Journal of High Institute of Public Health 2017;47(1):29-38.

Original Article

Evaluation of Different Diagnostic Approaches for Detection of *Cryptosporidium* in Stools of Diarrheic Children

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Abstract

Background: Cryptosporidiosis is of utmost importance especially in vulnerable age groups in developing countries. Malnourished children are more susceptible to recurrent diarrheal episodes, which can lead to chronic nutritional and cognitive sequelae or even death.

Objective(s): to evaluate four different diagnostic approaches for *Cryptosporidium* infection in stools of diarrheic children.

Methods: One hundred stool specimens were collected from diarrheic children in Alexandria University Children Hospital (El-Shatby). All samples were investigated by four techniques; directly by microscopic detection of *Cryptosporidium* oocysts using modified Ziehl-Neelsen (MZN) stain. Indirectly, through detection of coproantigen by enzyme linked immunosorbent assay (ELISA) and rapid strip test. *Cryptosporidium* DNA was detected by conventional polymerase chain reaction (PCR).

Results: Using the four methods, 65% of examined children had *Cryptosporidium* infection, while *Cryptosporidium* oocysts were shown by MZN stain technique among 41%. However, by rapid strip test, ELISA, and PCR the percentages were 45%, 48%, and 59%, respectively. PCR elicited the highest diagnostic efficiency (64%) among the three diagnostic non-microscopic techniques when the MZN technique was used as the gold standard test. However, rapid strip test showed the least diagnostic efficiency (48%) when compared to PCR that was considered as the gold standard test. Meanwhile, ELISA was of moderate performance when compared to either PCR or to MZN technique used as gold standard test.

Conclusion: PCR was more sensitive than rapid strip test and ELISA. It is time saving, but not cost effective. The rapid strip test could be considered as a complementary (additional) tool rather than a substitute for microscopic examination. It could be used for screening in cases of outbreaks of diarrhea for faster management of the problem.

Keywords: *Cryptosporidium*, PCR, ELISA, diarrheic children, diagnostic performance, rapid strip test

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Suggested Citation: Eassa SM, Flefel W, El-Masry SA, Abdul-Fattah AH. Evaluation of different diagnostic approaches for detection of cryptosporidium in stools of diarrheic children. JHIPH. 2017;47(1):29-38.

INTRODUCTION

Cryptosporidiosis is of major public health concern in both developed and developing countries. *Cryptosporidium* has become one of the most commonly reported enteric pathogens in both immunocompetent and immunocompromised persons worldwide. [(1)]Immunocompetent hosts can control and eliminate the infection, which typically causes acute self-limited watery diarrhea lasting 5 to 10 days. However, in patients with defects in cellular immune responses (e.g., AIDS, malnutrition, or defects in the CD40-CD154 system) *Cryptosporidium* frequently causes persistent or chronic diarrhea and may also involve the biliary tract. [(2)] Malnourished children are more susceptible to recurrent diarrheal episodes, which can lead to chronic nutritional and cognitive sequelae or even death. Thus, the host immune response plays a critical role in the control of human cryptosporidiosis. [(3)] Cryptosporidiosis is more common in children aged between 1 and 5 years, although outbreaks occur worldwide in all age groups. The young age was a risk factor for developing *Cryptosporidium* associated

diarrhea. It was found that breastfeeding had a role in the protection against infection. The epidemiology of *Cryptosporidium* associated diarrhea in the Nile River Delta of Egypt was studied over two years period, 17% of examined children were found to be infected with *Cryptosporidium*.[4] In the United States in 2005, the youngest age group (0-9 years) had the lowest prevalence rates (0-3%), and the rates increased with increased age up to 7-9% at 80-89 years.[5] Misdiagnosis of parasitic infections may be a leading factor for public health problem in developing countries.[6] In the majority of laboratories, the diagnosis of unusual protozoa as *Cryptosporidium*, *Isospora*, Microsporidium, *Cyclospora* spp and *Blastocystis hominis* is not a part of the routine microscopic examination. They involve the use of special staining techniques that have been greatly improved in the last years[7], where electron microscopy and molecular biology facilities for the diagnosis of opportunistic infections are lacking.[8] Non-microscopic methods include antigen detection in feces; immunoassays have become a well-established aid to microscopic examination for the diagnosis of cryptosporidiosis. Good sensitivities and specificities have been reported for some of these tests in several comparative studies. However, considerable progress has been made in the molecular characterization of *Cryptosporidium* since the development of these tests, resulting in the identification of at least seven human-infecting species.[9] The accurate identification of a parasite at the species and/or genotype level has major implications for various aspects of human parasitology, including the diagnosis, the treatment, and the control. The advent of molecular techniques, in particular those based on the in vitro amplification of nucleic acids, has dramatically improved the ability to detect infections.[10] In infectious dose, studies and models suggested that low infective dose, even a single oocyst or cyst carriers; have some probability of causing an infection. Finally, most feces that contain oocysts end up in the environment and can be spread to food by irrigation or by direct contact, and can persist in the water, as routine treatments eliminate only a fraction of these stages. This situation explains the growing interest towards the development of methods that allows such stages to be detected with the highest sensitivity and specificity. [11] The present study aimed to compare four different diagnostic approaches; MZN stain, ELISA, rapid strip test and PCR for *Cryptosporidium* infection among diarrheic children by using diagnostic accuracy tests.

METHODS

Study Setting and Design: A cross-sectional study was conducted. Stool samples were collected from Alexandria University Children's Hospital at EL-Shatby district. Examination of samples was carried out in the laboratory of Tropical Health Department at the High Institute of Public Health.

Sampling: Assuming the prevalence of cryptosporidiosis in children= 37.3[12], a sensitivity of ELISA in relation to IFA=93 %, specificity=99%, and desired precision =2%with confidence level=95%, the minimal required sample size was found to be 97 (increased to 100). The required sample was calculated directly online.[13]

Study Procedure: Two visits per week were carried out for collection of stool samples from these children until reaching the sample size. A predesigned interview questionnaire was used to collect the data from mothers of the examined children, after taking their verbal consent. It included the socio-demographic data such as age, sex, residence.

One hundred stool samples were collected from children less than 36 months of age suffering from diarrhea. They were examined by four tests; microscopically by MZN technique to detect *Cryptosporidium* oocysts, and indirectly, to detect coproantigen by ELISA and rapid strip test. Conventional PCR was done to detect *Cryptosporidium* DNA.

Stool samples were collected from diarrheic children; each sample was divided into four parts, one part was preserved in 10% formalin to be prepared by Formol ether concentration technique (FEC) [14] for later staining with MZN method. [15] The remaining parts were preserved in special tubes at -20 °C to be tested by the following techniques:

1. Rapid strip test (Frankfurt, Germany) for the detection of *Cryptosporidium* coproantigen[16], a single-step, immunochromatographic test. Specific *Cryptosporidium* antibodies are bound to these strips.
2. ELISA technique RIDASCREEN® (Frankfurt, Germany), for the detection of *Cryptosporidium* coproantigen in which, specific antibodies are used in a sandwich-type method.[17]
3. Conventional PCR to detect *Cryptosporidium* DNA.[18] The QIAamp DNA Stool Mini Kit (Frankfurt, Germany) is designed for rapid purification of total DNA from 180 up to 220 mg stool and is suitable for both fresh and frozen samples. The fast and easy procedure comprises the following steps:

- Lysis of stool samples
- Adsorption of impurities
- Purification of DNA on QIAamp Mini spin columns (Frankfurt, Germany), according to the manufacturer's instructions. For best results in downstream PCR, the minimum amount of eluate possible in PCR was used; the volume of eluate used as template did not exceed 10% of the final volume of the PCR mixture i.e. 1.5ul. Also, the high amounts of template DNA may inhibit the PCR so DNA yield was typically 15–60 µl keeping the eluate at –20°C. The amplified DNA & DNA ladder were separated on agarose gel electrophoresis. The bands were visualized using UV transilluminator (320nm). Figure 1

Statistical Analysis

Data were tabulated and analyzed using Statistical Package for Social Science (SPSS) version (16.0) and the statistical threshold was set at p=0.05.To assess diagnostic performance of the four different adopted approaches for

diagnosis of cryptosporidiosis, various diagnostic accuracy tests were calculated. Such accuracy tests included sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic efficiency, likelihood ratio for positive result (PLR), likelihood ratio for negative result (NLR), discriminating ability (DA) and receiver operating characteristic (ROC) curve and the area under the curve (AUC). Accuracy tests were used two times; once to test the three methods versus MZN which was a gold standard and another time diagnostic tests when PCR was used as a gold standard. [19, 20] AUC is one of the diagnostic accuracy tests performed in order tovalidate the predictors of cryptosporidiosis. Discrimination is important while distinguishing the outcomeeither infected or not. A discriminatory power of 0.90 or more is considered an excellent performance, while 0.80-0.89 is considered as a good performance and 0.70-0.79 is considered as a fair discriminatory performance. [21]

Ethical Considerations

The study protocol was approved by the Institutional Review Board and the Ethics Committee of High Institute of Public Health, Alexandria University. The study conformed to the International Guidelines for Research Ethics and that of Helsinki Declaration. A verbal consent was obtained from all mothers before enrollment in the study after explanation of the purpose and benefits of the research. Anonymity and Confidentiality of the participants' data were ensured.

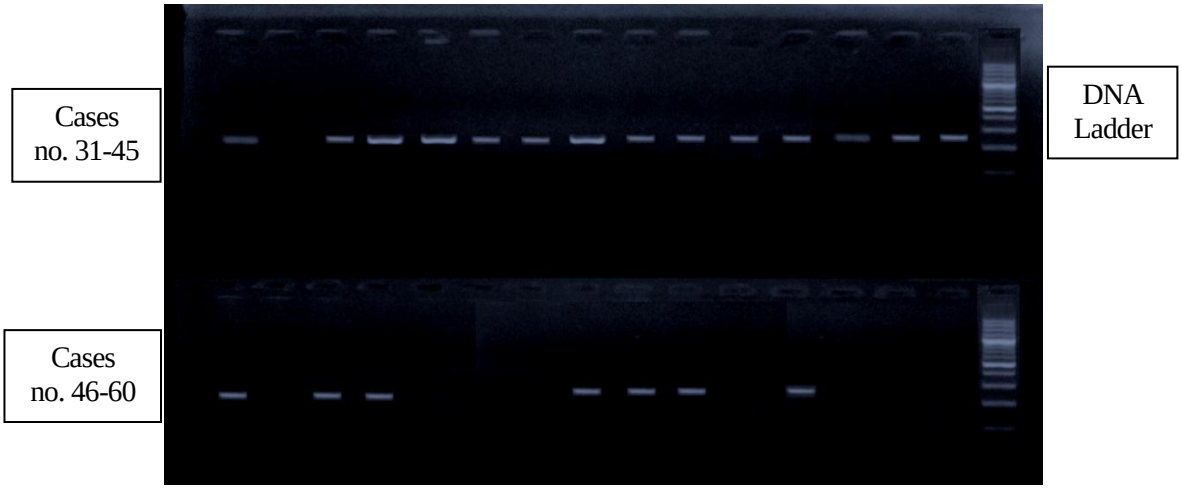


Figure (1): PCR results for casesno. 31 to 60

RESULTS

The highest percentage of infection was found among children aged more than 24 months compared to those aged from 12 to 24 months, and those who were less than 12 months (47.06%, 39.29%, and 36.84%, respectively). *Cyclospora cayetanensis* was the most prevalent coccidian followed by *Cryptosporidium*, and lastly *Blastocystis hominis* among the infected children. The children residing urban areas have higher infection rate than those residing rural areas (48.39% versus 37.68%), but the difference was statistically insignificant (X^2=1.01, *P*> 0.05). The rate of infection was directly proportional to crowding index; the higher the crowding index, the higher was the infection rate, the difference was statistically significant (Fisher exact test 49.52, *P*=0.0001). (Not shown) Diagnostic performance of methods used in the diagnosis of cryptosporidiosis among diarrheic children. MZN method as a reference test(Table 1, 2 and Figure 2) showed the following results:

Regarding rapid strip test, among 100 diarrheic children, 45 (45%) had cryptosporidiosis. Its sensitivity was 56%, specificity 62.70%, PPV 51.10%, NPV 67.20% with overall diagnostic efficiency 60%, the PLR 1.5, NLR 0.7, DA 18.30% and AUC 0.586.i.e. Discriminatory performance is less than fair. The diagnostic performance of the ELISA; Out of 100 diarrheic children, 48 (48%) had cryptosporidiosis with a sensitivity 63.40%, specificity 62.70%, PPV 54.20%, NPV 71.20% with overall diagnostic efficiency 63%. Meanwhile PLR, NLR, DA and AUC were 1.69, 0.58, 25.40% and 0.631, respectively. The diagnostic performance of the PCR; Out of 100 diarrheic children, 59 (59%) had cryptosporidiosis with a sensitivity 78.0%, specificity 54.20%, PPV 54.20%, NPV 78.0% with overall diagnostic efficiency 64%, while the PLR 1.7, NLR 0.40, DA 32.20% and AUC 0.661.

Table (1): The percentage of *Cryptosporidium* infection among diarrheic children by different techniques

Techniques	Infection (%)
MZN	41.0
Rapid Strip	45.0
ELISA	48.0
PCR	59.0

Table (2): Diagnostic performance of the rapid strip test, ELISA and PCR as diagnostic methods for cryptosporidiosis compared to the MZN technique as the gold standard test among diarrheic children

	Diagnostic accuracy tests MZN as a gold standard								
Variables	Diagnostic efficiency%	Sensitivity% (95%Cl)	Specificity% (95%Cl)	PPV% (95%Cl)	NPV% (95%Cl)	PLR (95%Cl)	NLR (95%Cl)	DA%	AUC
Rapid strip	60	56 (39.8-71.1)	62.70 (49.1-74.6)	51.10 (35.9-66)	67.20 (531-78.9)	1.5 (98.1-2.30)	0.7 (48.5-1)	18.30	0.586 (0.471-0.70)
ELISA	63	63.40 (46.9- 77.4)	62.70 (49.1- 74.6)	54.20 (39.3- 68.3)	71.20 (56.7- 82.4)	1.69 (1.13- 2.54)	0.58 (0.38- 0.89)	25.40	0.631 (0.519-0.742)
PCR	64	78 (61.9- 88.8)	54.20 (40.8- 67.0)	54.20 (40.8- 67.0)	78.00 (61.9-88.8)	1.7 (1.2-2.3)	0.4 (0.22-0.74)	32.20	0.661 (0.554-0.769)

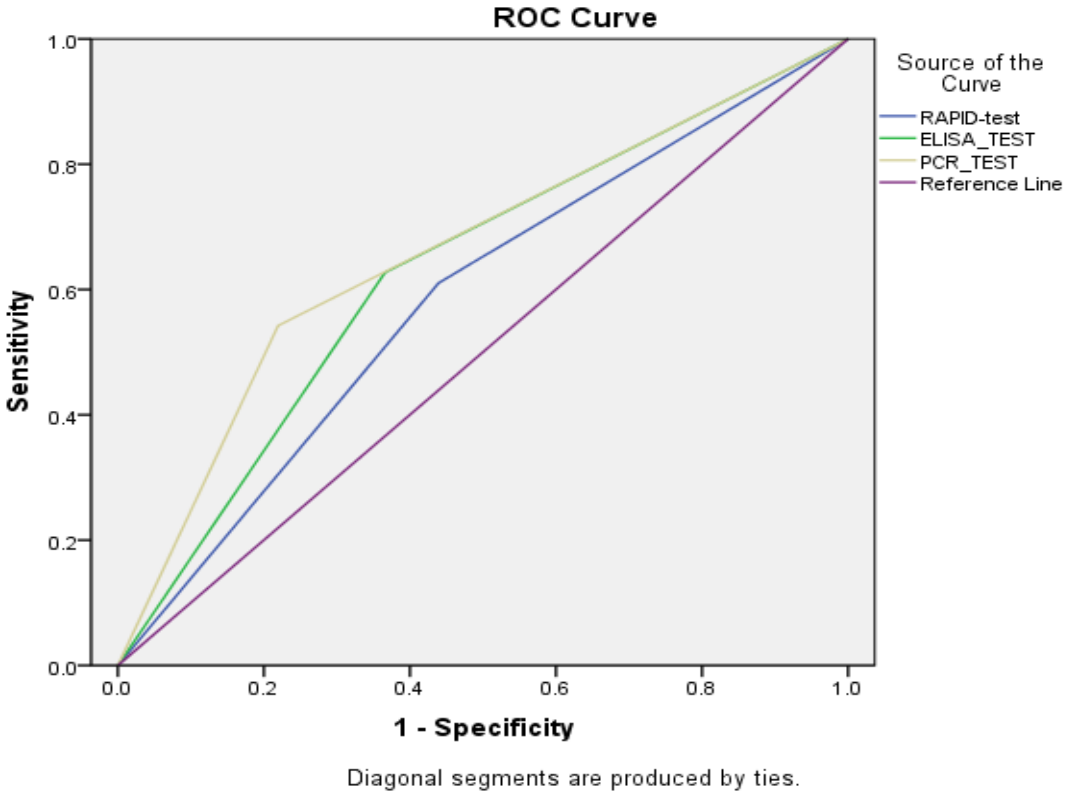
PPV, Positive predictive value; NPV, Negative predictive value; PLR, likelihood ratio for positive results; NPL, likelihood ratio for negative results; DA, Discrimination ability (PPV+ NPV-100)100%; AUC Area under the curve

Table (3): Diagnostic performance of the rapid strip test, ELISA and MZN as diagnostic methods for cryptosporidiosis compared to the PCR technique as the gold standard test among diarrheic children

	Diagnostic accuracy tests PCR as a gold standard method								
Variables	Diagnostic efficiency%	Sensitivity% (95%Cl)	Specificity% (95%Cl)	PPV% (95%Cl)	NPV% (95%Cl)	PLR (95%Cl)	NLR (95%Cl)	DA%	AUC
Rapid strip	48	44 (31.3- 57.5)	53.60 (37.6- 69.0)	57.70 (42.2-72.0)	40 (27.3-54.0)	0.94 (0.61-1.47)	1.04 (0.79-1.36)	16.40	0.497 (0.381-0.613)
ELISA	59	55.90 (42.4- 68.6)	63.40 (46.9-77.4)	68.70 (53.6-80.9)	50 (35.9-64.0)	1.52 (0.96-2.42)	0.69 (0.50-0.95)	18.70	0.597 (0.484-.710)
MZN	64	54.20 (40.8-67.0)	78 (61.9-88.8)	78 (61.9-88.8)	54.20 (40.8-67.0)	2.46 (1.32-4.60)	0.58 (0.43-0.78)	32.20	0.661 (0.554-0.769)

PPV, Positive predictive value; NPV, Negative predictive value; PLR, likelihood ratio for positive results; NPL, likelihood ratio for negative results; DA, Discrimination ability (PPV+ NPV-100)100%; AUC Area under the curve

Figure (2): Receiver Operating Characteristics (ROC) curve of predictors of cryptosporidiosis among diarrheic children, by using MZN as a gold standard

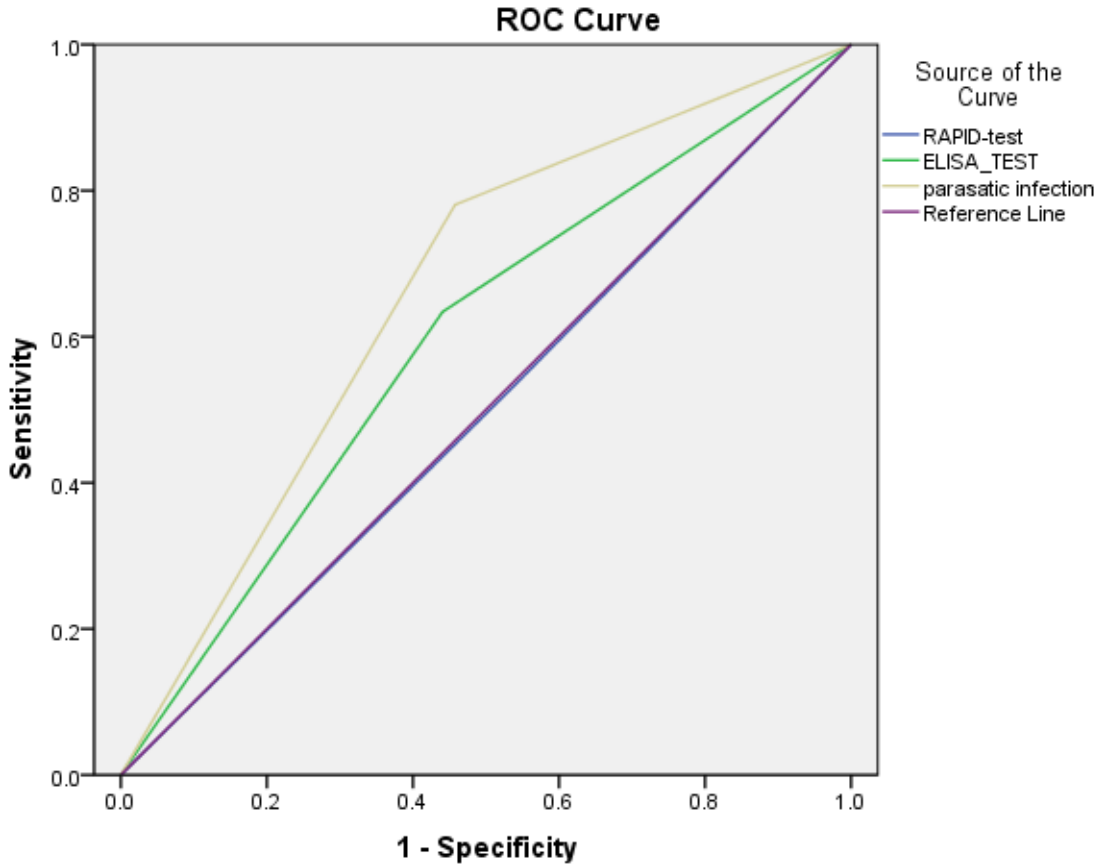


Asymptotic 95% Confidence Interval	
Lower Bound	**Lower Bound**
.471	.471
.519	.519
.554	.554

There was a significant difference between the sensitivities of the three tests, rapid strip test, ELISA and PCR (56%, 63.4% and 78% respectively) p=0.0336. However, the differences between the specificities of the used tests were not statistically significant. No statistical significance was found between the values of the PPV of the rapid strip test; ELISA and PCR that were 51.10%, 54.20%, and 54.20 respectively. As well as the difference between the values of the NPV of the rapid strip test, ELISA, and PCR (67.20%, 71.20% and78.0 percentage respectively) was insignificant. PCR technique as a gold standard (Table 1, 2, and Figure 3) illustrate the following results: The diagnostic performance of the rapid strip test; Among 100 diarrheic children, 45 (45%) had cryptosporidiosis, the sensitivity 44%, specificity 53.60%, PPV 57.70%, NPV 40.0% with overall diagnostic efficiency of 48%, while the PLR 0.94, NLR 1.04, DA 16.40% and AUC 0.497. The diagnostic performance of ELISA; Out of 100 diarrheic children, 48 (48%) had cryptosporidiosis. ELISA's sensitivity of 55.90%, specificity of 63.40%, PPV of 68.70%, NPV of 50.0% with overall diagnostic efficiency of 59%. The values of PLR, NLR, DA and AUC were 1.52, 0.69, 18.70% and 0.597 respectively.

The diagnostic performance of the MZN; Among 100 diarrheic children, 41 (41%) had cryptosporidiosis. The sensitivity was 54.20%, specificity 78.0%, PPV 78.0%, NPV 54.20% with overall diagnostic efficiency 64%, while the PLR 2.46, NLR 0.58, DA 32.20% and AUC 0.661. There was no significant relationship between the sensitivities of the three tests, rapid strip test, ELISA, and PCR. The specificities of the three tests were 53.60%, 63.40%, and 78.0% respectively and the difference was statistically significant. p= 0.0015. The values of the PPV of the rapid strip test, ELISA, and MZN were 57.70%, 68.70%, and 78.0 respectively. The difference was statistically significant. *p*=0.015. The values of the NPV of the rapid strip test, ELISA, and MZN were 40.0%, 50.0%, and 54.20% respectively. The difference was not statistically significant.

Figure (3): Receiver Operating Characteristics (ROC) curve of predictors of cryptosporidiosis among diarrheic children, by using PCR as a gold standard.



Diagonal segments are produced by ties.

Asymptotic 95% Confidence Interval	
Lower Bound	**Lower Bound**
.381	.381
.484	.484
.554	.554

DISCUSSION

Diarrhea is one of the major causes of infant and childhood mortality in developing countries as reported by the World Health Organization (WHO).[22] In the current study *Cyclospora cayetanens*is ranked the first among the diarrheic children followed by *Cryptosporidium*, this is in agreement with a previous study done in Iraq.[23] In contrast, *Cryptosporidium* among Nigerian infected children was the most prevalent coccidian followed by *Cyclospora cayetanensis*.[24]The comparison between assays described in this study for the one has the potential for accurate diagnosis in patients who do not presently know the reason for their diarrhea is of utmost importance. Microscopical analysis of stained fecal smears is the most widely used method for screening stool samples for *Cryptosporidium* in clinical diagnostic laboratories.[25]In the present study, the percentage of cryptosporidiosis among diarrheic children using rapid strip test was 45%. The diagnostic performance of the rapid strip test in the present study revealed a moderate sensitivity (56%) and specificity (62.70%). On the other hand, despite being valuable, field applicable method, rapid strip test showed lower PPV and NPV (51.1% and 67.2%, respectively) which may be a disadvantage for the epidemiological application of assay. So it could be considered as a complementary tool rather than a substitute

for microscopical examination. The rapid strip test could not detect all the positive samples; this may be due to the species specific type of anti-body used for *Cryptosporidium parvum* (*C. parvum*). However, rapid strip test could be used for screening in case of outbreaks of diarrhea for faster management of the problem.

Similarly, in a previous study done for the diagnosis of C. parvum, it was found that the percentage of infection by using rapid strip test was 56.23 %.[25]

The diagnostic accuracy test indicated a moderate performance (60%) as an indirect method for the diagnosis of cryptosporidiosis among diarrheic children.

Regarding ELISA, the present study showed that out of 100 diarrheic children, 48(48%) were ELISA positive. Sensitivity, specificity, PPV and NPV were 63.4%, 62.7%, 54.2%, and 71.2%, respectively. Such lower values could be due to inaccessible antigen (i.e. not recognized by antisera) as species of *C. parvum* has different strains. This is in accordance with previous studies, which found that immunologically based detection methods are not significantly more sensitive than conventional microscopy.[26,27]

On the contrary, Rosenblatt and Sloan, reported that the sensitivity, specificity and PPV of ELISA(in relation to IFA) were 93%, 99% and 99%, respectively. They attributed that sensitivity was slightly lower than the other values to the fact that the specimens were not processed before performing the ELISA, but were concentrated prior to IFA procedure.[28] However, it was concluded that the ease of use and costs are important criteria in determining the choice of the technique.[29]

Again, on the contrary to the present results, a study was done in Gaza, Palestine, among children attending Al-Nasser Pediatric Hospital, where single stool specimens from each of 416 children were examined by MZN (gold standard technique) and ELISA for the presence of *Cryptosporidium* oocysts. It was shown that 14.9% of children were diagnosed by acid-fast staining technique and 16.3% by using ELISA kit.[30]

The diagnostic efficiency level of ELISA was moderate (63%). The PLR was 1.69, which indicates that the probability of children who have the diarrhea was about two times more likely to have cryptosporidiosis than non-diarrheic children. AUC showed a sufficient level (0.631).Those diagnostic accuracy tests indicated a moderate performance as an indirect diagnosis of cryptosporidiosis among diarrheic children. This could be due to antigenic variability of clinical isolates of *Cryptosporidium.*

Using PCR in the current study, the percentage of cryptosporidiosis among diarrheic children was 59%, in relation to the sensitivity and specificity; they were 78% and 54.20%, respectively. PCR showed higher PPV and NPV (54.20% and 78.0%, respectively) i.e. it can detect four fifths of negative cases. Moreover, PCR has the advantage of being less influenced by observer variation bias as compared to microscopy-based methods.

In support of the current study, it was reported that PCR is a very sensitive (88.59%) and specific method. The authors also, reported that the sensitivity of microscopy was as less as 60% and microscopic method cannot distinguish pathogenic strains of intestinal protozoa from non-pathogenic ones.[25] However, PCR demands a more sophisticated laboratory, more complex operational efforts, and high cost. In addition it was reported that PCR sensitivity was 50% regarding schistosomiasis.(Farahat et al., 2009)[31], The reported missed cases after DNA amplification assay could be attributed to many factors such as: inhibition of the amplification reaction by fecal compounds and ⁄or DNA degradation during transportation from the field, variation in egg output and uneven distribution in feces. (Pontes et al., 2003). [32] Other authors also confirmed this finding even by using multiplex real-time PCR, Ten Hove et al., (2008).[33]

The diagnostic performance of the PCR in the present study revealed a relatively high sensitivity and moderate specificity (78.0% and 54.20%, respectively). The PLR was 1.7, which indicates that the probability of children who have the diarrhea was about two times more likely to have cryptosporidiosis than those who have no diarrhea. The diagnostic efficiency was relatively of high level (64%). Those diagnostic accuracy tests indicated a moderate performance as an indirect method for diagnosis of cryptosporidiosis among diarrheic children.

PCR technique as a reference test; in the present study, the diagnostic performance of the rapid strip test revealed poor sensitivity and moderate specificity (44.0% and 53.60%, respectively). The PLR and NLR were 0.94 and 1.04, respectively of no significant value. The Discriminating ability (DA) was of poor level (16.4%) and AUC was 0.497, which indicates that this test is not a useful test. Those diagnostic accuracy tests indicated a poor performance as an indirect indicator for diagnosis of cryptosporidiosis among diarrheic children.

Discordant to this study, it was reported that the PPV of crypto-strip was 85%. The sensitivity of crypto-strip was 78%, while its specificity was never lower than 98 % where, PCR was considered as a gold standard method for the detection of *C. parvum* infection.[25] Such discrepancy might be due to the use of different kits, or the coated antibodies to strips were non-specific.

The present study showed that ELISA sensitivity, specificity, PPV and NPV were 55.9%, 63.4%, 68.7% and 50 %, respectively. Similar results were obtained by authors who recommended the use of ELISA, as it is a fast, easy-to-read, and accurate method for the detection of *Cryptosporidium* in stool specimens.[30]

Higher results were shown in a study done for detection of *C. parvum* infection, it was reported that the PPV and sensitivity of ELISA were 99% and71%, respectively when PCR was considered as a reference method.[25] Another

study reported that ELISA sensitivity was 82% compared to microscopic methods as reference. They attributed decreased sensitivity to repeated freezing and thawing of specimens. [34]

The diagnostic performance of the ELISA in the present study revealed moderate sensitivity and specificity. The PPV, which indicates the probability of having cryptosporidiosis among diarrheic children was relatively high (68.7%).

Using MZN technique, the percentage of cryptosporidiosis among diarrheic children was 41% in the current study. The diagnostic performance of the MZN in the present study revealed a moderate sensitivity and high specificity (54.20% and 78.0%, respectively). Such higher values of both PPV and NPV (78.0% and 54.20%) were because MZN method confirms the diagnosis by tracing the parasites. On the contrary to the present work, it was found that the percentage of cryptosporidiosis was 26.86%. It was concluded that the microscopy is very specific but less sensitive method for the laboratory detection of *C. parvum* in feces.[25]

In Saudi Arabia, using a composite reference standard, in this case, true positives and negatives were considered as those with at least two positive or negative results out of the three tests. The microscopic test of a single stool specimen was less sensitive than either ImmunoCard STAT! or PCR methods of 85.7% (CI=56.2-97.5%) for *C. parvum*.[35]In the present work, the diagnostic efficiency of MZN was moderate (64%). The PLR was 2.46, which indicates that the probability of children who have the diarrhea was about 2.5 times more likely to have cryptosporidiosis than those without diarrhea. Those diagnostic accuracy tests indicated a relatively good value as a direct method for diagnosis of cryptosporidiosis among diarrheic children. Corroborating to that reported by Laude et al.,(2016)[36] microscopy is the reference standard for routine laboratory diagnosis in faecal parasitology, but there is a growing need for alternative methods to overcome the limitations of microscopic examination, e.g. the high dependence on expertise of the operator.

The variation of the validity of the diagnostic performance could be due to two main reasons. Firstly, the present study was limited since only one stool specimen was collected and examined. Previous studies had shown that repeated examination of more than one stool specimen over three consecutive days improve oocysts detection.[37]Secondly, the low sensitivity observed in all diagnostic tests could be due to the low intensity of the infection among the studied children.

CONCLUSIONAND RECOMMENDATIONS

Overall, the diagnostic performance of the three diagnostic methods used for the diagnosis of cryptosporidiosis among diarrheic children was of moderate values when using MZN as a reference technique. On the other hand the diagnostic performance of such tests ranged between relatively good values (MZN), moderate performance (ELISA) and a poor performance (rapid strip test) when using PCR as a reference test. The widely used microscopy is a less sensitive but very specific, cheaper method for the laboratory detection of *C. parvum* in faeces. However, PCR is particularly amenable to automation and large throughput processing. Moreover, modifications to the technique to reduce hands-on time, for PCR setup and amplification, e.g. tube test, loop isothermal amplification (LAMP) the cost could easily be further substantially reduced, thereby making PCR a more attractive option financially.

Conflict of Interest: None to declare.

***Funding*:** No financial support was received.

Acknowledgements

The authors wish to thank all the mothers of children who participated in the study. We wish to express our gratitude to the administrative officials of El-Shatby hospital who were very cooperative during the execution of the study. Thanks are also extended to the local health authority and those who work in the laboratories for supplying all the needed equipment and materials for field study and for supplying the required medications to treat infected children.

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