

## Identification of *Cryptosporidium* Antigens in Stool Specimen Using Enzyme Linked Immunosorbent Assay (ELISA) in Al-Diwanyia Province- Iraq

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### الخلاصة

تم جمع 156 عينة براز من المرضى الذين يعانون من الاسهال، تم تصنيف العينات حسب منطقته السكن (الريف والمدينة) ، الجنس، العمر، والحالة المناعية للمرضى (الحمل، مرض السكري، المعالجون بالمثبطات المناعية). اجري الفحص الروتيني لكل العينات باستخدام الصبغة المقاومة للحمض كما وقد اجري عليها فحص المقايسة المناعية المرتبطة بالأنزيم للكشف عن المستضدات بعينات البراز. اظهرت تقنية الصبغة المقاومة للحمض ان نسبة العينات الموجبة المتمثلة بوجود كيس البيض لطفيلي البوغى الخبئ كانت 14.7% بينما كانت نتيجة فحص المقايسة المناعية المرتبطة بالأنزيم (52.5%) وكانت نسبة الاصابة بالمناطق الريفية اعلى من المدينة و نسبة الاصابة في الذكور (18.7%) كانت اعلى من الاناث، بينما اظهرت النتائج ان اقل نسبه اصابة (5.2%) كانت بالفئة العمرية 16-20 سنة واعلى نسبة اصابة (29.4%) بالفئة العمرية لأقل من خمسة سنوات. اظهرت الحالة المناعية للمصابين (الحمل، مرض السكري، المعالجون بالمثبطات المناعية) دور هام في نسبة انتشار الطفيلي وكانت النتائج (17.6%، 29.8%، 27.6%) على التوالي. باعتماد هذه النتائج تكون مستوى الحساسية والخصوصية لفحص المقايسة المناعية المرتبطة بالأنزيم لطفيلي البوغى الخبئ هي 95% و 100% على التوالي. استنتجت الدراسة ان تشخيص الطفيلي بطريقة المقايسة المناعية المرتبطة بالأنزيم هي عالية الحساسية والخصوصية بالمقارنة مع بتقنية الصبغة المقاومة للحمض.

### Abstract

One hundred fifty six of stool samples were collected from patients suffering form diarrhea; the samples were classified according to the rural and urban areas, gender, age and immune status (pregnancy, diabetes, and corticosteroid therapy).

Routine parasitological examinations were done to all samples using acid-fast stain technique. Antigens detection method was also done by using ELISA.

The acid fast technique results showed that 23 /156 (14.7%) were positive cases for identify *Cryptosporidium* oocyte in stool specimen, while the ELISA showed 82/156 (52.5%) were positive cases for identify *Cryptosporidium* antigen in stool specimen.

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The rural areas showed higher positive cases(17.5%) than urban areas and males' positive cases were higher results ( 18.7%) than females, while the results showed that lowest positive case were in age group 16-20 years (5.2%) and the highest case were in age group less than 5years (29.4%).

Immune status (pregnancy, diabetes, and corticosteroid therapy) of patient showed an important role in the cases disruptions of the infection with *Cryptosporidium* spp.(17.6%, 29.8 % 27.6 %) respectively.

The sensitivity, specificity of ELISA for identification *Cryptosporidium* antigens in stool specimen in comparison with the modified acid-fast staining method were determined 95%, 100% respectively, so it can be concluded that acid-fast technique which can be used as a referential diagnostic method has some limitations while the ELISA is more sensitive and specific for identification of the parasite antigens in stool specimens.

## Introduction

*Cryptosporidium* spp. is a coccidian parasite infecting mammals, birds, reptiles, and fish (1). It is known to spread through fecal-oral routes and it may cause life threatening diarrhea in immunocompromised patients, while in immunocompetent persons, *Cryptosporidium* spp. causes short-term diarrhea (2). The *Cryptosporidium* is not new species, but strong evidence suggests its emergence as an important community distributed protozoan, since this parasite has been frequently detected in untreated surface water, as well as in swimming and lakes, day-care centers, and hospitals (3). Furthermore, this coccidian represents the leading cause of persistent diarrhea in HIV-infected individuals in developing countries, which additionally contributes for community dissemination (4).

The diagnosis of the etiological agent of diarrhea can be performed in the laboratory only, because clinical signs do not enable one to differentiate between the different microorganisms, the acid-fast technique method for diagnosis cryptosporidiosis is now accepted as a golden standard test (5). However, it is a time-consuming, requires expertise, unpractical and may prove inadequate in diagnosis of a small number of parasites (6). These conventional techniques can be replaced by ELISA for its simplicity and the limited laboratory tools requirements and also the use of immunological methods has increased recently, the ELISA technique is rapid and reliable and particularly suited to the analysis of large numbers of samples (7).

The aims of this study were

- 1- Evaluate the sensitivity and specificity of ELSIA method for identification of *Cryptosporidium* antigens in stool samples in comparison with acid fast stain.
- 2- Detection the infectious rate of cryptosporidiosis in the rural and urban area.

- 3- Compare cryptosporidiosis infection in male and female.
- 4- Discover the relationship of some immunological factor in cryptosporidiosis infection.

### Materials and methods

One hundred fifty six of stool samples were collected from patients suffering from diarrhea who attended to the Maternity and Childhood Teaching Hospital and Al-Dewania Teaching Hospital in Al-Dewania governorate from the first of March till the end of October 2012.

The samples were classified according to the rural and urban area, gender, age of patients, immune status (pregnancy, diabetes, corticosteroid therapy).

A routine parasitological examination were done to all samples using acid-fast technique (5) after being concentrated with modified formalin ethyl acetate procedure (8), then examined under light microscope (100x).

Antigen Detection Methods using ELISA was done according to the kit instructions of (Diag. Auto. INC, USA).

Statistical analyses were computer assisted using SPSS version 13 (Statistical Package for Social Sciences). Frequency distribution for selected variables was done first.

A chi-square test was performed for the correlation between the cases and parasite determination. A t-test was performed for the mean ages. In comparing the ELISA methods with acid-fast technique, sensitivity and specificity were taken into consideration. Sensitivity and Specificity were done according to the following formula (9).

Sensitivity % = Number of true positive/ Number of diseased people  $\times$  100%

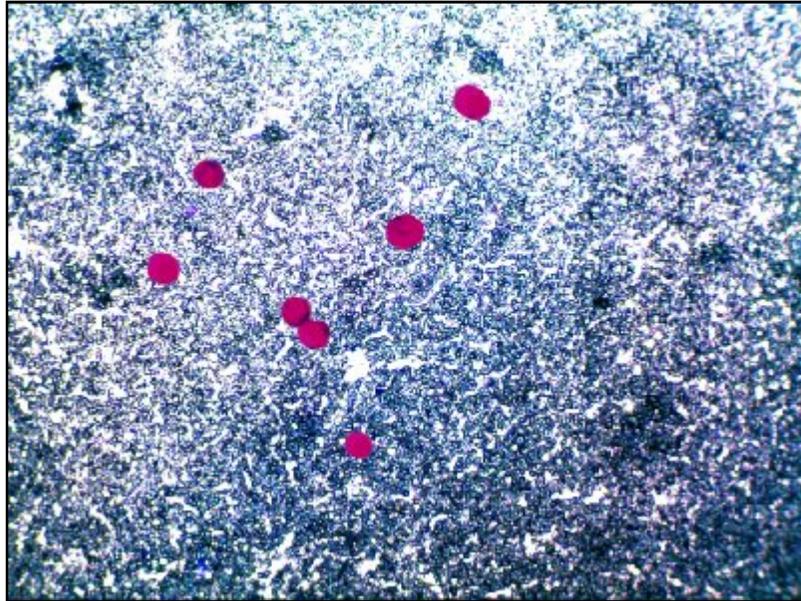
Specificity% = Number of true negative / Number of non- diseased people  $\times$  100%

### Results

In the present study, the acid fast technique and microscopic results showed that *Cryptosporidium* spp. was positive in 23out of 156 (14.7%) cases while the ELISA showed 82out of 156 (52.5%) positive cases (Table 1and Fig. 1).

**Table1: The percents of acid fast stain technique and ELISA positive cases in patients suffering from diarrhea (No. =156).**

Type of test	Positive cases	Percentage
Acid fast stain technique	23	14.7%
ELISA	82	52.5%



**Figure 1: *Cryptosporidium* oocysts in stool stained with acid fast stain technique**

According to the rural and urban areas, the acid fast stain technique showed that 7 out of 65 positive cases in urban areas and 16 out of 91 positive cases in rural area, while the ELISA showed that 21 out of 65 positive cases in rural areas and 61 out of 91 positive cases in urban areas (Table 2).

**Table 2: Distribution of acid fast stain technique and ELISA positive cases based on rural and urban areas.**

Type of area	Total of samples	Acid fast Positive cases %	ELISA Positive cases %
Urban	65	7 (10.7%)	21 (32.3%)
Rural	91	16 (17.5%)	61 (67.03%)
Total	156	23 (14.7%)	82 (52.5%)

According to the gender distribution the acid fast stain technique results showed that 15 out of 80 (18.7%) were positive case in males and 8 out of 76 (10.5%) were positive case in females while the ELISA results showed 55 out of 80 (68.7%) were positive case in males and 27 out of 76 (35.5%) positive case in females (Table 3).

**Table 3: Distribution of acid fast stain technique and ELISA to positive cases based on gender.**

Gender	No. of samples	Acid fast (%) Positive cases	ELISA (%) Positive cases
Males	80	15 (18.7 %)	55 (68.7%)
Females	76	8 (10.5 %)	27 (35.5%)
Total	156	23(14.7%)	82(52.5%)

The age play an important role in the cases distribution, the acid fast stain technique results showed that lowest positive case in (10-15, 16-20 and 21-25) years were (5.8%,5.2% and 6.6%) respectively, while the highest case were in age group less than 5 and more than 30 years (29.4% and 24%) also the ELISA results showed that the lowest positive case were same age groups (52%, 36% and 53.3%) and highest positively were recorded in the same age groups (60% and 58.8%)respectively (Table 4).

**Table 4: Distribution of acid fast stain technique and ELISA in positive cases based on age groups.**

Age group(year)	Total of samples	Acid fast Positive cases %	ELISA Positive cases %
5 □	25	10 (29.4 %)	15 (60 %)
5-9	24	2 (8.3 %)	14 (58.3 %)
10-15	17	1 (5.8 %)	9 (52.9 %)
16-20	19	1 (5.2 %)	7 (36.8 %)
21-25	15	1 (6.6 %)	8 (53.3 %)
26-30	22	2 (9 %)	9 (40.9 %)
30 >	34	6(24%)	20 (58.8 %)
Total	156	23(17.7)	82 (52.5%)

The distribution of infection according to the some condition affecting on immune state the acid fast stain technique showed that pregnant women, diabetes patients and corticosteroids therapy were 17.6%, 29.8%, 27.6% positive respectively; while ELISA results showed that pregnant women, diabetes patients and corticosteroids therapy were 55.8 %, 61.4 %, 90.7 % positive respectively (Table 5).

**Table 5: Distribution of infection with *Cryptosporidium* based on immune status using acid fast stain and ELISA technique; some patients had more than one complaint.**

condition	Total of cases	Acid fast stain Positive cases %	ELISA Positive cases %
Pregnant women	34	6 (17.6 %)	19 (55.8 %)
Diabetes	57	17 (29.8 %)	35 (61.4 %)
Corticosteroids therapy	65	18 (27.6%)	59 (90.7%)

During this study, 23 out of 156 stool specimens were positive by the modified acid-fast staining technique, and 22 out of these 23 were positive by ELISA, while 74 out of 156 stool samples were negative by ELISA, although all of these samples gave negative results by modified acid-fast staining technique. According to these findings; sensitivity, specificity of ELISA for the diagnosis *Cryptosporidium* oocyst and compatibility with the modified acid-fast staining method were found 95%, 100% (Table 6).

**Table 6: The sensitivity and specificity of the Diagnostic automation INC. ELISA kit.**

	Acid fast stain technique +	Acid fast stain technique -
ELISA +	22	0
ELISA -	1	74

Sensitivity  $22/23 = 95\%$

Specificity  $74/74 = 100\%$

**Discussion**

*Cryptosporidium* spp., accompanied by diarrhea, is more frequently seen in bad hygiene, malnutrition cases, among the old and child category, and in immunocompromised patients (10). Waterborne *Cryptosporidium* outbreak are seen commonly in recent years, and revealed a serious public health problem (11). The identification of *Cryptosporidium* spp. is important to reveal the epidemiology of *Cryptosporidium* infections in humans and animals (3).

Acid fast staining is able to detect *Cryptosporidium* spp. oocytes with agreeable sensitivity and it was accepted as a golden standard test (5). ELISA for the detection of *Cryptosporidium* spp. antigens in stool samples have recently been developed in several laboratories and some are now commercially available to calculate its helpfulness in clinical and epidemiological locations (12,13, 14 and 15).

In the present study the percent of acid fast stain technique in patients suffering from diarrhea who attended to the Maternity and Childhood Teaching Hospital and Al-Dewanyia Teaching Hospital in Al-Dewanyia province was 14.7%. This finding is reach agreement with the results obtained in Los Angeles (16) who reported 14%, also a previous epidemiological study reveled the infectious rate of cryptosporidiosis was 15% (17), while a previous surveys studies carried out in numerous parts of the world revealed that the prevalence rate of cryptosporidiosis was anywhere from 3-50% (18 & 19). Also past a number of studies showed that the prevalence of cryptosporidiosis was in the range of 15% or below (20).

In the present study, cryptosporidiosis rate showed a statistically differences between the rural and urban center. *Cryptosporidium* spp. infection was seen in rural population higher than urban area (17.5%). Thus, returns of living in the rural could have been related by such factors as problems of drainage, lack of enough clean water sources, fecal droppings from both animals and humans are found in most places and unselective defecation. The rural population's also shows that person-to-person transfer through food or water was high and this confirms that there is a high level of contamination by human feces. This result similar with previous study that carries out the percent rate of Cryptosporidiosis in rural area was 20 % (21). Also a prior study revealed that the rural area was more infected with *Cryptosporidium* spp. 55% (17).

In relation to gender, the present study showed the males were higher percent than females in cryptosporidiosis rate, this is may be due to the male in Iraqi population were more frequenter to restaurant and coffee shop and thus make them more deal with the contaminated water, vegetable and contaminated instrument, this result were nearly similar to previous study in Brazil revealed that 9.33% of samples were positive for *Cryptosporidium*, with higher frequency of cases in male patients (22).

Cryptosporidiosis is accompanying with age and immunity, in this study, the common of the positive cases was frequently seen in child less than 5 years. The occurrence of high infection rates in this category may be attributed to the immature immunity, most of them contacted with soil and playing with contaminated materials and also with a bad hygiene , this finding was conformed with other study who revealed that the child were more susceptible than other age group in *Cryptosporidium* infection(23) .

*Cryptosporidium* spp. associated with immunocompromised, can be life threatening in immunocompromised patients. In current study was more often seen in those with corticosteroid therapy, diabetes cases and pregnant women, this result was semi similar to the anther study in Brazil (22).

Indeed, *Cryptosporidium parvum* infection was similar to previous reports (17, 23). However, a previously reported result in Brazil, showed higher rates

(7%) of *Cryptosporidium* in patients with acquired immunodeficiency syndrome (22).

The present study was evaluated the ELISA kits (Diagnostic Automation INC, USA) comparing with acid fast stain technique in stool samples, the ELISA showed markedly high sensitivity and specificity (95 % & 100%) respectively. The technical properties of an ELISA mean that many specimens can be processed and read by a single technician in a short period of time, by this means maintaining a level standard. Therefore the ELISA is potentially more suitable than microscopy in certain settings, especially in epidemiological surveys and in follow up examinations of patients known to be *Cryptosporidium* positive. Thus, the *Cryptosporidium* spp. ELISA appears to be more sensitive and specific than the acid-fast stain technique. These findings support the reports of others using this technique (24 and 25).

It was recommended that efforts should be made to routinely search for this parasite especially in immunocompromised individuals. Secondly, the high prevalence of this parasite in apparently healthy populations confirms the high levels of morbidity and mortality associated with it when the immune system is compromised, and also should be intensified towards the establishment of suitable and clean water, public education on better-quality personal and environmental hygiene as these will go a long way in reducing the morbidity and mortality associated with cryptosporidiosis.

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