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The prevalence of the *Cryptosporidium parvum* among children in Ramadi city

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ABSTRACT

The purpose of this study was to determine the prevalence of *Cryptosporidium parvum* among children in the city of Ramadi and made a comparison between ziehl -Neelsen method and the ELISA method, which is usually used to diagnose this parasite in Ramadi hospital (administration of Obstetrics and Gynecology Hospital and Ramadi Teaching Hospital).

Fecal samples were collected from 813 children under 5 years. Oocysts of *Cryptosporidium parvum* parasite were determined by using ELSIA and Ziehl-Neelsen (acid-fast stain, ZN).

Our study was shown that the rate of prevalence of the *C. parvum* was 7.87% from the total of 813 samples of diarrhea by using Zeihl-Neelsen method while ELISA technique result was 11.56% From our results, ELISA technique more specific than Ziehl-Neelsen method in diagnosis of *C. parvum* and the different is significant (P <0.05)..

Introduction

One of the significant causes of diarrheal disease worldwide is *C. parvum* its gastrointestinal tract pathogen, an obligate enteric parasite [1] According to the studies, these parasites infected children under five years of age in developing countries and causes death [2] Most children who are immunosuppressed are more susceptible to infection with the *Cryptosporidium*, unlike children with immunity, health, and good nutrition, they suffer from self-limited diarrhea [3].

Cryptosporidium transported by contaminated food and water therefore it's considered as a waterborne disease[4] Many studies show that development countries have a high prevalence of *C. pravum* [5] Most of the studies conducted in Iraq found the highest incidence of *Cryptosporidium* oocysts in animal and human feces samples, these due to drinking tap water that increasing the likelihood of infection with this parasite [6].

For diagnosis *Cryptosporidium*, most of the laboratories used microscopic methods for diagnosis only or in combination with other methods such as genetic and immunological methods [6Many studies proved that using ELISA method is the better in diagnosis *C.parvum* **[7]** therefore, Our study aimed to find out the effectiveness of these two techniques used in the Ramadi city Hospital laboratory and to find out how prevalent parasites are in Ramadi city children.

Material and method

Collecting samples

The study included the age groups of children under five years who suffer from diarrhea and attend the Al-Ramadi Teaching Hospital for Women and Children for the period between January 2013 and January 2014

Fecal samples were collected from 813 children under 5 years suffering from diarrhea .To diagnosis *C.parvum* two methods were used Modified Ziehl-Neelsen (mZN) staining and Enzyme linked Immunosorbent assay (ELISA)

Parasite cryptosporidium parvum diagnosis

Modified Ziehl-Neelsen staining (mZN)

A drop of the stool sample is placed on a glass slide and left to dry, then the smear is fixed for three minute with methanol then added alkaline fashion on smears and heated to evaporate, without boiling, the smear washed with distill water after five minute to decolorization stage, After using (2.5% H_2SO4) for one minute, the slide was washed in distilled water. For one minute 1% methylene blue was added, then washed perfectly and dried[8]**.**

Microscopic examination of test slides: By using a light microscope, twenty five to thirty five fields were

tested under the 40x objective lens to study the presence of oocyst in the feces samples, to confirm the internal morphology the oil immersion objective was used [9].

Cryptosporidium oocysts: isolation and calculation

By using the flotation method with Sheather's sugar solution [10,11], parasite-
oocysts originating from human feces can be isolated, purified, and quantified. The haemocytometer slide u sed for white blood count can be used for measuring the parasite oocyst number cells calculation[12]

Enzyme linked Immunosorbent assay [ELISA]

For each sample, we made a stool supernatant by mixing 1 gram of stool with a similar volume of distilled water. We centrifuged the mixture 5 minutes at 1500 rpm .We transferred the supernatant to another tube to preserve the parasitic antigen. We mixed 0.2% menthol with the supernatant and then stored it till we could test it for Cryptosporidium-specific antigen [13]. An examination of the presence of the *Cryptosporidium* parasite antigen in human stool samples was performed in our study using a commercial kit [14] to detect the presence of the *Cryptosporidium* parasite antigen in children's stool samples.

Statistical analysis: The Z test is used to determine the effectiveness of the immunoassay method [ELISA] for diagnosis of cryptosporidiosis and microscopically test the stool, to compare oocysts shed by symptomatic and asymptomatic patients, When we used the Wilcoxon W test. P values less than 0.05 were considered significant [15, 16].

Results

Microscopical examination of the oocysts

Oocysts can be detected by light microscopy, they are seen as smooth, spherical, shining red bodies, with size measured 6 µm (fig 1).

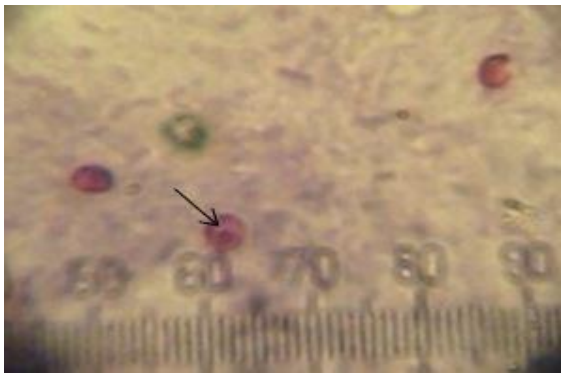

Fig. 1: Oocyst under light microscopy, spherical shape, shining red colored.

After staining with the (mZN) stain, microscopic examination of the stool containing oocysts shows that they are shiny red, and spherical in shape (Figure 1). Isolated oocysts from humans were measured to have an average diameter of 6 µm using the ocular micrometer[18] .

We determined the shedding rate of oocysts in ten samples from ten patients (symptomatic and asymptomatic) attended by the Obstetrics and Gynecology Hospital, and the oocysts were counted in 100 microliters of each sample. We observed significant differences between the shedding rate of oocysts in the symptomatic and asymptomatic patients **(**table 1).

Table 1: Oocysts shedding from symptomatic patients in terms of total and mean numbers.

prepared slices	Total number of oocysts on slide in 100 µl	The total number of oocysts on a slide in 1ml
Slide 1	189	1890
Slide 2	182	1820
Slide 3	178	1780
Slide 4	177	1770
Slide 5	156	1560
Mean ± SD	176.40 ±12.34	

Table 2: The total number and mean of oocysts shedding from asymptomatic patients.

The preparation of slides	Total number of oocysts on slide in 100 µl	The total number of oocysts on a slide in 1ml
Slide 1	146	1460
Slide 2	145	1450
Slide 3	134	1340
Slide 4	112	1120
Slide 5	109	1090
Mean± SD	129.20 ± 17.74	

Table 3: Wilcoxon Test Statistics

Wilcoxon W	15.000
Z test	-2.611
Asymp. Sig. [2-tailed]	0.009

The Z-test shows that there are significant differences in shedding of oocyst between symptomatic and asymptomatic patients at a lower level of significance (0.05), and the arithmetic mean of the symptomatic may reach 176.4 with a standard deviation of 12.3, while the arithmetic mean of the asymptomatic is 129.2 with a standard deviation of 17.7.

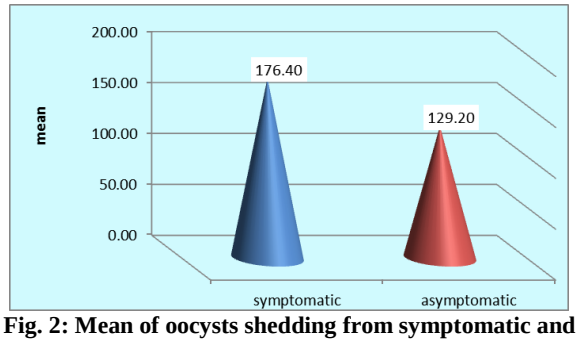

Fig. 2: Mean of oocysts shedding from symptomatic and asymptomatic patients. There were significant differences in the numbers of shedding oocyst between symptomatic and asymptomatic children.

Tests using enzyme-linked immunosorbent assays (ELISA)

Based on ELISA results, ninety-four individuals had cryptosporidiosis [the infection percentage (11.56%),

while 719 fecal samples were negative (88.4%), whereas results of the (mZN) stain revealed that sixty-four individuals (7.87%) had cryptosporidiosis, and 749 fecal samples were negative (92.13%).

Table 4: Two types of tests were used to diagnose samples isolated from Obstetrics and Gynecology Hospital in Ramadi, both of which found infected samples

The number of diagnostic samples	Diagnosis technique	Positive	Infection rate%]]	Negative	Infection rate%]]
813	Ziehl-Neelsen	64	7.87%	749	92.13%
	The ELISA method	94	11.56%	719	88.4%

A statistical analysis of Figure 3 shows that the two diagnostic methods showed significant differences at the 0.05 level ($p < 0.05$).

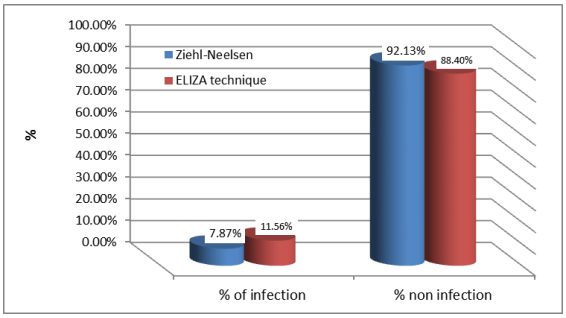

Fig. 3: Comparison between Ziehl-Neelsen method and ELISA.

Discussion

To determine the shape and measurements oocysts of *C.parvum*, which were Isolated from the stool samples of symptomatic patients and asymptomatic patients, the Ziehl- Neelsen staining method was used, the oocysts appeared red spherical bodies, 6 micrometer. This result is agree with another study found that the size of oocyst ranging between 5 to 7 µm **[19]**.Other study found that the size of oocysts ranged between 4.5-5 µm **[20].**

In a comparison of two ways to detect parasite presence in patients' stool samples, both of which are commonly used in hospitals, it was found that the ELISA method was more accurate than the Ziehl-Neelsen method(table 2). This result is in agree with the results of [13].

Diagnosis by using Zeihl-Nelseen stain depends on examining the slides under a microscope and relying on the shape and size of the oocysts . Thus, the examined slides may show remnants of undigested food or yeast in the patient's stool samples that may interfere with the diagnosis of oocysts and give a negative result. Therefore, this method is considered not accurate and specific in diagnosis, and this was confirmed by similar study**[17].** Because of the low cost of materials used to diagnose the parasite by microscopic examination, it is one of the preferred and important methods for diagnosing *C.parvum*, but this method requires high expertise in coloring and visual examination**[5].**

It was found through the results of the research that the rate of infection with the *C.parvum* was 7.87% of the total samples examined, which is a percentage close to a similar study in Wasit city, where the infection rate was 6.17%[22].

Through the results of preparing oocysts shedding by symptomatic and asymptomatic patients, there is a significant difference between these results, and this result is identical to another study conducted previously[21] Where our study showed an increase in the number of oocysts shedding by patients who developed symptoms of diarrhea (176.4) more than patients who did not develop symptoms of diarrhea (129.2) figure (2)

Studies conducted in different regions and environments of Iraq indicated a high rate of infection with this parasite due to drinking tap water, which facilitates the transmission of the parasite from animal to human[5, 22].In a study conducted in India, it was found that the infection rate is equal and high among children who drink tap water or bottled water, which indicates that the transmission of infection is not from water alone[23].

Conclusion

The prevalence of the *C.parivum* among the children of Ramadi city attending the hospital is considered a high rate if compared to other countries, but a percentage close to the rates of its prevalence in other cities of Iraq. The use of immunological methods ELISA in the diagnosis of the C.parvum is more rapid, easy, sensitive and specific than the use of the (mZN) dye method.

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انتشار طفيل الابواغ الخبيئة بين الاطفال في مدينة الرمادي

خديجة خليف عبدالله الدليمي

كلية طب الاسنان ، جامعة الانبار

الملخص

الغرض من هذه الدراسة هو تحديد انتشار طفيل الابواغ الخبيئة *Cryptosporidium parvum* بين الاطفال في مدينة الرمادي واجراء مقارنة بين طريقة الزيل نلسن المحورة وطريقة الاليزا التي تستخدم عادة لتشخيص هذا الطفيل في مستشفى الرمادي (ادارة التوليد ومستشفى امراض النساء ومستشفى الرمادي التعليمي).

تم جمع 813 عينة براز للاطفال دون سن الخامسة وتم تحديد بيوض طفيل الابواغ الخبيئة باستخدام الاليزا وصبغة الزيل نلسن المحورة.

اظهرت دراستنا ان نسبة انتشار طفيل الابواغ الخبيئة 7.87% من اجمالي 813 عينة من الاسهال عند استخدام طريقة زيل نلسن بينما نتج عن تقنية الاليزا 11.56% , تقنية الاليزا اكثر كفاءة من طريقة الزيل نلسن المحورة في تشخيص هذا الطفيل والاختلاف معنوي ($P < 0.05$).