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 zielh-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 Zielh-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 Zielh-Neelsen method while ELISA technique result was 11.56%. From our results, ELISA technique more specific than Zielh-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. parvum [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 [6]Many 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 Zielh-Neelsen (mZN) staining and Enzyme linked Immunosorbent assay (ELISA)

Parasite cryptosporidium parvum diagnosis
Modified Zielh-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% H2SO4) for one minute, the slide was washed in distilled water. For one minute 1% methylene blue was added, then washed perfectly and dried

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 used 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.](image)

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>
<thead>
<tr>
<th>Table 1: Oocysts shedding from symptomatic patients in terms of total and mean numbers.</th>
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<tbody>
<tr>
<td>prepared slices</td>
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<tr>
<td>Slide 1</td>
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<tr>
<td>Slide 2</td>
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<td>Slide 3</td>
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<td>Slide 4</td>
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<td>Slide 5</td>
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<td>Mean ± SD</td>
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<table>
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<th>Table 2: The total number and mean of oocysts shedding from asymptomatic patients.</th>
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<tr>
<td>The preparation of slides</td>
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<tr>
<td>Slide 1</td>
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<td>Slide 2</td>
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<td>Slide 3</td>
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<td>Slide 4</td>
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<td>Slide 5</td>
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<td>Mean ± SD</td>
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</tbody>
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<th>Table 3: Wilcoxon Test Statistics</th>
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<tr>
<td>Wilcoxon W</td>
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<tr>
<td>Z test</td>
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<td>Asymp. Sig. [2-tailed]</td>
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</table>

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.
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

<table>
<thead>
<tr>
<th>The number of diagnostic samples</th>
<th>Diagnosis technique</th>
<th>Positive</th>
<th>Infection rate%</th>
<th>Negative</th>
<th>Infection rate%</th>
</tr>
</thead>
<tbody>
<tr>
<td>813</td>
<td>Ziehl-Neelsen</td>
<td>64</td>
<td>7.87%</td>
<td>749</td>
<td>92.13%</td>
</tr>
<tr>
<td></td>
<td>The ELISA method</td>
<td>94</td>
<td>11.56%</td>
<td>719</td>
<td>88.4%</td>
</tr>
</tbody>
</table>

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.](Image)

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 Ziehl-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.parvum* 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.
References
انتشار طفيل الإبواغ الخبيئة بين الأطفال في مدينة الرمادي
خديجة خليف عبدالله الدليمي
كلية طب الأسنان، جامعة الآداب

الملخص
الغرض من هذه الدراسة هو تحديد انتشار طفيل الإبواغ الخبيئة Cryptosporidium parvum بين الأطفال في مدينة الرمادي وإجراء مقارنة بين طريقة الزيل نلسن المحررة وطريقة الألبيزا التي تستخدم عادة لتشخيص هذا الطفيل في مستشفيات الرمادي (إدارة التوليد، مستشفى أمارس النساء، مستشفى الرمادي التعليم).

تم جمع 813 عينة للاطفال دون سن الخامسة وتم تحديد بروز طفيل الإبواغ الخبيئة باستخدام الألبيزا وصبغة الزيل نلسن المحررة. أظهرت دراستنا أن نسبة انتشار طفيل الإبواغ الخبيئة 7.8% من إجمالي 813 عينة من الألفان عند استخدام طريقة زيل نلسن بينما نتج عن تقنية الألبيزا 11.56% تقنية الألبيزا أكثر كفاءة من طريقة الزيل نلسن المحررة في تشخيص هذا الطفيل والاختلاف معنوي (P<0.05).

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