children whose families breeding animals in their homes was found highly infected with *Cryptosporidium* (22.8%) but no statistically significant association was found between animal breeding and acquisition of *Cryptosporidium* in these children.

Previous research has associated farm animal contact with outbreaks of *Cryptosporidium*; moreover, calf contact and lamb contact have been identified as risk factors for sporadic infection **[116]**. Other studies indicate that pets are not a major risk factor for acquiring *Cryptosporidium* **[53]**. Another study of additional interest was the reduced likelihood of zoonotic transmission of *Cryptosporidium* spp. to residents of the Loch Lomond supply area because they were less likely to be exposed to farm animals **[109]**. In the present study (16.4%) of children, eating vegetables infected with *Cryptosporidium*. *Cryptosporidium* can be transmitted through food and **[32]**. Raw ingredients may be contaminated, as may the water used for irrigation **[137]** and food processing, particularly in developing countries **[135]**. During production and processing, raw salad can be contaminated with water containing oocysts **[118]**. it was found that (16.2%) of children had eaten white cheese was found infected with *Cryptosporidium* this may be due to dairy products having caused outbreaks when the milk was improperly pasteurized **[51]**.

5.1.6 The relationship between cryptosporidiosis and breast-feeding

Most previous studies on the prevalence of cryptosporidiosis in children associated with breast-feeding, especially exclusive breast-feeding; found lower infection rates **[1]**. In the present study (18%) of children breast feeded was found infected with *Cryptosporidium* but no statistically significant association was found between breast-feeding and *Cryptosporidium*. In spite, other opinions reported that exclusive breast-feeding protects children from exposure to diarrhea- causing pathogen **[124]**.

5.1.7 The relationship between cryptosporidiosis, education and occupation

In the present study regarding education the infected children belong to mothers with secondary level education were found to be high infected with *Cryptosporidium* (19.7%) compared to other levels of education due to better-educated mothers tended to enjoy a relatively higher standard of living. In addition, education was reflected in child rearing and child health care practices during illness. Our study disagreement with previous study shows that cryptosporidiosis was not associated with the educational achievement of either parent **[100]**. In children who have employee, mother or student mother they showed high prevalence of (28.7%), (25%) respectively compared to house wife (7.1%). Due to that employee mother leave here children a long time thus living conditions of these children will not be safe and they lack of self awareness at this critical age and may need to care in this age or may has been less childcare.

5.1.8 Other protozoan parasites

In the present study the prevalence of other protozoan parasites detected *E. histolytica and Giardia lamblia* were similar & close (5%) and (5.7*%)* respectively. Four cases of *Ascaris lambricoides* (1.3%)**,** 3 cases of *Enterobius vermicularis* (1%) and 1 case of *Trichuris trichiura* and *Hymenolypes nana* (.3%).

5.1.8.1 Co- infection related to *Cryptosporidium*

Infections with two or more pathogens can occur, particularly in people returning from developing countries. It is thought to represent exposure to sources of contamination that contain multiple pathogens such as sewage or animal waste **[102]**. In present study Co-infection between *Cryptosporidium* and *Giardia* results from the existence of a common source of infection. Our study is agreement with previous study that reported significant association between *Giardia* and *Cryptosporidium* **[70]**. In contrast, other study found no association between *Giardia* and *Cryptosporidium* **[63]**.

5.1.9 Correlation between stool ELISA and microscopic examination of *Cryptosporidium* oocysts

An ELISA test with high sensitivity and specificity for the coprodiagnosis of Cryptosporidial antigen, which is not dependent on the skill of the technicians. Where the identification of the *Cryptosporidium* was based on the staining and identification of the oocyst with a size of 4-6 µm with a pink color on a green or blue according to the counter stain. It was stated that the morphological detection and identification of the oocyst, offers a diagnostic alternative to the laborious conventional direct microscopy **[89]**. In present study at least one of the ELISA-positive, microscopy-negative samples suggesting either that microscopically undiagnosed *Cryptosporidium sp.* was still present or that the ELISA detected parts or components organisms or fragments, simply mean that presence of cryptosporidial antigens in the stool without the presence of whole oocysts. One unconfirmed positive microscopic examination, in this case that ELISA confirmed that presence of *Cryptosporidium.* Three samples were negative while microscopic positive one possibility due to amount of oocyst in sample very few or oocysts may simply mean that these samples contained an amount of free antigen below the sensitivity of the assay. This ELISA is quite practical. Samples are easy to prepare for the ELISA and particularly useful to diagnosing cryptosporidiosis often in epidemiology.

Chapter SIX

Conclusions and Recommendations

6.1 Conclusions

1- Cryptosporidiosis still exists among children in Gaza strip.

2- Intestinal parasitic infection is an important public health problem in camps and villages areas.

3- The prevalence of *Cryptosporidium* is high when compared to that in developed countries.

4- It was found that 54 of children attending Al-Nasser, European hospital, Gaza strip were infected with *Cryptosporidium* with a prevalence of (18%).

5- There was a difference in the prevalence of *Cryptosporidium* among males (9.4%) and (27.7%) among females.

6- It was observed that the highest prevalence with *Cryptosporidium* (24.4%) was in the age group (12-24 month old**)** while the lowest prevalence with *Cryptosporidium* (5.9%) was in the age groups (25-36 month old).

7- In the present study, the detection of 54 cases infected with *Cryptosporidium* was proved using modified acid fat stain but no case was detected by direct smear technique. Also during the research in the local literature and the annual reports of ministry of health no mention for such parasite.

8- The most frequent symptoms was found to be abdominal pain (40.7%), vomiting (64.3%), nausea (34.6%), fever (14%), dehydration (25.8%). In children with only cryptosporidiosis and this very statistically significant (P< 0.05**)**. This association between *Cryptosporidium* and these symptoms represent a strong evidence for the pathogenicity of *Cryptosporidium.*

9- Control measures depend on improved public health, including personal hygiene and sanitation, awareness program should be launched.

10- ELISA test is simple, reliable, for screening a large number of samples in short time.

6.2 Recommendations

There some suggestions that may help us move in the right direction

1- Our findings suggest the necessity of the application newer instructions to apply the routine testing for *Cryptosporidium* on all children with diarrhea and these pathogens should be taken into account.

2- *Cryptosporidium* should be considered, as it is now neglected or misdiagnosed.

3- Measures should be taken to ensure the delivery of clean uncontaminated drink water to people especially who are living in camps and a villages areas.

4- Developing proper sewage systems instead of septic tanks.

5- Future studies should address the drinking water issue and its contamination with *Cryptosporidium*.

6- Further studies need to be done with a larger sample to understand risk factors associated with zoonotic transmission of *Cryptosporidium spp.* in the population.

7– In epidemiological surveys, its better use ELISA technique due to its simplicity, reliability and screening a large number of specimens in short time.

REFERENCES

[1] Abdel-Messih I.A., Wierzba T.F., Abu-Elyazeed R., Ibrahim A.F., Ahmed S.F., Kamal K.,Sanders J., Frenck R., 2005 - Diarrhea associated with *Cryptosporidium parvum* among young children of the Nile River Delta in Egypt. J. Trop. Pediatr. 51: 154–159.

[2] Abu-Alrub S.M., Abusada G.M., Farraj M.A., Essawi T.A., 2008 - Prevalence of *Cryptosporidium* spp. in Children with Diarrhea in the West Bank, Palestine. Infect Developing Countries 2(1):59-62.

[3] Aksoy U., Erbay A., Akısu Ç., Apa H., Özkoç S., Öztürk S., 2003 - Intestinal parasites in children with neoplasm. Turk. J. Pediatr. 45(2):129-132.

[4] Akyon Y., Erguven S., Arikan S., Yurdakok K., Gunalp A., 1999 - *Cryptosporidium parvum* prevalence in a group of Turkish children. Turk. J. Pediatr. 41:189-196.

[5] Al- Braiken F.A., Amin A., Beaching N.J., Hommel M., Hart C. A., 2003 – Detection of *Cryptosporidium* among diarrhoeic and asymptomatic children in Jeddah, Saudi Arabia. Ann. Trop. Med. Parasitol. 97 (5).

[6] AL-Hindi A.I., Elmanama, A. A., Elnabris, K. J., 2007- Cryptosporidiosis Among children attending Al-Nasser pediatric hospital, Gaza, Palestine. Turk. J. Med. Sci. 37(6): 367-372.

[7] Alpers D.H. D.W., Yang C., Powell D.W., Silverstein F.E., 1995 - Textbook of gastroenterology vol (2), 2 nd editions, Library of congress cataloging in Publication Data.

[8] Anon, 1995 - *Cryptosporidium* in water supplies second report of the group of experts Department of the environment and department of health. London. HMSO.

[9] Angus K. W. D., Sherwood G., Hutchinson I., Campbell l., 1982 - Evaluation of the effect of 2-aldehyde-based disinfectants on the infectivity of faecal cryptosporidia for mice. Res. Vet. Sci. 33**:**379-381.

[10] Areeshi M.Y., Beeching N.J., Hart C.A., 2007 - Cryptosporidiosis in Saudi Arabia and neighboring countries. Ann. Saudi. Med. 27(5):325-32.

[11] Arrowood M., Fayer R., 1997 - *Cryptosporidium* and cryptosporidiosis. CRC Press, Boca Raton, New York, Tokyo. J. Diagnosis. 43 – 64.

[12] Baghdadi A. M., 1996 - Comparison between the prospect *Cryptosporidium* microtiter assay and the immunofluorescent test in the diagnosis of cryptosporidiosis. J. Egypt. Soc. Parasitol. 26:243-247.

[13] Balatbat A.B., Jordan G.W., Tang Y.J., Silva J.R. J., 1996 - Detection of *Cryptosporidium parvum* DNA in Human Feces by Nested PCR. J. Clin. Microbiol. 34 (7):1769 – 1772.

[14] Baxby D., Blundell N., Hart C. A., 1984 - The development and performance of a simple, sensitive method for the detection of *Cryptosporidium* oocysts in feces. J. Hyg. (Cambridge), 92:317–323.

[15] Bentley C., Laubach H., Spalter J., Ginter E., Jensen L., 2004 - Relationship of cryptosporidiosis to abdominal pain and diarrhea in Mayan Indians. Rev. Inst. Med. Trop. Sao. Paulo. 46(4):235-7.

[16] Blackman M. J., Bannister L. H., 2001 - Apical organelles of Apicomplexa: biology and isolation by subcellular fractionation. Mol. Biochem. Parasitol. 117**:**11-25.

[17] Blagburn B. L.; Soave R., 1997 - Prophylaxis and chemotherapy. In: Fayer R., editor. Cryptosporidium and *cryptosporidiosis*. Boca Raton, Fla: CRC Press; 111–123.

[18] Bonnin A., Dubremetz J.F., Camerlynck P., 1991 - Characterization and immunololization of an oocyst wall antigen of *Cryptosporidium parvum* (Protozoa: Apicomplexa). Parasitol. 103:171 - 177.

[19] Brown W. C., McElwain T. F., Hotzel I., Suarez C. E., Palmer G. H., 1998 - Helper T- cell epitopes encoded by the *B*abesia *b*igemina rap-1 gene family in the constant and variant domains are conserved among parasite strains. Infect. Immun. 66:1561–1569.

[20] Casemore D., P., Armstrong M., Sands R. L., 1985 - Laboratory diagnosis of cryptosporidiosis. J. Clin. Pathol. 38, 1337–1341.

[21] Casemore D.P., Wright S.E., Coop R., 1997 - Cryptosporidiosis - human and animal epidemiology. In: Ed. R Fayer. *Cryptosporidium* and cryptosporidiosis. CRC Press, Boca Raton, 65-92.

[22] Cegielski J.P., Ortega Y.R., McKee S., Madden J.F., Gaido L., Schwartz D.A., Manji K., Jorgensen A.F., Miller S.E., Pulipaka U.P., Msengi A.E., Mwakyusa D.H., Sterling C.R., Reller L.B., 1999 - *Cryptosporidium, Enterocytozoon*, and *Cyclospora* infections in pediatric and adult patients with diarrhea in Tanzania. Clin. Infect. Dis. 28: 314–321.

[23] Chacin-Bonilla L., Barrios F., Sanchez Y., 2008 - Environmental risk factors for *Cryptosporidium* infection in an island from Western Venezuela. Mem. Inst. Oswaldo Cruz [online]. v. 103, n. 1, pp. 45-49. ISSN 0074-0276.

[24] Chichino G., Bruno A., Cevini C., Atzori C., Gatti S., Scaglia M., 1991 - New rapid staining methods of *Cryptosporidium* oocysts in stools. J. Protozool. 38 (Suppl.), 2125–2145.

[25] Clancy J. L., Gollnitz W. D., Tabib Z., 1994 - Commercial labs: How accurate are they? J. Am. Water Works Assoc. 86:89–97.

[26] Clark D.P., 1999 - New insights into human cryptosporidiosis. Clin. Microbiol. Rev. 12:554-563.

[27] Clark D. P., C. L., Sears 1996 - The pathogenesis of cryptosporidiosis. Parasitol. Today 12**:** 221-225.

[28] Cook G.C., 1987 - Opportunistic parasitic infections associated with the acquired immune deficiency syndrome (AIDS): parasitology, clinical presentation, diagnosis, and management Q. J. Med. 65:967-983.

[29] Collings S., & Highton J. 2004 - *Cryptosporidium* reactive arthritis. N. Z.Med. J. 117(1200), one p following U1023.

[30] Crabb J. H., 1998 - Antibody-based immunotherapy of cryptosporidiosis. Adv. Parasitol. 40**:**121–149.

[31] Current W.L., 1985 - Cryptosporidiosis. J. Am. Vet. Med. Assoc. 187:1334-1338.

[32] Dawson D., 2005. Foodborne protozoan parasites. Int. J. Food Microbiol. 103, 207–227.

[33] Delaat A.N.C., 1979 - Microbiology for the allied health professions. 2 nd ed. London: Henry Kimpton Publishers.

[34] Dillingham R.A., Lima A.A., Gruerrant R.L., 2002 - Cryptosporidiosis: epidemiology and impact. Microbes and Infect. 4 (10): 1059 – 1066.

[35] DuPont H.L., Chappell C.L., Sterling C.R., Okhuysen P.C., Rose J.B., Jakubowski W., 1995 - The infectivity of *Cryptosporidium parvum* in healthy volunteers. N. Engl. J. Med. 332:855–9.

[36] Duffy G., Moriarty E.M., 2003 - *Cryptosporidium* and its potential as a food-borne pathogen. Anim. Health Res. Rev. 4, 95–107.

[37] Dziuban E.J., Liang J.L., Craun G.F., Hill V., Yu P.A., Painter J., et al., 2006 - Surveillance for waterborne disease and outbreaks associated with recreational water United States, 2003–2004. MMWR. Surveill. Summ. 55:1–30.

[38] EL-Mohamady H., Abdel-Messih I.A., Youssef F.G., Said M., Farag H., Shaheen H.I., et al., 2006 - Enteric pathogens associated with diarrhea in children in Fayoum, Egypt. Diagn. Microbiol. Infect. Dis. 56(1): 1-5.

[39] Entrala E., Rueda-Rubio M., Janseen D., Mascaro C., 1995 - Influence of hydrogen peroxide on acid-fast staining of *Cryptosporidium parvum* oocysts. Int. J. Parasitol. 25:1473–1477.

[40] Fayer R., Ungar B.L.P., 1986 - *Cryptosporidium* spp. and cryptosporidiosis. Microbiol. Rev. 50**:** 458-483.

[41] Fayer R., Speer C. A., Dubey J.P., 1997 - The General Biology of *Cryptosporidium*. In*:* Fayer R *Cryptosporidium* and Cryptosporidiosis CRC. Press, Boca Raton, New York, Tokyo.1 – 41.

[42] Fayer R., Morgan U., Upton S.J., 2000 - Epidemiology of *Cryptosporidium*: transmission, detection and identification. Int. J. Parasitol. 30**:** 1305-1322.

[43] Feltus D.C., Giddings C.W., Schneck B.L., Monson T., Warshauer D., McEvoy J.M., 2006 - Evidence supporting zoonotic transmission of *Cryptosporidium* spp. in Wisconsin. J. Clin. Microbiol. 44:4303-4308.

[44] Flanigan T.P., Soave R., 1993 - "Cryptosporidiosis." Prog. Clin. Parasitol. 1-20.

[45] Foudraine N. A. G. J., Weverling T., van Gool M. T., Roos F., de Wolf P. P., Koopmans P. J., van den Broek P. L., Meenhorst R., van Leeuwen J. M., Lange P. Reiss. 1998 - Improvement of chronic diarrhoea in patients with advanced HIV-1 infection during potent antiretroviral therapy. AIDS 12**:**35–41.

[46] Fretz R., Svoboda P., Ryan U.M., Thompson R.C.A., Tanner M., Gartner A.B., 2003 - Genotyping of *Cryptosporidium* spp. isolated from human stool samples in Switzerland. Epidemiol. Infect. 131: 663-667.

[47] Fricker C. R., J. H., Crabb 1998 - Water-borne cryptosporidiosis: detection methods and treatment options. Adv. Parasitol. 40**:**241-278.

[48] Frost F.J., Tollestrup K., Craun G.F., Fairley C.K., Sinclair M.I., & Kunde T.R., 2005 - Protective immunity associated with a strong serological response to a Cryptosporidium-specific antigen group, in HIV infected individuals. J. Infect. Dis. 192(4), 618-21.

[49] Garcia L.S., Bruckner D.A., Brewer T.C., et al., 1983 - Techniques for the recovery and identification of *Cryptosporidium* oocysts from stool specimens. J. Clin. Microbiol. 18:185-190.

[50] Gatei W., Wamae C.N., Mbae C., Mulinge E., Waithera T., Gatika S.M., Kamwati S.K., Revathi G., Hart C. A., 2006 - Cryptosporidiosis: prevalence, genotype analysis and symptoms associated with infections and children in Kenya. AM. J. Trop. Med. Hyg. 75(1):78-82.

[51] Gelletlie R., Stuart J., Soltanpoor N., Armstrong R., Nichols G., 1997 - Cryptosporidiosis associated with school milk. Lancet. 350, 1005 and 1006.

[52] Gennari-Cardoso M.L., Costa-Cruz J.M., Castro E., Lima L.M.F.S., Prudente D.V., 1996 - *Cryptosporidium* sp. in children suffering from acute diarrhea at UberlAndia city, State of Minas Gerais, Brazil. Mem Inst Oswaldo Cruz 91: 551-552.

[53] Glaser C., Safrin S., Reingold A., Newman T.B., 1998 - Association between *Cryptosporidium* infection and animal exposure in HIV-infected individuals. J. Acquir. Immune. Defic. Syndr. Hum. Retrovirol. 17:79–82.

[54] Goldstein S.T., Juarez D.D., Ravenholt O., Hightower A.W., Martin D.G., Mesnik J.L., Griffiths S.D., Bryant A.J., Reich R.R., Herwaldt B.L., 1996 - Cryptosporidiosis: an outbreak associated with drinking water despite state-of-the-art water treatment. Ann. Intern. Med. 124:459–468.

[55] Goodgame R.W., 1996 - "Understanding intestinal spore-forming protozoa: cryptosporidia, microsporidia, isospora, and cyclospora." Ann. Intern. Med. Feb 15; 124 (4): 429-41.

[56] Gookin J. L., S. K., Nordone R. A., Argenzio 2002 **-** Host responses to *Cryptosporidium* infection. J. Vet. Intern. Med. 16:12-21.

[57] Greenberg P.D., Cello J.P., 1997- Treatment of severe diarrhea caused by *Cryptosporidium parvum* with oral bovine immunoglobulin concentrate in patients with AIDS. AIDS Weekly Plus. Jan 20:31.

[58] Griffiths J.K., 1998 - Human cryptosporidiosis: epidemiology, transmission, clinical disease, treatment, and diagnosis. Adv. Parasitol. 40: 38-87.

[59] Harris J.R., Petry F., 1999 - *Cryptosporidium parvum:* Structural components of the oocyst wall. J. Parasitol. 85, 839-349.

[60] Henderson C., 1998 - Role of Receptor Binding Motifs Present in Thrombospondin Related Adhesive Proteins 1 and 2(TRAP-C1 and TRAP- C2) in the invasion Mechanisms of *Cryptosporidium parvum*. AIDS Weekly Plus. Dec 21:NA.

[61] Henriksen S.A., Pohlenz J.F., 1981 - Staining of *Cryptosporidium* by a modified Ziehl- Neelsen technique. Acta. Vet. Scand. 22:594– 596.

[62] Hiavsa M.C., Watson J.C., & Beach M.J. 2005 - Cryptosporidiosis surveillance--United States 1999-2002. MMWR Surveill. Summ. 54(1), 1-8.

[63] Holley H.P., J.R., Dover C., 1985 - Letter to the editor concerning the association between *Cryptosporidium* and *Giardia*. N. Engl. J. Med. 313:1019-1020.

[64] Huang D.B., Chappell C., Okhuysen P.C., 2004 - Cryptosporidiosis in children. Semin. pediatr. Infect. Dis. 15 (4):253-259.

[65] Hunter P.R., Hughes S., Woodhouse S., Syed Q., Verlander N.Q., Chalmers R.M., et al. 2004 - Sporadic cryptosporidiosis case-control study with genotyping. Emerg. Infect. Dis.

[66] Hunter P.R., Nichols G., 2002 - Epidemiology and clinical features of *Cryptosporidium* infection in immunocompromised patients. Clin. Microbiol. Rev. 15, 145–154.

[67] Iqbal J., Hra P.R., Al-Ali F., Philip R., 2001 - Cryptosporidiosis in Kuwaiti children: seasonality and endemicity. Clin. Microbial. Infect. 7: 261-266.

[68] Jenkins M. B., D. D., Bowman W. C., Ghiorse 1998 - Inactivation of *Cryptosporidium parvum* oocysts by ammonia. Appl. Environ. Microbiol**.** 64: 784-788.

[69] Johnston S.P., Ballard M.M., Beach M.J., Causer L., Wilkins P.P., 2003 - Evaluation of three commercial assays for detection of *Giardia* and *Cryptosporidium* organisms in fecal specimens. J. Clin. Microbiol. 41(2): 623 - 626.

[70] Jokipii L., Pohjola S., Jokipii A.M.M., 1985 - Cryptosporidiosis associated with traveling and giardiasis. Gastroenterol. 89:838-842.

[71] Keusch G.T., Hamer D., Joe A., Kelley M., Griffiths J., Ward H., 1995 - "Cryptosporidia--who is at risk?" Schweiz Med Wochenschr, May 6; 125 (18): 899-908.

[72] Korich D.G., Mead J.R., Madore M.S., Sinclair N.A., Sterling C.R., 1990 - Effects of ozone, chlorine dioxide, chlorine and monochloramine on *Cryptosporidium parvum* viability. Appl. Environ. Microbiol. 56:1423–8.

[73] Kvac M., Kvetonova D., Puzova G., Ditrich O., 2003 - Comparison of selected diagnostic methods for identification of *Cryptosporidium parvum* and *Cryptosporidium andersoni* in routine examination of faeces. Med. B.Infect. Dis. Vet. Public. Health. 50(8): 405 - 411.

[74] Laupland K.B., Church D.L., 2005 - Population based laboratory surveillance for *Giardia* sp. and *Cryptosporidium* sp. infections in a large Canadian health region. BMC Infect. Dis. 5:72.

[75] Leach C.T., Koo F.C., Kuhls Th.L., Hilsenbeck S.G., Jenson H.B., 2000 - Prevalence of *Cryptosporidium parvum* infection in children along the Texas Mexico border and associated risk factors. Am. J. Trop. Med. Hyg. 62(5): 656–661.

[76] Leav B.A., Mackay M., Ward H.D., 2003 - *Cryptosporidium* species: new insights and Old challenges. Clin. Infect. Dis. 36(7): 903 - 908.

[77] Leger 1911- Establishes the family Cryptosporidiidae. Arch. Protistenkd 22: 71-88

[78] Le Moing V. F., Bissuel D., Costagliola Z., Eid F., Chapuis J. M., Molina D., Salmon-Ceron P., Brasseur C., Leport 1998 - Decreased prevalence of intestinal cryptosporidiosis in HIV-infected patients concomitant to the widespread use of protease inhibitors. AIDS 12:1395– 1397.

[79] Levine N.D., 1973 - Introduction, history and taxonomy. In: The Coccidia (Hammond D.M., Long, P.L., editors), University Park Press, Baltimore, Maryland, USA. pp. 1-22.

[80] Levine N.D., 1985 - Phylum II. Apicomplexa **Levine 1970 -** in Illustrated Guide to the Protozoa, Lee J.J., Hutner S.H., Bovee E.C., Eds., Society Protozool. Lawrence, Kansas, 322–374.

[81] Levine N. D., 1984 - Taxonomy and review of the coccidian genus *Cryptosporidium* (protozoa, apicomplexa). J. Protozool. 31:94-98.

[82] Lorntz B., Soares A.M., Moore S.R., Pinkerton R., Gansneder B., Bovbjerg V.E., Guyatt H., Lima A.M., & Guerrant R.L., 2006 - Early childhood diarrhea predicts impaired school performance. Pediatr. Infect. Dis. J. 25(6), 513-20.

[83] Ma P., Soave R., 1983 - Three-step stool examination for cryptosporidiosis in 10 homosexual men with protracted watery diarrhea J. Infect. Dis. 147, 824–828.

[84] Mackenzie W.R., Schell W. L., Blair K.A., et al., 1995 - Massive outbreak of waterborne *Cryptosporidium* infection in Milwaukee, Wisconsin: recurrence of illness and risk of secondary transmission. Clin. Infect. Dis. 21: 57–62.

[85] Maggi P., A. M., Larocca M., Quarto G., Serio, O., Brandonisio G., Angering G., Pastore 2000 - Effect of antiretroviral therapy on cryptosporidiosis and microsporidiosis in patients infected with human immunodeficiency virus type 1. Eur. J. Clin. Microbiol. Infect. Dis. 19**:**213–217.

[86] Mahdi N. K., Al-Sadoon I.. A., Mohamed A. T., 1996 - First report of cryptosporidiosis among Iraqi children. Med.J. of Basra Univer. 2:115-120.

[87] Mahgoub E.S., Almahbashi A., Abdulatif B., 2004 - Cryptosporidiosis in children in a north Jordanian paediatric hospital. East Mediator Health J. 10(4-5): 494-501.

[88] Marshall M.M., Naumovitz D., Ortega Y., Sterling C.R., 1997 - Waterborne protozoan pathogens. Clin. Microbiol. Rev. 10 (1): 67 - 85.

[89] Mehta P. L., 2002 - laboratory diagnosis of cryptosporidiosis. J Postgrad Med. 48:217.

[90] Mercado R., Buck G.A., Manqué P.A., Ozaki L.Sh., 2007 - *Cryptosporidium hominis* infection of the human respiratory tract. Emerg.Infect. Dis., 13: 462- 464.

[91] Michel M. Y., Khalifa, A. M., Ibrahim, I. R., 2000 - Detection of the *Cryptosporidium parvum* antigen by co-agglutination test and ELISA. Eastern Mediterranean Health Journal. 6(5): 898-907.

[92] Miron D., Colodner R., Kenes Y., 2000 - Age-related seroprevalence of *Cryptosporidium* in northern Israel. Isr. Med. Assoc. J. 2(5): 343-5.

[93] Molbak K., et al., 1992 - Persistent and acute diarrhoea as the leading causes of child mortality in urban Guinea-Bissau. Trans. Roy. Soc. Trop. Med. and Hyg. 86:216–20.

[94] Moodley D., Jackson T. F., Gathiram V., van den Ende J., 1991 - A comparative assessment of commonly employed procedures for the diagnosis of cryptosporidiosis. S*.* Afr*.* Med*.* J.

79, 314-317.

[95] Morgan-Ryan U. M., A., Fall L. A., Ward, N., Hijjawi I., Sulaiman R., Fayer R. C., Thompson M., Olson A., Lal L., Xiao – 2002 –*Cryptosporidium hominis* n. sp. (Apicomplexa: Cryptosporidiidae) from Homo sapiens. J. Eukaryot. Microbiol. 49:433-440.

[96] Morgan U. M., L., Pallant B. W., Dwyer D. A., Forbes G., Rich R. C. A., Thompson 1998 **-** Comparison of PCR and microscopy for detection of *Cryptosporidium parvum* in human fecal specimens: clinical trial. J. Clin. Microbiol. 36**:**995-998.

[97] Nahrevanian H., Assmar M., Samin M.Gh., 2007 - Cryptosporidiosis among immunocompetent patients gastroenteritis in Iran: a comparison with other enteropathogenic parasites. J. Microbiol. Immunol. Infect. 40:154-156.

[98] Nanduri J., Williams S., Aji T., Flanigan T.P., 1999 - Characterization of an immunogenic glycocalyx on the surfaces of *Cryptosporidium parvum* oocysts and sporozoites. Infect. Immun. 67, 2022-2024.

[99] Naumova E.N., Christodouleas J., Hunter P.R., Syed Q., 2005 - Effect of precipitation on seasonal variability in cryptosporidiosis recorded by the North West England surveillance system in 1990–1999. J. Wat. Health. 3, 185–196.

[100] Nchito M., Kelly P., Sianongo S., Luo N.P., Feldman R., Farthing M., Baboo K.S., 1998 - Cryptosporidiosis in urban Zambian children and analysis of risk factors. Am. J. Trop. Med. Hyg. 59(3): 435-437.

[101] Newman R.D., Jaeger K.L., Wuhib T., Lima A.A., Guerrant R.L., Sears C.L., 1993 - Evaluation of an antigen capture enzyme-linked immunosorbent assay for detection of *Cryptosporidium* oocysts. J. Clin. Microbiol. 31(8):2080-2084.

[102] Nichols G., 1992 – The Biology, Epidemiology and Typing of *Cryptosporidium* spp. Ph.D. thesis. Surrey University.

[103] Nichols G., 2003 - Using existing surveillance based data. In Hunter P.R., Wait M., Ronchi E., Eds., Drinking Water and Infectious Disease - Establishing the Links. CRC Press, Boca Raton, FL, pp. 131–141.

[104] O'Donoghue P.J., 1995 - *Cryptosporidium* and cryptosporidiosis in man and animals. Int. J. Parasitol. 25(2): 139 - 195.

[105] Peng M.M., Xiao L., Freeman A.R., et al., 1997 - Genetic polymorphism among *Cryptosporidium parvum* isolates: evidence of two distinct human transmission cycles. Emerg. Infect. Dis. 3: 567–73.

[106] Pereira M.D., Atwill E.R., Barbosa A.P., Silva S.A., García-Zapata M.T., 2002-

Am. J. Trop. Med. Hyg. 66(6):787-93.

[107] Piper M.B., Bankier A.T., Dear P.H .A., 1998 - Happy Map of *Cryptosporidium Parvum*. Genome Research. 8 (12): 1299 – 1307.

[108] Pohjola S., Jokipii L., Jokipii A. M. M., 1984 - Dimethylsulphoxide-Ziehl-Neelsen staining technique for the detection of cryptosporidial oocysts. Vet. Rec. 115, 442–443.

[109] Pollock K.G.J., Young D., Smith H.V., Ramsay C.N., 2008 - Cryptosporidiosis and filtration of water from Loch Lomond, Scotland. Emerg. Infect. Dis. 14:115-119.

[110] Quintero-Betancourt W., Peele E.R., Rose J.B., 2002 - *Cryptosporidium parvum* and *Cyclospora cayetanensis:* a review of laboratory methods for detection of these waterborne parasites. J. Microbiol. Methods 49:209–224.

[111] Ramirez N.E., Ward L.A., & Sreevatsan S., 2004 - A review of the biology and epidemiology of cryptosporidiosis in humans and animals. Microbes Infect. 6(8), 773-5.

[112] Reduker D.W., Speer C.A., Blixt J.A., 1985a - Ultrastructure of *Cryptosporidium parvum* oocysts and excysting sporozoites as revealed by high resolution scanning electron microscopy. J. Protozool. 32(4): 708-711.

[113] Reducker D.W., Speer C.A., Blixt J.A., 1985b - Ultrastructural changes in the oocyst wall during excystation of *Cryptosporidium parvum* Apicomplexa: Eucoccidiorida). Can. J. Zool. 63, 1892-1 896.

[114] Riggs M. W., Stone A. L., Yount P. A., Langer R. C., Arrowood M. J., Bentley D. L., 1997 - Protective monoclonal antibody defines a circumsporozoite-like glycoprotein exoantigen of *Cryptosporidium parvum* sporozoites and merozoites. J. Immunol. 158:1787–1795.

[115] Rivera W.L., Yason J.A.D.L., Rivera P.T., 2005 - Serological detection of cryptosporidiosis among Filipino cancer patients. Parasitol. Res. 98: 75-76.

[116]Robertson B., Sinclair M.I., Forbes A.B., Veitch M., Kirk M., Cunliffe D., et al., 2002 - Case-control studies of sporadic cryptosporidiosis in Melbourne and Adelaide, Australia. Epidemiol. Infect. 128:419–31.

[117] Robertson L.J., Campbell A.T., Smith H.V., 1993a - In vitro excystation of *Cryptosporidium parvum.* Parasitol. 106, 13-29.

[118] Robertson L.J., Gjerde B., 2001b - Occurrence of parasites on fruits and vegetables in Norway. J. Food *Prot*. 64, 1793–1798.

[119] Robertson L.J., Greig J.D., Gjerde B., Fazil A., 2005 - The potential for acquiring cryptosporidiosis or giardiosis from consumption of mung bean sprouts in Norway, a preliminary step-wise risk assessment. Int. J. Food Microbiol. 98, 291–300.

[120] Rose J.B., Huffman D.E., Gennaccaro A., 2002 - Risk and control of waterborne cryptosporidiosis. FEMS Microbiol. Rev. 26:.113–23.

[121] Sallon S., el Showwa R., el Masri M., Khalil M., Blundell N., Hart C.A., 1990 - Cryptosporidiosis in children in Gaza. Ann Trop Paediatr 11: 277–281.

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

[123] Samie A., Bessong P.O., Obi C.L., Sevilleja J.E., Stroup S., Houpt E., Guerrant R.L., 2006 – *Cryptosporidium* species: preliminary descriptions of the prevalence and genotype distribution among school children and hospital patients in the Venda region, Limpopo Province, South Africa. Exp. Parasitol. 114(4):314-22.

[124] Sastry N., Burgard S., 2005 - The prevalence of diarrheal disease among Brazilian children: trends and differentials from 1986 to 1996. Soc. Sci. Med. 923-35.

[125] Semenza J.C., Nichols G., 2007 - Cryptosporidiosis surveillance and water-borne outbreaks in Europe. Euro. Surveill. 12(5):E13-4.

[126] Sevinc F., Irmak K., Sevinc M., 2003 - The prevalence of *Cryptosporidium parvum* infection in the diarrhoiec and non- diarrheic calves. Revue. Méd. Vét. 154**:** 357-361.

[127] Smith T. G., Walliker D., Lisa C., Ranford-Cartwright L.C., 2002 - "Sexual Differentiation and Sex Determination in the Apicomplexa." Trends in Parasitol. 18(7):315-323.

[128] Soave R., Ruiz J., Garcia-Saucedo V., Garrocho C., Kean B.H., 1989 - Cryptosporidiosis in a rural community in central Mexico [letter]. J. Infect. Dis. 159*:* 1160–1162.

[129] Spano F., Crisanti A., 2000 - *Cryptosporidium parvum*: the many secrets of a small genome. Int. J. Parasitol. 30(4): 553 - 565.

[130] Stockman M., M., Fromm H., Schmitz W., Schmidt E. O., Riecken J. D., Schulzke 1998 - Duodenal biopsies of HIV-infected patients with diarrhea exhibit epithelial barrier defects but no active secretion. AIDS 12**:**43-51.

[131] Strickland G.T., 2000 - Hunter's tropical medicine and emerging infectious disease. 8th edition. The W.B. Saunders company.

[132] Sturdee A.P., Chalmers R.M., Bull S.A., 1999 - Detection of *Cryptosporidium* oocysts in wild mammals of mainland Britain. Vet. Parasitol. 80**:** 273-280.

[133] Sulaiman I.M., Hira P.R., Zhou I., Al-Ali F.M., Shelahi F.A., Shweiki H.M., Iqbal J., et al., 2005 - Unique endemicity of cryptosporidiosis in children in Kuwait. J. Clin. Microbiol. 43: 2805-9.

[134] Sunnotel O., Lowery C.J., Moore J.E., Dooley J.S.G., Xiao L., Millar B.C., Rooney P.J., Snelling W.J., 2006 - *Cryptosporidium*. Lett. Appl. Microbiol. 1:7-16

[135] Sutthikornchai C., Jantanavivat C., Thongrungkiat S., Harnroongroj T., Sukthana Y. 2005 - Protozoal contamination of water used in Thai frozen food industry. SE Asian J. Trop. Med. Pub. Health. 36 (Suppl. 4), 41–45.

[136] Tetley L., Brown S.M.A., McDonald V., Coombs G.H., 1998 - Ultrastructural analysis of the sporozoite of *Cryptosporidium parvum.* Microbiol. 144(Pt 12): 3249 - 3255.

[137] Thurston-Enriquez J.A., Watt P., Dowd S.E., Enriquez R., Pepper I.L., Gerba C.P., 2002 - Detection of protozoan parasites and microsporidia in irrigation waters used for crop production. J. Food Prot. 65, 378–382.

[138] Tumwine J.K., Kekitiinwa A., Bakeera-Kitaka S., Ndeezi G., Downing R., Feng X., Akiyoshi D.E., Tzipori S., 2005 - Cryptosporidiosis and microsporidiosis in Ugandan children with persistent diarrhea with and without concurrent infection with the human immunodeficiency virus. Am. J. Trop. Med. Hyg. 73: 921–925.

[139] Tumwine J.K., Kikitllnwa A., Nabukeera N., Akiyoshi D.E., Rich S.M., Widmer G., and Feng X., Tzipori S., 2003 - *Cryptosporidium parvum* in children with diarrhea in Mileage hospital Kampala, Uganda. Am. J. Trop. Med. Hyg. 68(6): 710-715.

[140] Tyzzer E.E. A., 1910 - extracellular coccidian, *Cryptosporidium muris* (Gen. et., Sp. Nov.)of the gastric glands of the common mouse. J. Med. Res. 23: 487-509.

[141] Tzipori S., 1998 - Cryptosporidiosis: laboratory investigations and chemotherapy. Adv. Parasitol. 40**:**187–221.

[142] Upton S. J., 2000 - Suborder Eimeriorina Léger, 1911, In J. J. Lee, G. F. Leedale, and P. Bradbury (ed.), the illustrated guide to the protozoa, 2nd ed. Soc. Protozool. Allen Press, Lawrence, Kans. p. 318-339.

[143] Vetterling J. M., H. R., Jervis T. G., Merrill H., Sprinz. 1971 - *Cryptosporidium wrairi* sp. n. from the guinea pig Cavia porcellus, with an emendation of the genus. J. Protozool. 18:243-247.

[144] Ward H., Cevallos A.M., 1998 - *Cryptosporidium:* molecular basis of host parasite interactions. Adv. Parasitol. 40, 151-178.

[145] Weir S. C., N. J., Pokorny, R. A., Carreno J. T., Trevors H., Lee 2002 - Efficacy of common laboratory disinfectants on the infectivity of *Cryptosporidium parvum* oocysts in cell culture. Appl. Environ. Microbiol. 68**:** 2576-2579.

[146] Wetzel R. 1938 - Ein neues Coccid (*Cryptosporidium vulpis*. sp. Nov.) aus dem Rotfuchs. Arch. Wissenschaft. Prak. Tier. 74:39-40.

[147] White D.C., Alugupalli S., Schrum D.P., Kelly S.T., Sikka M.K. Fayer R., Kaneshiro E.S., 1997 - Sensitive quantitative detection / identification of infectious *Cryptosporidium parvum* oocysts by signature lipid biomarker analysis. In: International Symposium on Waterborne *Cryptosporidium* Proceedings, Newport Beach, California. (Fricker, C.R., Clancy, J.C. and Rochelle, P.A., editors) American Water Works Association, Colorado, USA. pp. 53-60.

[148] Whiteside M.E., Barkin J.S., May R.G., et al., 1984 - Enteric coccidiosis among patients with the acquired immunodeficiency syndrome. Am. J. Trop. Med. Hyg. 33:1065-1072.

[149] WHO (1994): Bench Aids for the diagnosis of intestinal parasites, WHO, Geneva.

[150] www.dpd.cdc.gov/dpdx/HTML/ Cryptosporidiosis.htm

[151] Xiao L., Fayer R., Ryan U., Ubton S., 2004 - *Cryptosporidium* taxonomy: recent advances and implications for public health. Clin. Microbiol. Rev. 17(1):72-97. **Tyzzer, E. 1907 -** A sporozoon found in the peptic glands of the common mouse. Proc. Soc. Exp. Biol. Med. 5:12-13. **Tyzzer E.E., 1912 -** *Cryptosporidium parvum* (sp. Nov.), a coccidian found in the small intestine of the common mouse. Arch. Protistenkd 26:394-412.

[152] Yoder J.S., Beach M.J., 2007 - Cryptosporidiosis surveillance--United States, 2003-2005. MMWR Surveill. Summ. 7; 56(7):1-10.

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

[154] Zhu G., M. J., Marchewka J. G., Ennis J. S., Keithly 1998 - Direct isolation of DNA from patient stools for polymerase chain reaction detection of *Cryptosporidium parvum*. J. Infect. Dis. 177:1443-1446

Appendix (1)

التاريخ:2008 and 2007 / /

استبانة عن طفيل *Cyptosporidium*

رقم الاستبانة ☐

1. الاسم.....

2.الجنس **ذكـــر** ☐ **أنثـى** ☐

3. العمر بالسنوات أو بالأشهر.....

4. منطقة السكن **مدينة** ☐ **معسكر** ☐ **قرية** ☐

5. مهنة الأب.....

6. المستوى التعليمي للأب **أساسي** ☐ **ثانوي** ☐ **جامعي** ☐

7. مهنة الأم.....

8. المستوى التعليمي للأم **أساسي** ☐ **ثانوي** ☐ **جامعي** ☐

أعـــراض طـبية

9. وجود إسهال **نعم** ☐ **لا** ☐

10. وجود جفاف **نعم** ☐ **لا** ☐

11. وجود حمى **نعم** ☐ **لا** ☐

12. وجود غثيان **نعم** ☐ **لا** ☐

13. وجود تقيؤ **نعم** ☐ **لا** ☐

14. وجود ألم في البطن **نعم** ☐ **لا** ☐

15. مشاكل في الجهاز التنفسي **نعم** ☐ **لا** ☐

الصرف الصحي والصحة العامة

16. مصدر مياه الشرب **فلتر** ☐ **حنفية** ☐

17. نوع الرضاعة للأطفال **طبيعية** ☐ **صناعية** ☐ **كلاهما** ☐

18. هل يتناول الطفل الخضروات نعم □ لا □

19. هل يتناول الطفل شرائح الجبنة البيضاء نعم □ لا □

20. ما نوع الصرف الصحي في المنزل مجاري مغلقة □ مجاري مفتوحة □ براميل □

21. هل يوجد قمامة حول المنزل نعم □ لا □

22. وجود أراضي زراعية حولكم نعم □ لا □

23. ري الأراضي الزراعية على مياه الأمطار نعم □ لا □

24. ري الأراضي الزراعية على مياه البرك نعم □ لا □

25. ري الأراضي الزراعية على مياه المجاري نعم □ لا □

26. هل يوجد حيوانات في المنزل أو قريب منه نعم □ لا □

27. هل أصيب الطفل بديدان نعم □ لا □

28. نوع الديدان أنتميبا □ جارديا □ إسكارس □ دبوسيه □ غير ذلك □

29. هل تلقى العلاج اللازم نعم □ لا □

Appendix (2)

Palestinian National Authority
Ministry of Health
Helsinki Committee

السلطة الوطنية الفلسطينية
وزارة الصحة
لجنة هلسنكي

Date: 30/4/2007

التاريخ: 2007/4/30

Name: Ahmed Tabash

الاسم: أحمد طبش

I would like to inform you that the committee has discussed your application about:

نفيدكم علماً بأن اللجنة قد ناقشت مقترح دراستكم حول:-

Epidemiology of cryptosporidiosis infection among individuals from Gaza strip.

In its meeting on April 2007

و ذلك في جلستها المنعقدة لشهر ابريل 2007

and decided the Following:-

و قد قررت ما يلي:-

To approve the above mention research study.

الموافقة على البحث المذكور عاليه.

Signature
توقيع

Ministry of Health
Helsinki Committee

Member
عضو

Member
عضو

Chairperson

Conditions:-

- Valid for 2 years from the date of approval to start.
- It is necessary to notify the committee in any change in the admitted study protocol.
- The committee appreciate receiving one copy of your final research when it is completed.

Gaza Etwam – Telefax 972-7-2878166

Appendix (3)

Materials

Apparatus

- Light microscope, ELISA, vortex mixer, centrifuge and refrigerator

Equipments

- Slides, glass tube**,** wood sticks, gauze pads, conical tubes, gloves, sterile disposable plastic container**,** labeled**,** cotton, plastic droppers, beads, permanent marker and plastic funnels**.**

Reagent and stain

- Sodium acetate acetic acid formaldehyde (SAF) (10%), ether**,** ethyl alcohol (70%), modified acid-Fast staining kit, ELISA kit and immersion oil.

Appendix (4): The examination of C*ryptosporidium* by modified acid fast stain

Cryptosporidium	Number	%
with *Cryptosporidium*	54	(18)
without *Cryptosporidium*	246	(82)
Total	300	100.0

Appendix (5): The result of C*ryptosporidium* staining with relation to sex among the children

Sex	The sex of the children				P value
	Males		Females		
	No.	%	No.	%	
Infected	15	(9.4)	39	(27.7)	0.001
Not infected	144	(90.6)	102	(72.3)	
Total	159	100.0	141	100.0	

Appendix (6): Distribution of stool sample with *Cryptosporidium* infection

Seasonal variation	Positive for *Cryptosporidium*		Negative for *Cryptosporidium*		Total	P value
	No.	%	No.	%		
June n=(53)	5	(9.4)	44	(89.8)	49	0.4
July n=(50)	5	(14)	45	(90)	50	
Aug n=(45)	13	(24.4)	38	(74.5)	51	
Jan n=(53)	11	(20.8)	39	(78)	50	
Feb n=(49)	10	(20.4)	40	(80)	50	
March n=(50)	10	(20)	40	(80)	50	

Appendix (7): Distribution of *Cryptosporidium* due to sewage and garbage prevalence

Parameter	With *Cryptosporidium*		Without *Cryptosporidium*		P Value
	No.	%	No.	%	
Closed sewage(n=200)	15	(7.5)	185	(92.5)	
Opened sewage(n=65)	37	(56.9)	28	(43.1)	
Tank septic n= 35	2	(5.7)	33	(94.3)	0.001
(Prevalence of garbage)					
There is garbage n=(182)	35	(19.2)	147	(80.8)	
There is no garbage n=(118)	19	(16.1)	99	(83.9)	0.5

Appendix (8): Distribution of *Cryptosporidium* due to irrigation of lands

Irrigation of lands with pool	With *Cryptosporidium*		Without *Cryptosporidium*		P Value
	No.	%	No.	%	
Irrigation of lands with pool n=(50)	14	(28)	72	(36)	0.044
Irrigation of lands without pool n=(250)	40	(16)	210	(84)	

Appendix (9): Distribution of *Cryptosporidium* due to breeding animals

Breeding animals	With *Cryptosporidium*		Without *Cryptosporidium*		P Value
	No.	%	No.	%	
There is breeding animals n=(114)	26	(22.8)	88	(77.2)	0.09
There is no breeding animals n= (186)	28	(15.1)	158	(84.9)	