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DETECTION OF CRYPTOSPORIDIUM INFECTION BY DIFFERENT METHODS IN CHILDREN WITH DIARRHEAL SAMPLES IN MISURATA

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Abstract

Background: Cryptosporidiosis is an intestinal infection caused by an intracellular protozoan parasite Cryptosporidium; which is one of the critical causes of diarrhea in children around the world. **Objective:** to detect Cryptosporidium infection in clinical samples from pediatrics suffering from diarrhea in Misurata, Libya, and evaluate three methods of diagnosis. **Methods:** 150 stool samples were collected from children with diarrhea, in the age group (1 month – 15 years) in both genders who attended Misurata Central Hospital. The stool samples were inspected by microscopic examination Modified Ziehl Neelsen (MZN), crypto-strip test and enzyme-linked immunosorbent assay (ELISA). **Results:** indicated that 17 children in the study group (11.3%) were positive for Cryptosporidium infection using MZN, while 19 (12.7%) were positive using the Crypto-strip test and ELISA, bearing in mind that there was no significant difference between male and females nor between urban and rural areas. The higher infection appeared in age group < 5-year-old, the sensitivity, specificity of the Crypto-strip test and ELISA were 89.47%, 100% respectively. **Conclusions:** The high prevalence rate of Cryptosporidium infection among children of less than 5 years old in both genders were sensitive to be infected with Cryptosporidium in both rural and urban areas, the three methods proved to be a useful mean for diagnosis of cryptosporidiosis in stool samples.

Keywords: Cryptosporidium, Modified Ziehl Neelsen, Crypto-Strip Test, and ELISA

BACKGROUND

Cryptosporidium species are protozoan parasites that cause infection and diarrheal illness in a wide range of human and animals (Ryan et al., 2016). Diarrheal diseases are extremely common in the developed and developing worlds and are major causes of morbidity and mortality, affecting millions of individuals each year (Liu et al., 2012), and as reported by the World Health Organization (WHO), Diarrheal remains one of the most common illnesses among children and is one of the major causes of infant and childhood mortality in developing countries (Boschi-Pinto et al., 2008).

Humans can acquire Cryptosporidium infections through several transmission routes; such as direct contact with infected persons or animals, and ingestion of contaminated food (foodborne transmission) and water (waterborne transmission) (Xiao, 2010; Santin, 2020).

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Cryptosporidiosis manifests as a self –imited diarrhea in immunocompetent individuals whereas, in immunocompromised individuals causes severe and chronic diarrhea that if untreated, can lead to death Children due to their low immunity are more susceptible to infection, but most immunocompetent children regarding to nutritional status, health and other environmental factors suffer a self-limited diarrhea (Tahvildar-Biderouni and Salehi, 2014; Mahdavi et al., 2021).

There are different methods to diagnose Cryptosporidiosis, including Microscopic examination, staining, immunological and molecular methods. Microscopic examination can help to identify oocysts through several techniques, one of which is the Formalin–ethyl acetate concentration method (Graczyk et al., 2000). A large number of staining techniques have been used to recognize Cryptosporidium oocysts, the modified acid-fast procedures; which are the gold standard for the detection of Cryptosporidium spp. is the most widely used technique (Taha et al., 2022). Immunological techniques by ELISA used for detecting antigens and Crypto-strip test have been developed based on the detection of parasite antigens (Regnath et al., 2006).

AIM OF THE STUDY

The aim of this study is the detection of Cryptosporidium infection in clinical samples from diarchic children in Misurata city by using microscopic examination Modified Ziehl-Neelsen (MZN), Crypto-strip test and enzyme-linked immunosorbent assay (ELISA). And evaluate three methods to determine the best method for detection of Cryptosporidium spp. in stool samples in children.

METHODS

This study was carried out during the period from May 2018 to December 2018 in Central Hospital of Misurata city, Libya. A total of 150 stool samples were taken from children aged one month to 15 years who are suffering from acute or chronic diarrhea. Fifty healthy children with matched age and gender were included as a control group. A questionnaire containing demographic, clinical and environmental data was obtained from each case.

Samples were collected into clean, leak-proof containers, and each container was labelled with a number and the name of the patient. Samples then transferred to the Misurata Central Laboratory to examination. Stool samples were collected and immediately divided into three parts.

The first part were examination by prepared of fecal smears by using Formalin-Ethyl Acetate Sedimentation Concentration, and stained using the modified acid-fast staining technique (Cheesbrough, 2016), then Examine the smear by using an oil immersion objective.

The second part the fresh stool samples were then subjected to copro antigen detection for cryptosporidium using Crypto-strip test RIDA®QUICK Quick cryptosporidium from R-Biopharm Germany. Following the manufacturer's recommended procedures.

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The third part was preserved at -20°C until used for ELISA from R-Biopharm, Germany, following the manufacturer's recommended procedures. The RIDA®QUICK Cryptosporidium Test employs specific antibodies in a sandwich type method, these specific antibodies to Cryptosporidium parvum and Cryptosporidium hominis are attached to the well surface of the micro well plate

Statistical Analysis

All data were subjected to statistical analysis using SPSS win statistical package version 17. A p-value below 0.05 was regarded as statistically significant, according to Pearson's chi-square test results. To assess the diagnostic performance of the four different adopted approaches for diagnosis of cryptosporidiosis by using MEDCALA program.

RESULTS

A total of 150 stool samples were collected from children patients at Misurata Central Hospital (MCH) and then were examined by three different methods; the total infectivity with Cryptosporidium was (11.3%) when examined by microscopic examination (modified Ziehl-Neelsen stain). While when examining the samples by Crypto-strip test and ELISA the infective rate was (12.7%), as illustrated in (Figure1).

Figure 1: The infection rate of Cryptosporidium in infected and non-infected children diagnosed by different methods

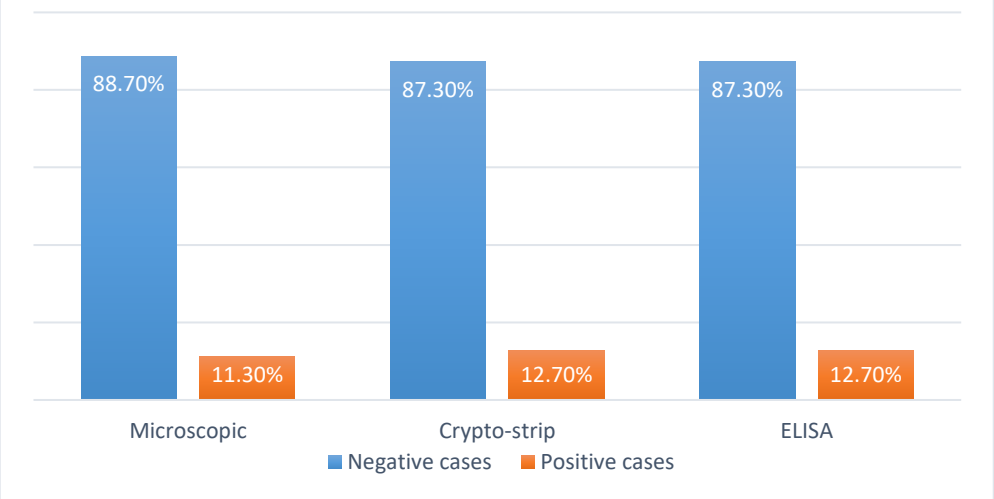

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Table 1: Distribution of Cryptosporidium among Children Regarding Gender

Methods	Gender	Results Mean (%)		P-value
		Negative	Positive	
MZN	Female	(72) 85.7%	(12) 14.3%	0.152
	Male	(61) 92.4%	(5) 7.6%	
Crypto-strip test	Female	(70) 83.3%	(14) 16.7%	0.077
	Male	(61)92.4%	(5) 7.6%	
ELISA	Female	(70)83.3%	(40)16.7%	0.077
	Male	(61) 92.4%	(5) 7.6%	

Table 1 represents the distribution of Cryptosporidium infection according to gender. The recorded infection rate was 7.6% in males and 14.3% in females by MZN, While the infection rate by Crypto-strip test and ELISA was 7.6% in males and 16.7% in females by Crypto-strip test and ELISA as illustrated in table (1), with no significant difference in the total rate of infection with Cryptosporidium between males and females (P>0.05). There was not a significant difference found between the rate of infection among the children of urban areas (14.0%) and children of rural areas (7.8%) by MZN. Moreover, the infection rate among children of rural areas (7.8%) and (16.3%) by Crypto-strip test and ELISA (table 2).

Table 2: Prevalence of Cryptosporidium infections in rural and urban areas

Methods	Area	Results Mean (%)		P-value
		Negative	Positive	
MZN	Rural	(59) 92.2%	(6) 7.8%	0.181
	Urban	(70)86.0%	(12) 14.0%	
Crypto-strip test	Rural	(59)92.2%	(6)7.8%	0.096
	Urban	(72)83.7%	(14)16.3%	
ELISA	Rural	(59)92.2%	(7)7.8%	0.096
	Urban	(72)83.7%	(14)16.3%	

In below, the highest prevalence of Cryptosporidium infection using MZN method appeared in the age group, (<1 years) and (1-5 years) respectively, at a rate of (14.0%), while the lowest infection rate appeared in the age group (6-10 years) at a rate of (3.1%).

The infection rate by Crypto-strip test and ELISA in The age group (<1 year) showed the highest at a rate of (17.5%) followed by the age group (1-5 years) at a rate of (14.0%) then lowest infection rate appeared in age group (6-10 years) at a rate (3.1%). Moreover, no Cryptosporidium infection in the age group (11-15 years) by using three methods, were not statistically significant (P = 0.209). From the total 150 stool samples that were examined by the three methods, the positive samples had a percentage rate of 100% for children who have direct and indirect contact with animals. Statistically, there was from Table 3, 4 illustrate the relationship between the infection rate and the children who have contact with animals (P-value < 0.05).

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Table 3: Distribution of Cryptosporidium among children regarding to age group

Methods	Age	Results Mean (%)		P-value
		Negative	Positive	
MZN	>1	(49)86.0%	(8)14.0%	0.323
	1-5 Y	(49)86.0%	(8)14.0%	
	6-10 Y	(31)96.9%	(1)3.1%	
	11-15 Y	(4)100.0%	(0)0.0%	
Crypto-strip test	>1	(47)82.5%	(10)17.5%	0.209
	1-5 Y	(49)86.0%	(8)14.0%	
	6-10 Y	(31)96.9%	(1)3.1%	
	11-15 Y	(4)100.0%	(0)0.0%	
ELISA	>1	(47)82.5%	(10)17.5%	0.209
	1-5 Y	(49)86.0%	(8)14.0%	
	6-10 Y	(31)96.9%	(1)3.1%	
	11-15 Y	(4)100.0%	(0)0.0%	

Table 4: Distribution of Cryptosporidium among children who have a contact with animal

Methods	Results	Contact with animal		P-value
		No	Yes	
MZN	Positive	0.0%	100.0%	0.000
	Negative	4.5%	95.5%	
Crypto-strip test	Positive	0.0%	100.0%	0.000
	Negative	3.1%	96.9%	
ELISA	Positive	0.0%	100.0%	0.000
	Negative	3.1%	96.9%	

Diagnostic performance of methods used in the diagnosis of cryptosporidiosis among diarrheic children, MZN method as a reference test, showed the following results: Regarding Crypto-strip test and ELISA among 150 diarrheic children (12.7%) had cryptosporidiosis. Its sensitivity was 89.47%, specificity 100%, PPV 100%, NPV 96.5 %.). (Table 5).

Table 5: Comparison of ELISA and crypto-strip test with microscopic examination (MZN) method as a standard golden test among diarrheic children

Method	Result	Microscopic Examination (MZN)		Sensitivity	Specifics	PPV	NPV
		Positive	Negative				
ELISA	Positive	17	2	89.47%	100%	100%	96.5%
	Negative	0	131				
Crypto-strip test	Positive	17	2	89.47%	100%	100%	96.5%

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DISCUSSION

The current study explores the prevalence of Cryptosporidium infections among children <15 years old in Misurata, Libya. The study group (n=150) cryptosporidiosis was revealed (11.3%) using the MZN, and (12.7%) using the Crypto-strip test and ELISA, while in the control group, there was not any infection of Cryptosporidium was detected. In Libya, the first report of Cryptosporidium infection was published by (Ali et al., 2005) in Zlitin, where it was found that the prevalence rate of Cryptosporidium infection was 13%, and in Sebha, (Mabroka et al., 2017) reported a prevalence rate of 10% for Cryptosporidium associated diarrhea in 150 children using the MZN and Giemsa staining methods.

The prevalence rate of Cryptosporidium infection among children was higher in neighboring countries than Libya; such as Egypt Reports by (Shalaby and Shalaby, 2015) stated that in Egypt 25% positive cases of cryptosporidiosis among 120 randomly chosen schoolchildren aged 4-16 years. On the other hand, (Iqbal et al., 2001) found that the prevalence of cryptosporidiosis infection at a local hospital in Kuwait was the highest (73%) in children above 2 years of age.

The Disparity in infection prevalence of Cryptosporidium in Libya in comparison with other countries may be due to the differences in samples size, living conditions, socio economic criteria, nutritional status, immune status and personal hygiene and variation in the nature of areas type of the test, which used for diagnosis.

Concerning the present study revealed that no significant difference in the prevalence of Cryptosporidium infection according to gender. it was noted the prevalence between males (7.6%) and females (14.3%) using MZN, and the recorded infection rate was 7.6% in males and 16.7% in females using the Crypto-strip test and ELISA; which may indicate that both genders have an equal chance of being infected. This result agrees with the results of (Saneian et al., 2010; Gatei et al., 2006; Tigabu et al., 2010). Alternatively, (Mumtaz et al., 2010) found that the rate of infection in males was higher than females may be due to more exposure of males to the sources of contamination. As regards the distribution of positive cases according to children.

The data show no significant differences were found in the prevalence rate between children residing in rural areas (7.8%) and those in urban (14.0%) by using MZN. While by Crypto-strip test and ELISA techniques the rate infection of cryptosporidiosis among children in rural areas was 7.8% and 16.3% in urban areas may be due to that all children in both areas have close contact with animals, which appears to be a contributing factor that increase the risks infection with Cryptosporidium.

This agrees with (Tamomh et al., 2021); which disagree with the result of (Ali and Ali, 2013) who reported that there is difference in prevalence of Cryptosporidium infection between rural and urban areas. This may be attributed to the increased exposure to zoonotic infections, low socioeconomic standard and close contact with animals and soil.

The present study observed the difference between the prevalence rates of Cryptosporidium infection among the age group of children used the three methods. The

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present study observed the difference between the prevalence rates of Cryptosporidium infection among the age group of children used the three methods. The high prevalence rate of cryptosporidiosis among children in less than 5 years was detected using MZN examination (14%), this may be due to their low immune functions, and therefore, a low dose of infection may result in cryptosporidiosis.

These results agreed with (Abdel-Messih et al., 2005); where it was reported, a prevalence of Cryptosporidium infection in children below 5 years of age was (17%). Moreover, Crypto-strip test and ELISA techniques, the most cases of cryptosporidiosis occurred among children less than 1-year-old may explain this explained by the weak immunity in this age group, consuming infected colostrum (animal milk) and creeping on contamination ground. This agrees with the results stated in (Golan, 2019), where they reported that prevalence of Cryptosporidium infection was higher in children < 1-year-old in Ramadi city in Iraq.

Regarding as the history to animal contact with positive cases, our results revealed that 100% had a history of animal contact. This concludes that a close contact with animals is the main reason of infectivity with Cryptosporidium spp. This result was confirmed by (Sarkar et al., 2014) that found in children lived close to animals. The present study is performed to compare MZN, Crypto-strip test and ELISA for the detection of Cryptosporidium in clinical stool samples.

MZN method is considered as the gold standard for detection of Cryptosporidium in stool samples, the diagnostic performance of the Crypto-strip test and ELISA in the present study revealed sensitivity (89.47%) and specificity (100%). Both methods showed high PPV and NPV (100% and 96.5% respectively).

According to the result, the three techniques are useful methods in Cryptosporidium diagnosis, but the ease of use and costs are important criteria in determining the choice of a technique. The Crypto-strip method is a rapid test as it takes only 10 minutes to give as accurate result, however, regardless to its high cost, this method may be an advantage rapid strip test as it could be used for screening in case of outbreaks of diarrhea for faster management of the problem. Regarding ELISA, it is a reliable and widely used detection method, however, it takes two and half hours for providing complete result in addition to its expensive cost.

ELISA method is recommended to detect and monitor Cryptosporidium infection in epidemiological studies and scientific research. Microscopic examination technique is regarded as useful when using concentration method and Modified Ziehl-Neelsen stain, due to its applicability and low cost.

In accordance with (El-Shazly et al., 2002) found that the MZN stain had the lowest sensitivity in relation to either ELISA or PCR. On the contrary, (Lee et al., 2000) reported that ELISA sensitivity was 82% compared to microscopic method as reference, they attributed the decreased sensitivity to reported freezing and thawing of samples. On the other hand, (Eassa et al., 2017) reported that the PCR was more sensitive than rapid strip

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test and ELISA. Also, (Kaushik et al., 2008) in India, evaluated the four methods including Modified Ziehl-Neelsen staining, safranin methylene blue staining, antigen detection ELISA and Nested-PCR for Cryptosporidium detection. The staining techniques were found less sensitive as compared to antigen detection and PCR for detection of Cryptosporidium.

CONCLUSIONS

The Cryptosporidium spp. is the most common enteric parasite that associate with persistent diarrhea in children, it demonstrated clearly a high prevalence rate of Cryptosporidium infection among children of less than 5 years old in both genders were sensitive to be infected with Cryptosporidium in both rural and urban areas. The present study is the first record of cryptosporidiosis among children in Misurata, Libya and resulted that the infection rate of Cryptosporidium positive cases was 11.3% using MZN method and 12.7% using ELISA and crypt-strip test. The three methods proved to be a useful mean for diagnosis of cryptosporidiosis in stool samples. MZN method considered to be low cost comparing to ELIZA and Crypto-strip; which make it accessible in all laboratories and hospitals، in addition to that it has enough accuracy for Cryptosporidium diagnosis with low risk to children.

List of Abbreviations

(MZN): Modified Ziehl-Neelsen, (ELISA): Enzyme-linked immunosorbent assay.
(MCH): Misurata Central Hospital

Ethics Approval and Consent to Participate

Before collecting samples from children, their mothers signed the approval letters.

Consent for Publication

Not applicable

Availability of Data and Material

The data that support the findings of this study are available from the corresponding author, (L.E).

Competing Interests

The authors declare that they have no competing interests

Funding

No funding was obtained for this study

Authors' contributions

A.M. conceived of the presented idea. A.M. And L.E developed the theory and performed the analysis. All authors read and approved the final manuscript.

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