

## The Role of Laboratory Techniques in Diagnosing *Cryptosporidium* Spp in Patients with Diarrhea in Al-Diwaniyah Governorate

Suhad Muhammad Ali Abbas and Ass. Prof. Dr. Khaled Thamer Al-Shaibani

Department of Life Sciences - College of Education - University of Al-Qadisiyah  
[Suhad.mohammad@qu.ed.iq](mailto:Suhad.mohammad@qu.ed.iq), [khaled.alshaebani@qu.ed.iq](mailto:khaled.alshaebani@qu.ed.iq)

### Abstract

This study was conducted on (300) stool samples taken from people with diarrhea of both sexes using laboratory techniques in diagnosis (Flukation technique with Scheather's sugar solution -Acid Fast Stain Staining Technology) to check infection with the parasite *Cryptosporidium* spp, And to know the effect of some general characteristics on the spread of the parasite. The results of this study showed that the total infection rate of the parasite amounted to 16.66%, with significant differences ( $P > 0.05$ ) for infection according to the method of flatting 40 samples by 13.33%, and according to the method of staining 50 samples, at a rate of 16.66%, as well as during the months of the study, when the highest infection rate was reached by method of flatting in November (16.36%), while the lowest percentage was in February (9.09%), the study also recorded significant differences in injury for age groups, as it recorded the highest rate of injury in the age group (1-14), as it reached (23.38%), and the lowest percentage of injury in the age group (43-56) years, when it reached (5.12%) Significant for infection with the parasite between males and females, the infection rate for males was (7.48%), while for females it was 18.95%), as for the nature of housing, the study showed significant differences, as the highest infection rate was reached in rural areas ((20%) and the lowest infection rate was recorded in The city is 6.2%. As for the pigmentation method, the highest rate of infection was in November (18.66%), while the lowest rate was in February (9.09%), the study also recorded significant differences in injury for age groups, as the highest infection rate was recorded in the age group 1-14)) As it reached (29.03%) and the lowest rate of infection in the age group (43-56) years, which reached (7.69%), a significant difference was observed for parasite infection between males and females, as the infection rate for males reached (9.52%), while it was for females (23.52%), as for the nature of housing, the study showed significant differences, as the highest rate of injury was in rural areas (24.18% and the lowest injury rate recorded in the city was 8.84%).

### Introduction

The parasitic protozoa, which are Unicellular, Eukaryotic cell organisms, are pathogenic and are no less important than other biological pathogens such as bacteria, viruses and fungi, The protozoans contain several types of living organisms, as 70,000 primary parasites have been found that infect humans and animals in several ways, such as drinking water and food that contains the infectious stages of the parasite. (Dalmasso, et. al, 2011), (Ayeh-kumi et al, 2009)

*Cryptosporidium* is one of the diseases that is widespread in various parts of the world, as it is common between humans and animals Zoonotic disease (Radostits et al, 2000). The

Cryptosporidium parasite is also a cause of diarrhea and comes in third place after Rota varum and Escherichia coli (Udaya & Prakash, 1997). This parasite also infects a wide range of hosts, up to 79 hosts, such as birds, reptiles, amphibians, fish, and mammals, including humans (Fayer, et.al, 2000), and due to the lack of research and studies on Cryptosporidium, its danger to humans, and its wide spread recently, and the emergence of many From the infections, the idea of this study came, which aimed at (investigating the extent of the spread of the Cryptosporidium parasite in Diwaniyah Governorate - studying the relationship between the factors affecting the spread of the parasite epidemic in the governorate (age - gender - areas of residence - months of the study) - Knowing the efficiency of some techniques Laboratory in diagnosing the parasite).

## **Material and methods**

### **First: the field study**

(300) faecal samples were collected from diarrhea sufferers suffering from intestinal disorders and arrivals to the Women's and Children's Teaching Hospital, Diwaniyah Teaching Hospital, and hospitals belonging to the districts and sub-districts in Diwaniyah governorate of all ages, starting from Sunday 6/9/2020 until On Monday (2/15/2021), faecal samples were collected in clean and sterile plastic containers containers with a volume of 20 ml with a wide mouth and a tight cover to maintain the sample's moisture and prevent its drying, containing on one of its sides a paper sticker to write down the questionnaire form number for the sample.

### **Second: The laboratory study: Examination of faecal samples by the following methods:**

#### **The method of flattening with a solution of sugar Sheather ( Anderson and Bulgin, 1981)**

Take 5 gm of feces with 15-20 cm<sup>3</sup> of distilled water, filter through 4 layers of gauze, and put in a centrifuge to spin 500 revolutions / minute for 10 minutes. The floating liquid was removed from the sediment and suspended from the sediment with Levitation glucose solution having a specific density of 1.27 g / cm and then the suspension turned again in the centrifuge at the same speed, after that a 7 mm thread metal wire was inserted to lift the liquid from the concave surface and put on the slide Then he placed the slide cover over it and examined with an optical microscope at the magnification of X100, X40.

#### **The acid fast Stain method**

Fecal samples were examined using the acid-fast stain (AFS) method, as follows (John & Petri, 2006)Fecal samples were examined using the acid-fast stain (AFS) method, as follows (John & Petri, 2006)

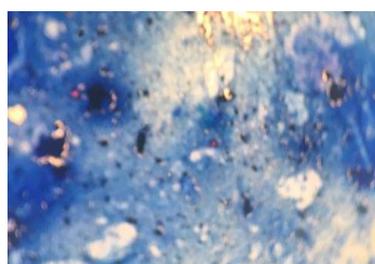
- Put a small drop of physiological saline solution on a glass slide, then add a small amount of faeces (0.1 g) to it and mix it gently using wooden sticks, then leave it to dry in the air at room temperature. Then it is dipped in methanol for the purpose of stabilization.

- Dip the slides with Carbol-fuchscin and run the flame for 5 minutes with a Bunsen burner until the steam appears.
- Wash the slides with tap water and shorten it with sulphuric acid at a concentration of 5% for two minutes, then wash it again with tap water.
- The slides were immersed in methylene blue for a minute, then washed with tap water and left to dry in the air.
- The stained samples were examined using a light microscope under the oily lens with a power of 100x to check for the presence of parasite egg cysts.



**Cryptosporidium oocyst**  
**Cryptosporidium**

**By flattening method**



**oocyst**

**By staining method**

## Results

The results of the current study showed by collecting and examining (300) stool samples for the injured, that the number of actually infected samples is (50) stool samples, and by (16.66%) of the total (75) stool samples are positive. As follows

### The distribution of the injury according to the examination method

The results of our study showed, as in Table (1), the positive samples according to the examination method, as the total number of samples reached 300 samples, distributed according to the method of flattening 40 samples at a rate of 13.33% and according to the staining method (the method of Zylsen) 50 samples at a rate of 16.66%, while the probability value It was calculated that there were significant differences between the two methods, as ( $P > 0.05$ ).

**Table (1) positive samples according to the examination method**

The Way	Total number positive number percentage	Total number positive number percentage	Total number positive number percentage
Flatting method	<b>300</b>	<b>40</b>	<b>13.33</b>
Staining	<b>300</b>	<b>50</b>	<b>16.66</b>

method (Zyl Nelson stain)	
Chi-square value / calculated likelihood value	<b>1.307/0.253</b>

**The distribution of the diagnosed injury by the Flatting method according to the studied characteristics**

The results of the current study indicated, according to Table (2), which shows the distribution of injury by Flatting method, according to the characteristics (gender, place of residence, age, and the duration of the month of collection). The results of the table showed that there was a significant increase ( $P < 0.05$ ) in the number of examined female samples with 153 samples, and the number of samples to be tested was 29 samples with a percentage of 29%, compared with the examined male samples of 147 samples, and the number of samples that needed to be tested was 11 with a percentage of 7.48%. . It was evident from Table (2) that there was a significant increase ( $P < 0.05$ ) for the rural population, with the number of tested samples 155, positive samples were diagnosed with 31 samples and a percentage of 20% compared with the city population with the number of examined samples amounting to 145 samples and the number of positive samples with 9 Sample, with a percentage of 6.2%. The age group characteristic also showed a significant increase for the age group between 1-14 years of age, 29 positive samples with a percentage of 23.38%, followed by the age group between 42-29 years with the number of positive samples 4 and a percentage of 5.97%, followed by the rest of the age groups with the number of positive samples (from 15-28 years with 3 samples, 56-43 with 2, and 70-57 samples with 2) respectively, and the total number of samples to be tested was 40 samples with a percentage of 13.33 out of a total of 300 examined samples. Significant differences between the months of the year according to the probability level ( $P > 0.05$ ) that showed the number of positive samples in the month (November 12 samples, December 11 samples, January 3 samples, October 9 samples, September 3 samples) respectively.

**Table (2) Distribution of the injury diagnosed by the flatting method according to the studied characteristics**

Properties		the number	Positive samples	percentage	Chi-square value / calculated likelihood value
Sex	Male	147	11	7.48	<b>8.53/0.003*</b>
	Female	153	29	18.95	
	the total number	300	40	13.33	
living address	City	145	9	6.2	<b>12.33/0*</b>
	Countryside	155	31	20	
	the total number	300	40	13.33	
Age (years)	14-Jan	124	29	23.38	<b>18.65/0.001*</b>
	15-28	47	3	6.38	

	<b>29-42</b>	<b>67</b>	<b>4</b>	<b>5.97</b>	
	<b>43-56</b>	<b>39</b>	<b>2</b>	<b>5.12</b>	
	<b>57-70</b>	<b>23</b>	<b>2</b>	<b>8.69</b>	
	<b>the total number</b>	<b>300</b>	<b>40</b>	<b>13.33</b>	
<b>The month of sample collection</b>	<b>September</b>	<b>20</b>	<b>1</b>	<b>5</b>	<b>25.48/0*</b>
	<b>October</b>	<b>55</b>	<b>14</b>	<b>25.45</b>	
	<b>November</b>	<b>75</b>	<b>18</b>	<b>24</b>	
	<b>December</b>	<b>69</b>	<b>4</b>	<b>5.79</b>	
	<b>January</b>	<b>59</b>	<b>2</b>	<b>3.38</b>	
	<b>February</b>	<b>22</b>	<b>1</b>	<b>4.54</b>	
	<b>the total number</b>	<b>300</b>	<b>40</b>	<b>13.33</b>	

**\* There are significant differences at the level of significance of 5%.**

### **Distribution of the diagnosed injury by staining method according to the studied characteristics**

The results of Table (3) indicated the results of the distribution of diagnosed and positive injuries according to the staining method for the characteristics (sex, place of residence, age, month of sample collection) and the presence of significant differences ( $P < 0.05$ ) for samples that positive for the sex characteristic, and the females recorded a significant increase in the positive samples sample 36 percent 36% compared to males who registered 14 samples, with 9.52% of the total number of positive samples, and 50 positive samples. As for the property of the place of residence, the rural population recorded a significant increase of 37 positive samples, by 24.18%, compared to the 13 sample population of the city, by 8.84%.

The age characteristic in years for the age group recorded a significant increase from (1-14 years) 36 samples at a rate of 29.03% compared to the rest of the groups, followed by the age group of 42-29 years with 6 samples and a percentage of 8.95%, followed by 15-28 and 56-43 years with 3 samples and a percentage 8.95% for both, and the category of 70-57 years with 2 samples and 8.69% of the total sample. As for the month of sample collection, there are clear significant differences between the positive samples for the months of collection according to the significant value ( $P > 0.05$ ).

**Table (3) Distribution of the injury diagnosed by the staining method according to the studied characteristics**

<b>Properties</b>		<b>the number</b>	<b>Positive samples</b>	<b>percentage</b>	<b>Chi-square value / calculated likelihood value</b>
<b>Sex</b>	<b>Male</b>	<b>147</b>	<b>14</b>	<b>9.52</b>	<b>10.58/0.001*</b>
	<b>Female</b>	<b>153</b>	<b>36</b>	<b>23.52</b>	

	<b>the total number</b>	<b>300</b>	<b>50</b>	<b>16.66</b>	
<b>living address</b>	<b>City</b>	<b>147</b>	<b>13</b>	<b>8.84</b>	<b>12.7/0*</b>
	<b>Countryside</b>	<b>153</b>	<b>37</b>	<b>24.18</b>	
	<b>the total number</b>	<b>300</b>	<b>50</b>	<b>16.66</b>	
<b>Age (years)</b>	<b>14-Jan</b>	<b>124</b>	<b>36</b>	<b>29.03</b>	<b>23.41/0*</b>
	<b>15-28</b>	<b>47</b>	<b>3</b>	<b>6.38</b>	
	<b>29-42</b>	<b>67</b>	<b>6</b>	<b>8.95</b>	
	<b>43-56</b>	<b>39</b>	<b>3</b>	<b>7.69</b>	
	<b>57-70</b>	<b>23</b>	<b>2</b>	<b>8.69</b>	
	<b>the total number</b>	<b>300</b>	<b>50</b>	<b>16.66</b>	
<b>The month of sample collection</b>	<b>September</b>	<b>20</b>	<b>2</b>	<b>10</b>	<b>23.7/0*</b>
	<b>October</b>	<b>55</b>	<b>17</b>	<b>30.9</b>	
	<b>November</b>	<b>75</b>	<b>20</b>	<b>26.66</b>	
	<b>December</b>	<b>69</b>	<b>6</b>	<b>8.69</b>	
	<b>January</b>	<b>59</b>	<b>4</b>	<b>6.77</b>	
	<b>February</b>	<b>22</b>	<b>2</b>	<b>9.09</b>	
	<b>the total number</b>	<b>300</b>	<b>50</b>	<b>16.66</b>	

\* There are significant differences at the level of significance of 5%.

## Dissociation

The current study recorded 50 cases of the *Cryptosporidium* parasite in people with cases of diarrhea, at a rate of (16.66%) of a total of (75) positive stool samples using the technique of flattening and staining with the stain Zyl Nelson, and this percentage is identical to many previous studies, including (Al-Alousi, TI & Mahmood, OI, 2012), which recorded (16.9%) in Mosul and El-Settawy, M. A. & Fathy, G. M., 2012), which recorded (18.6%) in Egypt, Also, it was identical to the study (Al-Shabani, 2008), which recorded a rate of (18.01%) in Diwaniyah Governorate. On the other hand, the current total infection rate was higher than what was found in the study (Al-Kaabi, 2006), as it recorded (6.6%) for children in the Diwaniyah governorate, and the study (Khalil, 2007), which recorded a rate (15.23%) in the Mosul governorate. It was obtained in the current study relatively high and poses a risk to the health of the injured in the governorate, and the researcher believes that it is due to many reasons, including the lack of health and environmental awareness for individuals, the spread of pathogens, and the decline in public services in the governorate. Either the reason for the congruence or contrast of the results obtained in this study with the results in other studies in Iraq or in other regions of the world is due to the variation in environmental conditions, population density, ways of living, examination

methods, the time period covered by this study, as well as the size of the sample that has A role in the contrast of the recorded results with the results of other studies as well as the difference in the ages of the injured, the geographical location, the nature of nutrition, the immune status, personal hygiene, and the temperature difference from year to year in the same region and between one country and another.

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