Journal of the Egyptian Society of Parasitology, Vol.52, No.2, August 2022
J. Egypt. Soc. Parasitol. (JESP), 52(2), 2022: 261 – 266
(Online: 2090-2549)

VALUE OF MICROSCOPY AND COPROANTIGEN FOR DETECTION OF CRYPTOSPORIDIUM AND GIARDIA IN STOOL SAMPLES

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Abstract

Giardiasis and cryptosporidiosis are common protozoa causing gastrointestinal troubles with symptoms ranged from asymptomatic to long-lasting diarrhea with mal-absorption. The present work was carried on a total of 96 cases; 62 of them presented with GIT symptoms with or without diarrhea, 50% of them was previously diagnosed as giardiasis or cryptosporidiosis infected and treated 1-2 months ago, 11 cases were free from GIT symptoms and 23 cases suffered from chronic debilitating disease as cancer or renal disease, some of them have previous history of gastro-intestinal disturbances.

The rapid immunochromatographic test (ICT) and copro-ELISA detected *G. lamblia* and *C. parvum* antigens in all fecal samples compared to formol ether concentration and Modified Ziehl-Neelsen (MZN) staining technique. The sensitivity of *G. lamblia* in stool samples was 60%, 98.3% & 96.6% using concentration technique, ICT & ELISA respectively, and specificity was 98.75%, 96.43% & 96.43% respectively. The sensitivity of *C. parvum* with MZN stain and antigen detection by ICT& ELISA was 46.2%, 96.4% & 100% respectively. Specificity of the tests in detecting *C. parvum* was better than *Giardia;* 96.4% -100% respectively. All tests have 100% PPV and 80-888% NPV for the immunological techniques with superiority of ICT over ELISA.

Keywords: Giardia, Cryptosporidium, ELISA, Coproantigen, ICT, MZN, formol ether concentration

Introduction

Giardia lamblia and *Cryptosporidium parvum* are common protozoa causing gastrointestinal infections (Ismail *et al*, 2018), manifested with a wide range of symptoms to asymptomatic with secretory diarrhea and malabsorption (El Bahnasawy *et al*, 2018). Both protozoa are transmitted through fecal-oral routes (Smith *et al*, 2006). In developing countries, poor water quality, crowded urban areas, and a lack of sanitation enhance infection transmission (Ramadan *et al*, 2020). O'connor *et al*. (2011) reported that cryptosporidiosis was identified in every continent except Antarctica. In USA, cryptosporidiosis incidence was relatively stable from 2008 to 2010, following a greater than threefold increase from 2004 to 2007 due to changing epidemiology or increased availability and applied diagnostic assays was unclear (CDC, 2012). Contraction of infections was more prevalent in children in one-day care centers (Núñez *et al,* 2003). These protozoa parasites are the main cause diarrhea with malabsorption that has a negative impact on growth and development among children (Ismail *et al,* 2018).

Conventional microscopic diagnosis of *Giardia* and *Cryptosporidium* infections is not accurate, time-consuming and relies mainly on the microscopic skills and experience. The rapid antigen detection techniques have the advantage of more dependent, precise, and simple with high sensitivity in patients with low parasitic numbers (Hooshyar *et al,* 2019).

The present study aimed to evaluate sensitivity and specificity of the formol ether concentration, MZN staining and both immunochromatographic assay and ELISA in detecting coproantigen of giardiasis and cryptosporidiosis.

Materials and Methods

A total of 96 outpatients attending clinics of 6th October University Hospital from April 2020 to December 2020 were enrolled. They were divided into 3 groups: GI: (symptomatic) included 62 cases complained with of gastrointestinal symptoms with or without diarrhea. 50% of them were previously dia-

gnosed as giardiasis or cryptosporidiosis infected ant treated 1-2 months ago. GII: (asymptomatic) included 11 cases that were free from GI manifestations attended outpatient clinics for other medical problems, and GIII: (immunocompromised) included 23 cases suffered from debilitating diseases as cancer or chronic renal failure. They were either active or with previous history of gastrointestinal disturbances.

Fresh fecal specimens were collected in dry, clean plastic containers with tight-fitting lids. Each container was labeled with the patient`s name, date of collection and a serial number. Stool samples were divided into three parts; first part was subjected to formol ether concentration technique (Hernández-Arango *et al,* 2019) to detect *Giardia* spp. and stained MZN stain to detect of *Cryptosporidium* spp. (Abdel Gawad *et al,* 2018).

Second part of stool samples was immunologically examined by antigen detection using immunochromatographic strip test (ICT): (RIDA QUICK *Cryptosporidium/Giardia* co mbo) (r-biopharm, Germany), commercially available as quick in vitro immunochromatographic assay to determine qualitative *C. parvum* and/or *G. lamblia* antigen in feces.

Third part of stool samples was examined by using commercially available *Cryptosporidium /Giardia* (REF) detection ELISA kit to detection their antigen in fecal specimens. Steps and interpretation of both immunological methods were done according to manufacturer's instructions.

Ethical considerations: The study design was approval by the Research Committee Unit, Faculty of Medicine, 6th October University. Patients were informed verbally about the study purpose and written consents were taken before collection of samples

Statistical analysis: Data were collected, tabulated and analyzed by using statistical package of social science (SPSS) software; version "PASW® Statistics 18" (SPSS Inc., Chicago). Numerical data were expressed as mean or range as appropriate, and qualitative ones were expressed as frequency and percentage. Chi-square test examined the relation between qualitative variables (Chan, 2003a). P-value < 0.05 was considered statistically significant association, but > 0.05 was insignificant (Chan, 2003b). Microscopic and immunologic results of *Giardia* and *Cryptosporidium* were compared with the reference standard for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) after (Galen, 1980). The true-positives of *Giardia, or Cryptosporidium* were the samples that gave positive results by at least two of the three methods; microscopic concentrated samples or MZN, ICT & ELISA. True negatives were samples negative by three methods specified for each one.

Test sensitivity = a/(a+c); test specificity = d/(b+d); PPV = a/(a+b); NPV = d/(c+d), where a = true positive, b = false positive; c = false negative; d = true negative.

Results

Giardiasis GI showed the highest efficacy (95.16%) antigen detection by ELISA followed by ICT (59.1%) and lastly (48.3%) by formol-ether concentrated test. Least was in GIII 8.7%, 78.26% & 47.82% by the three techniques respectively. GII showed 1-3 positive ones using the three techniques. *Cryptosporidium* infection rate percentage in GII was lower than in *Giardia* infection.

Cryptosporidium by using traditional MZT and ICT & ELISA antigens showed that GI infection rate was 4.8%, 35.48%, & 37.1%, respectively, and in GIII was 65.2%, 21.7%, & 4.35% respectively.

Sensitivity of the tests in detecting *Giardia* copro-antigen in stools was 60%, 98.3% & 96.6%, and specificity was 98.75%, 96.43% & 96.43% respectively. Sensitivity of MZN in detecting *Cryptosporidium* was 46.2%, and antigen detection was 96.4 & 100% respectively. Specificity tests in detecting *Cryptosporidium* was better (96.4%-100%) than for *Giardia*. All tests gave PPV (100%) and acceptable NPV (80-88.8%) for immunology with superiority of ICT over ELISA.

Giardiasis patients suffered from abdomenal pain (55.9%), followed by the distension

(42.3%), and then diarrhoea (25.4%). Cryptosporidiosis patients suffered from diarrhoea (66.6%), abdominal pain (59.2%) and least was abdominal distension, with more than one complaint.

Details were given in tables (1, 2, 3 & 4)

Table 1: Efficacy of concentration and immunological techniques in detecting *Giardia* in stools

Investigated groups	GI (n=62)		GII (n=11)		GIII (n=23)		Total (N= 9)	
Evaluated techniques	No. +v e	%	No. +ve	%	No. +ve	%	No. +ve	%
Formol-ether conc. Tech.	30	48.3	1	9.0	2	8.7	33	53.22
ICT (RIDA QUICk)	37	59.6	3	27.2	11	47.82	51	53.68
Antigen detection ELISA	59	95.16	3	27.2	18	78.26	80	84.2

Table 2: Efficacy of staining and immunological techniques in detecting *Cryptosporidium* in stools

Investigated groups	GI (n=62)		GII (n=11)		G III (n=23)		Total (N= 9)	
Evaluated techniques	No. +ve	%	No. +v e	%	No. +v e	%	No. +v e	%
MZN Technique	3	4.8	0.0	0.0	15	65.2	18	18.75
ICT (RIDA QUICk)	22	35.48	0.0	0.0	5	21.7	27	27.42
ELISA	23	37.1	3	27.2	1	4.35	27	27.42

Table 3: Sensitivity and specificity of both immunological techniques in diagnoses of infection

Variations	Giardiasis patients			Cryptosporidiosis patients		
	Conc. Tech	ICT	ELISA	MZN	ICT	ELISA
Sensitivity %	60%	98.3%	96.6%	46.2%	96.4%	93.1%
Specificity %	98.75%	96.43%	96.43%	100%	96.43%	100%
PPV	100%	100%	100%	100%	100%	100%
NPV	65.8%	88.8%	80.0%	73.0%	88.8%	80.0%

Table 4: Gastro-intestinal manifestations among *Giardia* and *Cryptosporidium* positive patients

Patient complaints	Giardiasis (n = 9)		Cryptosporidiosis (n=27)	
	No.	%	No.	%
Abdominal pain	33	55.9%	16	59.2 %
Abdominal distension	25	42.3 %	14	51.8%
Diarrhoea	15	25.4%	18	66.6%
P-value	0.112			

Discussion

Generally, giardiasis and cryptosporidiosis are the most frequent causes of intestinal protozoa infections (Ismail *et al,* 2018). Both parasites are zoonotic diseases in rural areas in developing countries due to canal water contamination with raw sewage of animals, lack of water treatment, and use of animal manure fertilizer in agriculture (Ramadan *et al,* 2020). The increase of contact between humans and animals in the rural community was responsible for the higher prevalence of infection by zoonotic *Giardia* assemblages from humans to animals or vice versa (Sabry *et al,* 2009). Diagnosis of infection by conventional microscopic techniques is time consuming and relies mainly on microscopes' skills. Also, accurate microscopic examination must be on three successive stool samples or more (Bayoumy *et al,* 2010). Development of new immunological techniques specially those depends on detection of the specific parasite antigens in stool have several advantage of being more precise, simple with high sensitivity especially for diagnosis of chronic infection with low parasitic numbers (Squire and Ryan, 2017).

In the present study, Diagnosis by immunological methods showed high superiority concerning the specificity in diagnosis of *Giardia* infection than the other traditional techniques, with high efficacy for ELISA (95.16%) than ICT (59.1%) associated with low values (48.3%) using formol-ether concentrated technique in patients suffered from digestive disturbances. Immunocompromised patients showed lower infections; 8.7%, 78.26% & 47.82% using the three techniques respectively. However, one-three cases were false diagnosed in control ones. Values were significantly different (P/0.05) using MZN techniques and the immunological methods previously used in the study in detection of *Cryptosporidium* in the same stool samples. It was 4.8% using MZN, 35.48% by ICT and 37.1% by ELISA. The infection rate was different in GIII as it was 65.2%, 21.7% & 4.35% using the above techniques

respectively. The *Cryptosporidium* detection percentage in GII was lower than in *Giardia*.

The sensitivity of copro-antigen to detect *Giardia* in stool samples was 60%, 98.3% and 96.6% using concentration technique, ICT & ELISA respectively, and specificity was 98.75%, 96.43% & 96.43% respectively. The sensitivity of MZN in diagnosis of Cryptosporidium was 46.2% while it was 96.4 for ICT and 100% for ELISA and their specificity was better (96.4%-100%) than that recorded for *Giardia*.

The high sensitivity and specificity of both ICT and ELISA tests against *Giardia* agreed with several authors. Pillai and Kain (1999) Toronto Hospital, Canada recorded the sensitivity and specificity for ICT compared to reference microscopy as 83% and 100% respectively in 40 diarrheic patients attended tropical unit. Garcia and Shimizu (2000) in California reported 100% sensitivity and specificity for ICT assay against *Giardia* in diarrheic patients in. Previously, Garcia and Shimizu (1997) reported sensitivities of Enzyme Immune Assays (EIAs) for antigen detection ranged from 94 to 97% and specificities ranged from 99 to100% against *Giardia.*

In the present study, specificity of *Cryptosporidium* infection diagnosis was better (96.4%-100%) than that for Giardia. This agreed with El Shazly *et al* (2002) who studied the evaluation of different methods for the detection of *Cryptosporidium* and they found that the MZN had the lowest sensitivity in relation to either ICT, ELISA. Also, Regnath *et al.* (2006) examined the RIDA Quick assays with 27 stool samples positive for *Cryptosporidium* by EIA or microscopy. They illustrated that all the specimens were also positive by RIDA Quick assays, weak visible bands were seen for samples with lower numbers of oocysts.

Also, Garcia and Shimizu (2000) in Canada found sensitivity of 98.8% and specificity of 100% when they examined *Cryptosporidium* using immunochromatographic assay. Also, Tuli *et al.* (2010) reported that ELISA had a sensitivity of 93.25% and specificity of 97% against *Cryptosporidium*.

In the present work, all the 3 tests had a very high applicability as they had absolute PPV (100%) and acceptable NPV (80-88.8%) for the immunological techniques mainly with superiority of ICT over ELISA. These results were applied for both *Giardia* and *Cryptosporidium.* Also, Abdel Hameed *et al. (2008)* investigated RIDA Quick ICT for diagnosis of cryptosporidiosis as compared to MZN. Sensitivity, specificity, PPV and NPV of RIDA Quick test were 96.4%, 100%, 100% & 98.8% respectively.

The high percentage of infection by both parasites might be related to the easy transmission of the infective stages to the patients via contaminated food (Sabry *et al*, 2009) and water (Almeida, *et al,* 2015). However, they referred the high percentage of *Giardia* and *Cryptosporidium* might be related to the infective stages were non-host specific and their infective stages had special ability to survive and tolerate wide range of temperature and humidity (Ehsan *et al*, 2015).

The superiority of immunological diagnostic test over the traditional microscopic methods might be related to similarity of symptoms with other causes of gastro-intestinal disturbance (Sabry *et al*, 2009) and the presence of low number of diagnostic stages in chronic cases. In addition, the experiences of the investigators might play important role in miss diagnosis of the parasites (Squire and Ryan, 2017). They related the high percentage of infection by *Giardia* in immuno-compromised cases to the un-apparent chronic infection specially most of the diagnosed cases were adults not children. Moreover, the immunological diagnostic kits used to diagnose antigens of both parasites might interact for a degree with the obtained results especially it gave false diagnosis for three cases in the control (non-infected) group.

In the present study, in giardiasis abdominal pain was the commonest one (55.9%) followed by abdominal distention (42.3%), but diarrhea was in 25.4%. These agreed

with Heresi *et al.* (2000) who reported that abdominal pain was 55-80% in *Giardia* infected patients. Muller and Allmen (2005) reported that abdominal pain was the commonest clinical one. Younas *et al.* (2008) added that this sign was in most cases. However, Gendrel *et al.* (2003) reported that giardiasis was asymptomatic self-limited disease.

In the present study, in cryptospordiosis diarrhea was the commonest one (66.6%) followed by abdominal pain (59.2%), and abdominal distention (51.8%). This agreed with Tumwine *et al.* (2003) in Uganda found that diarrhea in cryptospordiosis hospitalized children was 25%. Al Braiken *et al*. (2003) in Saudi Arabia detected oocysts in 32% of diarrheic children, but 4.7% were asymptomatic ones, and Gatei *et al.* (2006) who reported that cryptosporidiosis was significantly associated with persistent diarrhea and abdominal pain. El Shazly *et al.* (2007) in Egypt found oocysts in canals' water used for plant irrigation and animal consumption. Davies and Chalmers (2009) in UK reported that *C. hominis* infections were associated with foreign travel and in day-care cases, but *C. parvum* was associated with animal contact. Al-Shamiri *et al*. (2010) in Yemen found 38.4% of diarrheic patients. Helaly *et al*. (2012) in Cairo detected oocysts in 11.3% diarrheic patients. However, O'connor *et al*. (2011) reported that cryptosporidiosis was identified in all the continents except Antarctica.

Conclusion

The copro-antigen detection of *Giardia* and *Cryptosporidium* by immunological diagnostic kits (ICT & ELISA) proved to be an accurate diagnostic method than other traditional ones. Species and subtyping identification are important for outbreak, epidemiology, burden assessment, and transmission.

Nevertheless, specific stained microscopically is cheap protozoa in massive diagnosis.

Conflict of interests: The authors declared that they neither have conflict of interests nor received fund.

Authors' contribution: All authors equally contributed in the field and laboratory work.

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