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FEASIBILITY OF A RAPID LATERAL FLOW TEST FOR SIMULTANEOUS DETECTION OF *GIARDIA LAMBLIA* AND *CRYPTOSPORIDIUM PARVUM* IN DUODENAL ASPIRATES OF PATIENTS SUFFERING FROM CHRONIC LIVER DISEASES AND ELIGIBLE FOR UPPER ENDOSCOPY

By
MARWA A. ELMALLAWANY[1], MOUSA A. MOUSA ISMAIL[1], SAMAR S. ATTIA[1]*, RASHA AHMED[2], MOHAMED N. ALKADY[2], SHAIMAA ELKHOLY[3], AHMED SHAKER[3], DINA O. HELMY[4], AND REHAM K. NAHNOUSH[1]
Department of Medical Parasitology[1], Department of Tropical Medicine[2], Department of Internal Medicine[3], and Department of Pathology[4], Faculty of Medicine, Cairo University, Cairo, Egypt (*Correspondence: ssattia@kasralainy.edu.eg)

Abstract
Chronic liver diseases (CLDs) represent an important health issue in developing countries and are commonly associated with impaired immunity. This increases the susceptibility to various infectious agents including parasitic infections, which should be properly managed to avoid life threatening complications. This study assessed the feasibility of rapid, easy and applicable screening test for *Giardia* and *Cryptosporidium* within 150 CLDs patients suitable for upper endoscopic examination. Stool samples, duodenal aspirates and duodenal biopsies were examined for *G. lamblia* and *C. parvum* by different diagnostic techniques. The results showed stool microscopy (13.3% & 7.3%), duodenal aspirate microscopy (5.3% & 4.7%), rapid lateral flow immune-chromatographic assay (RLFIA) applied on duodenal aspirate samples (16.7% & 10%), duodenal biopsies histopathological examination (6.7% & 5.3%) and direct fluorescent antigen detection in stools (16.7% & 9.3%) for giardiasis and cryptosporidiosis respectively. The high sensitivity of lateral flow immune-chromatographic assay in detecting *Giardia* and *Cryptosporidium* in duodenal fluid samples proved a good screening test for these patients.

Key words: Egypt, Chronic liver disease patients, *Giardia*, *Cryptosporidium*, Immunofluocence, duodenal aspirate, rapid immune-chromatography.

Introduction

The liver is crucially involved with parasitic infections causing significant morbidity and mortality (Peters *et al*, 2021). Globally, more than 800' millions cases deprived of that blessing, suffering from liver diseases**.** Experiencing such distressing condition for a long period of time exposes these patients to serious complications, including infection with various infections, with about 2 million annually (Marcellin and Kutala, 2018). Also, chronic liver diseases (CLDs) commonly accompanied by reduced cell-mediated immunity which may intensify the susceptibility to infection (Yu *et al,* 2011; Mousa *et al,* 2014). The endoscopic modality was a must for CLDs complications in expected patients not only for diagnosis, but also urgent treatment interventions (Krystallis *et al,* 2012).

Giardia lamblia and *Cryptosporidium parvum* are waterborne pathogens associated with diarrhea mainly in developing countries (Yakoob *et al*, 2010). Both are among the commonest infectious protozoa infected the CLDs' patients**,** contributed significantly to complicated diarrheal illness (Savioli *et al,* 2006). Severity and prognosis of giardiasis and cryptosporidiosis depend on the host immunity comprising innate and adaptive immunity (Ryan *et al,* 2013). These protozoal infections in immunocompetent individuals were presented with self-limited diarrhea or short-term gastroenteritis (Squire and Ryan, 2017). Manifestations may extend up to 2 weeks followed by self-recovery without anti-*Cryptosporidium/Giardia* treatment (Ryan *et al,* 2016). Conversely, immunocompromised individual, without cryptosporidiosis treatment, usually endured intractable fatal diarrhea (Azcona-Gutie´rrez *et al,* 2017).

Conventional diagnosis of giardiasis and cryptosporidiosis often rely on morphologic al identification of *Giardia* cysts and/or trophozoites and *Cryptosporidium* oocysts in stool samples using microscopy whether directly or using stains including Lugol's iodine, and Acid Fast (Thompson and Ash, 2019). Fecal microscopic examination was the traditional diagnostic method long ago, but being time-consuming and needed experted technician (Mathison *et al,* 2020). Alternative immunodiagnostic tools were developed to diagnose giardiasis and cryptosporidiosis as immunofluorescence assays, fecal antigen ELISA and Rapid lateral flow immunochromatographic assays **(**Adeyemo *et al,* 2018). Besides, the duodenal aspirates and the intestinal biopsy aided protozoal diagnosing (El-Hady and Abd-Elmaged, 2018).

Few studies paid attention to the prevalence of giardiasis and cryptosporidiosis infection in CLDs patients subjected to upper endoscopy. So, this study aimed to determine the prevalence of these two gastrointest inal protozoal parasites affecting such patients, by using the rapid lateral flow immune-chromatographic assay on duodenal aspirates and comparing the outcomes results with variable diagnostic tools to find the dependable one.

Patients and Methods

This study was done in Endoscopy Unit, Faculty of Medicine, Cairo University between June 2018 and June 2019. Patients were 150 with chronic liver diseases (CLDs) eligible for upper endoscopy and thus enrolled, after signing informed consents.

Clinical sheets were filled out on each patient. Endoscopy standardization, endoscopic investigations and sample collection were done by the gastroenterologist using a conventional forward-viewing endoscope (GIF 140-Olympus, Hamburg, Germany).

For histopathological examination: Intestinal and duodeno-jejunal (About 1 to 2ml drained by the endoscopic tube) flexure biopsies were taken and fixed in 10% formalin.

Histopathological examination: Biopsied materials were processed for histopathological studies after staining with hematoxylin and eosin and modified Ziehl-Neelsen (ZN) stain (Wahnschaffe *et al,* 2007).

Microscopic examinations: Fresh stool samples collected from patients were examined as direct wet-mount smears with or without iodine stain and formalin-ethyl acetate (FEA) sedimentation concentration. The duodenal fluids wet mounts were immediately microscopical examined for *Giardia lamblia* trophozoites and/or cysts and *Cryptosporidium parvum* oocysts (Garcia *et al,* 2018).

Rapid lateral flow immunochromatographic assay (RLFIA): After the manufacturer's instructions. RLFIA kit (RIDA®QUICK *Cryptosporidium*/*Giardia* Combi (cassettes), R-Biopharm, Germany) was used. RLFIA technique was based on specific monoclonal mouse antibodies against *G. duodenalis* and *C. parvum* antigens existed in the developmental stages on the duodenal fluid samples.

Direct fluorescent antigen assay (DFA): *G. lamblia* cysts and *C. parvum* oocysts in stool samples were examined by fecal direct fluorescent antigen (DFA) detection Kit (IVD Research Inc. Carlsbad, CA 92010 USA), after the manufacturer instructions.

Statistical analysis: Data were analyzed using SPSS© Statistics version 24. Numerical as mean and difference between groups was compared by using Mann-Whitney test. Categorical variables were presented as number and percentage. Chi-square test, Fisher's exact test, and Likelihood ratio tests compared nominal data and the chi-squared test compared ordinal data. *P*-value was considered significant if $p < 0.05$.

Results

The CLDs patients were 94 males & 56 females with age range 17 to 77years with mean of 45.76. The highest diagnostic value was obtained by RLFIA in duodenal aspirate for giardiasis (16.7%) and cryptosporidiosis (10%), but lowest ones were by duodenal aspirates microscopic examination (5.3%) and (4.7%) for giardiasis and cryptosporidiosis respectively. Also, six cases were *Giardia & Cryptosporidium* positive by fecal microscopy and other diagnostic tests. Besides, microscopic examination of stools showed *Ent-*

amoeba histolytica in 13(8.67%) patients, *Entamoeba coli* in 5(33.33%) and *Blastocystis hominis* in 3(2%).

In the present study, direct fluorescent antigen assay (DFA) was used as the gold diagnostic standard. So, RLFIA giardiasis and cryptosporidiosis of duodenal aspirate gave high sensitivity 96% & 92.86%, and specificity 99.2% & 98.53% respectively. But, duodenal aspirate microscopy gave the least sensitivity 32% & 50% respectively, but 100% specificity, with significant differences (P <0.0001). Cohen's Kappa values showed a significant positive agreement between duodenal aspirates microscopic examination, stool microscopic examination, duodenal biopsies by histopathological examination or by RLFIA (Cohen's Kappa value 0.44, 0.526, 0.87 & 0.952) for giardiasis and for cryptosporidiosis (0.645, 0.707, 0.869 & 0.885). RLFIA showed an excellent agreement for giardiasis and cryptosporidiosis. The highest incidence for giardiasis and cryptosporidiosis was in patients of age group 40-60years, without significance differences (P 0.007).

Males showed high giardiasis and cryptosporidiosis incidence than females, but without significant difference (P= 0.546 & 0.571, respectively). Diarrhea, abdominal pain, nausea, vomiting, weight loss, fatigue and fever were encountered in giardiasis and cryptosporidiosis positive cases with significant difference than negative cases. Abdominal pain was the commonest clinical pictures in all. Liver enzymes (AST & ALT) & serum albumin didn't show significant difference among negative and positive cases. Endoscopic duodenal examination of the CLDs patients showed duodenitis in 6/25 (24%) giardiasis and in 5/14(35.7%) cryptosporidiosis with significant difference (P <0.0001).

Details were given in tables (1, 2, 3, 4 & 5) and figures (1 & 2).

Table 1: Diagnostic yield of different techniques for giardiasis and cryptosporidiosis.

Parasite	Stool microscopy	DFA (Stool samples)	Microscopy of duodenal aspirate	Histopathology of duodenal biopsies	RLFIA of duodenal aspirate
Giardia duodenalis	20/150 (13.3%)	25/150 (16.7%)	8/150 (5.3%)	10/150 (6.7%)	25/150 (16.7%)
Cryptosporidium parvum	11/150 (7.3%)	14/150 (9.3%)	7/150 (4.7%)	8/150 (5.3%)	15/150 (10%)

Table 2: Sensitivity, specificity, positive predicative value (PPV), negative (NPV) and Kappa agreement for giardiasis.

Diagnostic test used		DFA		Kappa Agreement value	*P-value*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		Negative	Positive						
Stool microscopy	Negative	12 (96.2%)	5 (3.8%)	0.87	<0.0001	80%	100%	100 %	96.15 %
	Positive	0 (0%)	20 (100%)						
microscopy of duodenal aspirate	Negative	125 (88%)	17 (12%)	0.44	<0.0001	32%	100%	100 %	88.03 %
	Positive	0 (0%)	8 (100%)						
histopathology of duodenal biopsies	Negative	125 (89.3%)	15 (10.7%)	0.526	<0.0001	40%	100%	100 %	89.29 %
	Positive	0 (0%)	10 (100%)						
RLFIA of duodenal aspirate	Negative	124 (99.2%)	1 (0.8%)	0.952	<0.0001	96%	99.2%	96%	99.2%
	Positive	1 (4%)	24 (96%)						

Table 3: Sensitivity, specificity, positive predicative value (PPV), negative (NPV) & Kappa agreement for cryptosporidiosis.

Diagnostic Test		DFA		Kappa agreement value	*P-value*	Sensitivity (%)	Specificity (%)	PPV (%)	NPP (%)
		Negative	Positive						
Stool microscopy	Negative	136 (97.8%)	3 (2.2%)	0.869	<0.0001	78.57%	100%	100%	97.84%
	Positive	0 (0%)	11 (100%)						
microscopy of duodenal aspirate	Negative	136 (95.1%)	7 (4.9%)	0.645	<0.0001	50%	100%	100%	95.1%
	Positive	0 (0%)	7 (100%)						
histopathology of duodenal biopsies	Negative	136 (95.8%)	6 (4.2%)	0.707	<0.0001	57.14%	100%	100%	95.77%
	Positive	0 (0%)	8 (100%)						
ICT of duodenal aspirate	Negative	134 (99.3%)	1 (0.7%)	0.885	<0.0001	92.86%	98.53%	86.67 %	99.26%
	Positive	2 (13.3%)	13 (86.7%)						

Table 4: Age distribution of patients (n=150)

Age groups	Giardiasis		Cryptosporidiosis	
	Negative	Positive	Negative	Positive
< 40 years	9 (7.2%)	7 (28%)	16 (11.8%)	0(0%)
40-60 years	75 (60%)	13(52%)	79 (58.1%)	9 (64.3%)
> 60 years	41 (32.8%)	5 (20%)	41 (30.1%)	5 (35.7%)

Table 5: Endoscopic findings following duodenal examination of cases (n=150)

	Giardiasis		Cryptosporidiosis	
	Negative	Positive	Negative	Positive
Duodenitis	109 (87.2%)	6 (24%)	110 (80.9%)	5 (35.7%)
Multiple red spots	6 (4.8%)	2 (8%)	8 (5.9%)	0 (0%)
Normal mucosa	10 (8%)	17 (68%)	18 (13.2%)	9 (64.3%)

Discussion

Chronic liver disease is prolonged pathological condition characterized by ongoing destruction of liver parenchyma with gradually replaced by fibrous tissue (Kurokawa and Ohkohchi, 2018). In Egypt, endemic zoonotic giardiasis was reported (Helmy *et al,* 2009), genotyping was done (El Shazly *et al,* 2004), and usefulness and reliability of simple species-specific primers was a must to genotype *Giardia* spp. and mixed infect-ions detection (Abd El-Latif *et al,* 2020). Also, zoonotic crypotosporidiosis was repo-rted in man (Youssef *et al,* 2008), immunocompetent patients (Massoud *et al,* 2008) as well as farm animals (Mahfouz *et al,* 2014).

In the present study, several techniques were used to detect *G. lamblia* and *C. parvum*. They included microscopic examination for stool samples and duodenal aspirates, direct fluorescent antigen technique for stool samples (DFA), rapid lateral flow immune-chromatographic assay (RLFIA) for duodenal aspirates and histopathological examination of duodenal biopsies. Weitzel *et al.* (2006) attributed the difficulty in comparing various diagnostic techniques without a factual reference standard. Microscopic examination of stool samples was the reference one for diagnosis of intestinal protozoal infections, but technique was time-consuming and require operators who were experienced and well trained (Autier et al, 2018).

In the present study, DFA achieved 100% sensitivity and specificity in validation human studies as reference standard for assessing other diagnostic techniques (Kehl *et al,* 1995; Garcia and Shimizu, 1997; Johnston *et al,* 2003, El-Nahas *et al,* 2013, Elsafi *et al,* 2014; Roellig *et al,* 2017).

In the present study, DFA was used as the gold standard test, 25/150 chronic liver disease were giardiasis positive (16.7%), & 14 cryptosporidiosis positive (9.3%). Roberts-Thomson *et al.* (1982) in Austria found giardiasis in liver biopsy of a patient with portal tracts granulomas and cholangitis with chronic diarrhea, weight loss, fever, hypoalbuminemia, and anemia. Aronson *et al.* (2001) in USA reported biliary giardiasis in an HIV patient. But, relatively higher cryptosporidiosis was among CLDs patients. Shrestha *et al.* (1993) found cryptosporidiosis in 20%. Yu *et al.* (2011) detected *Cryptosporidium* oocysts in 6% patients suffered from acute on chronic of liver failure and 0.8% of chronic HBV patients. Mousa *et al.* (2014) reported higher *Cryptosporidium* infection, (32%, 22% & 36%) among patients suffered from hepatocellular carcinoma, liver cirrhosis without ascites and liver cirrhosis with ascites respectively.

In Egypt, cryptosporidiosis was detected in 9.5 % & 2.5% of acute and chronic HBV patients respectively (Ramadan *et al,* 2015). Also, Shahat *et al.* (2020) reported cryptosporidiosis by molecular techniques in 3.3% patients with liver cirrhosis and hepatocellular carcinoma whom were microscopically negative. Variation of cryptosporidiosis prevalence in different studies was attributed to the patients' immune status (Yu *et al,* 2011).

Generally, liver disease as hepatitis don't increase susceptibility to infections without being associated with progressive liver cirrhosis, which causes impairment of cellular immune response rendering patients more susceptible to opportunistic parasite as cryptosporidiosis (Shahat *et al,* 2020).

In the present study, stool microscopy detected 20/25 proved giardiasis by DFA with 80% sensitivity, 100% specificity, 100% positive predictive value (PPV) & 96.15% negative predictive value (NPV). Doğruman *et al.* (2006**)** found that microscopic examination gave 85.7% sensitivity, 77.8% specificity, 81.1% PPV & 22.2% NPV when compared to DFA. Lower microscopic examination for giardiasis sensitivity was 66.7% (Hanson and Cartwright, 2001), 88% (Branda *et al,* 2006), and 82.2% (Baig *et al,* 2010). El-Nahas *et al.* (2013) used immunofluorescence as gold standard method, reported 76.9% sensitivity & 100% specificity. Jahan *et al.* (2014) for *Giardia* cysts reported a sensitivity of 46% for a single stool examination, which agreed with Rasmussen *et al.* (2016).

In the present study, stool microscopy for *Cryptosporidium* oocysts by ZN stained identified 3/14 confirmed DFA cases with 78.5% sensitivity, 100% specificity, 100% PPV & 97.84% NPV. Khurana *et al.* (2012) found 79.06% sensitivity & 100% specificity with MZN stained smears. Elsafi *et al.* (2014) reported 66.67% and 88.24% sensitivity and specificity respectively by acid fast stained smears compared to DFA. Blanshard *et al.* (1992**)** found that a single stool examination diagnosed only 30% of *Cryptosporidium* infection. Thus, DFA showed higher sensitivity than the traditional staining procedures in diagnosing cryptosporidiosis with or without concentrated fecal samples. The variable accuracies of acid-fast stain were attributed to variations in number of oocysts excreted in feces or stool specimens. The false negative of acid-fast technique data were in specimens with low parasite numbers (Elsafi *et al,* 2014). Also, false positives data were due to poor stain up taken by oocysts, caused the difficult of distinguishing *Cryptosporidium* oocysts from other spherical non-cryptosporidial acid-fast organisms (Khurana *et al,* 2012).

In the present study, microscopy of duodenal aspirate detected 8 giardiasis (32% sensitivity & 100% specificity), and 7 cryptosporidiosis (50% sensitivity & 100% specificity). Many studies showed that microscopic examination of duodenal aspirate gave lower sensitivity than stool microscopy for diagnosing giardiasis and cryptosporidiosis. Suzuki *et al.* (1994) detected *Giardia* trophozoites in 9 samples of duodenal aspirate and *Giardia* cysts in 20 stool samples. Wahnschaffe *et al.* (2007) diagnosed giardiasis by microscopy in 3 duodenal aspirates out 16 DFA-giardiasis confirmed cases with lower sensitivity (19%). They concluded that duodenal aspirate examination was inferior to stool microscopy in giardiasis & cryptosporidiosis diagnosis. But, Mahdi and Taha (2002) found that duodenal fluid microscopic examination for giardiasis and cryptosporidiosis was superior to stool examination, as *Giard ia* trophozoites were detected in duodenal aspirates of 15 patients, compared to 5 giardiasis positive for by stool examination. Also, they identified 4 cryptosporidiosis in duodenal aspirates compared to 2 cases by stool examination.

In the present study, histopathological examination of duodenal biopsies showed sensitivities of 40% & 57.14% in diagnosis of giardiasis and cryptosporidiosis respectively, with 100% specificity for both parasites. The histological examination sensitivities of duodenal biopsies for diagnosis of giardiasis were 22.2% (Grazioli *et al,* 2006), and 44% (Wahnschaffe *et al,* 2007) respectively. Santos *et al.* (2011) found that histological examination of duodenal biopsies was less effective than stool examination in diagnosing giardiasis and cryptosporidiosis.

In the present study, rapid lateral flow immune-chromatographic assay (RLFIA) gave a sensitivity of 96%, specificity of 99.2% in diagnosing giardiasis and a sensitivity of 92.86%, specificity of 98.53% in diagnosing cryptosporidiosis. However, this may be the first application of RLFIA cartridges on duodenal aspirate samples. In the present study, RLFIA proved to be rapid < 30 minute, easy done and interpreted. This agreed with Bitilinyu-Bangoh *et al.* (2019) who reported that rapid diagnostic tests for *C. parvum* and /or *G. duodenalis* infections in stool samples were rapid, without technical expertise and applicable in resource limited setting.

In the present study, duodenal biopsy technique was invasive, expensive, time consuming, with low sensitivity and needed expertise pathologist. This agreed with Grazioli *et al.* (2006), Wahnschaffe *et al.* (2007) and Santos *et al.* (2011). The microscopic stool examination has low cost and accessible in poor settings as compared to immunological and molecular ones (Elsafi et *al,* 2014), but time consuming, greatly affected by intermittent of parasites and required expertise technician (Cunha *et al,* 2019). The DFA, using monoclonal antibodies needed experience for interpretation, moderate intense labor and good turnaround speed and simple (Kehl *et al,* 1995; Garcia and Shimizu, 1997; Johnston *et al,* 2003), with high sensitivity and specificity (McHardy *et al,* 2014), but, higher costive for application on large scale (Cunha et al, 2019).

Conclusion

The rapid lateral flow immune-chromatographic assay of duodenal aspirated samples is simple, easily used, short time, high sensitivity, and recommended for screening duodenal aspirates in endoscopy patients.

References

Adeyemo, FE, Singh, G, Reddy, P, Stenström, TA, 2018: Methods for the detection of *Cryptosporidium* and *Giardia*: from microscopy to nucleic acid-based tools in clinical and environmental regimes. Acta Trop. 184:15-28.

Abd El-Latif, NF, El-Taweel, HA, Gaballah, A, Salem, AI, Abd El-Malek, AH, 2020: Molecular characterization of *Giardia intestinalis* detected in humans and water samples in Egypt. Acta Parasitol. 65, 2:482-9.

Aronson, NE, Cheney, C, Rholl, V, Burris, D, Hadro, N, 2001: Biliary giardiasis in a patient with human immunodeficiency virus J. Clin. Gastroenterol. 33, 2:167-70.

Autier, B, Belaz, S, Razakandrainibe, R, Gangneux, JP, Robert, F, 2018: Comparison of 3 commercial multiplex PCR assays for the diagnosis of intestinal protozoa. Parasite 25:48. doi: 10.1051/parasite/2018049.

Azcona-GutieÂrrez, JM, de Lucio A, Herna Ândez-de-Mingo, M, GarcõÂa, C, Soria-Blanco, LM, *et al,* 2017: Molecular diversity and frequency of the diarrhea genic enteric protozoan *Giardia duodenalis* and *Cryptosporidium* sp. in a hospital setting in Northern Spain. PLoS One 12, 6: e0178575. https://doi.org/10.1371/journal.pone.0178575

Baig, MF, Badvi, JA, Qadeer, SA, Kharal, S A, 2010: A comparison study of different methods used in the detection of *Giardia lamblia* on fecal specimen of children. Med. Channel 16, 3: 489-92.

Bitilinyu-Bangoh, J, Voskuijl, W, Thitiri, J, Menting, S, Verhaar, N, *et al,* 2019: Performance of three rapid diagnostic tests for detection of *Cryptosporidium* spp. and *Giardia duodenalis* in children with severe acute malnutrition and diarrhea. Infect. Dis. Poverty 8:96-104.

Blanshard, C, Jackson, AM, Shanson, DC, Francis, N, Gazzard, BG, 1992: Cryptosporidiosis in HIV-seropositive patients. Qatar J. Med. 85:813-23.

Branda, JA, Tai-Yuan, DL, Rosenberg, ES, Halpern, EF, Ferraro, MJ, 2006: A rational approach to the stool ova and parasite examination. Clin. Infect. Dis. 42:972-8.

Cunha, FS, Peralta, JM, Helena, R, Peralta, S, 2019: New insights into the detection and molecular characterization of *Cryptosporidium* with emphasis in Brazilian studies: A review. Rev. Inst. Med. Trop. São Paul, 61:e28.

Doğruman, AF, Kuştimur, S, Özekinci, T, Balaban, N, İlhan, MN, 2006: The use of enzyme linked immunosorbent assay (ELISA) and direct fluorescent antibody (DFA) methods for diagnosis of *Giardia intestinalis*. Türk. Parazitol. Derg. 30, 4:275-8.

El-Hady, H, Abd-Elmaged, S, 2018: Parasites in duodenal aspirate using endoscopy in Sohag, Egypt. Sohag Med. J. 22, 3:239-44

El-Nahas, HA, Salem, DA, El-Henawy, AA, El-Nimr, HI, Abdel-Ghaffar, HA, *et al,* 2013: *Giardia* diagnostic methods in human fecal samples: A comparative study cytometry. Part B, Clin. Cytomet. 84B:44-9.

Elsafi, SH, Al-Sheban, SS, Al-Jubran, KM, Abu Hassan, MM, Al Zahrani, EM, 2014: Comparison of Kinyoun's acid-fast and immunofluorescent methods detected an unprecedented occurrence of *Cryptosporidium* in the Eastern Region of Saudi Arabia. J. Taibah Uni. Med. Sci. 9, 4:263-7.

El-Shazly, AM, Mowafy, N, Soliman, M, El-Bendary, M, Morsy, ATA, *et al,* 2004: Egyptian genotyping of *Giardia lamblia*. J. Egypt. Soc. Parasitol. 34, 2:265-80.

Garcia, LS, Arrowood, M, Kokoskin, E, Paltridge, GP, Pillai, DR, *et al,* 2018: Laboratory diagnosis of parasites from the gastrointestinal tract. Clin. Microbiol. Rev. 31:e00025-17. https: // doi.org/10.1128 /CMR.00025-17.

Garcia, LS, Shimizu, RY, 1997: Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for detection of *Giardia lamblia* and *Cryptosporidium parvum* in human fecal specimens. J. Clin. Microbiol. 35:1526-.9.

Gawad, SS, Ismail, MA, Imam, NF, Eassa, A H, 2018: Detection of *Cryptosporidium spp*. in diarrheic immunocompetent patients in Beni-Suef, Egypt: Insight into epidemiology and diagnosis. Kor. J. Parasitol. 56, 2:113-9.

Grazioli, B, Matera, G, Laratta, C, Schipani, G, Guarnieri, G, *et al,* 2006: *Giardia lamblia* infection in patients with irritable bowel syndrome and dyspepsia: A prospective study. World J. Gastroenterol. 12, 12:1941-4.

Hanson, KL, Cartwright, CP, 2001: Use of an enzyme immunoassay does not eliminate the need to analyze multiple stool specimens for sensitive detection of *Giardia lamblia*. J. Clin. Microbiol. 39, 2:474-7.

Helmy, MMF, Abdel-Fattah, HS, Rashed, L, 2009: Real-time Pcr/rflp assay to detect *Giardia intestinalis* genotypes in human isolates with diarrhea in Egypt. J. Parasitol. 95:1000-4.

Jahan, N, Khatoon, R, Ahmad, SA, 2014: Comparison of microscopy and enzyme linked immunosorbent assay for diagnosis of *Giardia lamblia* in human faecal specimens. J. Clin. Diagn. Res., 8, 11:DC04-6.

Johnston, SP, Ballard, MM, Beach, MJ, Causer, L, Wilkins, PP, 2003: Evaluation of three commercial assays for detection of *Giardia* and *Cryptosporidium* organisms in fecal specimens. J. Clin. Microbiol. 41:623-6.

Kehl, KS, Cicirello, H, Havens, PL, 1995: Comparison of four different methods for detection of *Cryptosporidium* species. J. Clin. Microbiol. 33:416-8.

Khurana, S, Sharma, P, Sharma, A, Malla, N, 2012: Evaluation of Ziehl-Neelsen staining, auramine phenol staining, antigen detection enzyme linked immunosorbent assay and polymerase chain reaction, for the diagnosis of intestinal cryptosporidiosis. Trop. Parasitol. 2:20-3

Krystallis, C, Masterton, GS, Hayes, PC, Plevris, JN, 2012: Update of endoscopy in liver disease: More than just treating varices. Wld. J. Gastroenterol. F18, 5:401-11.

Kurokawa, T, Ohkohchi, N, 2018: Role of Platelet, Blood Stem Cell, and Thrombopoietin in Liver Regeneration, Liver Cirrhosis, and Liver Diseases (Eds.): Yun-Wen Zheng, Stem Cells and Cancer in Hepatology, Academic Press.

Mahdi, NK, Taha, SA, 2002: The efficiency of duodenal aspirate in the diagnosis of parasitosis and candidiasis. Qatar Med. J. 11, 2:27-9.

Mahfouz, ME, Mira, N, Amer, S, 2014: Prevalence and genotyping of Cryptosporidium spp. in farm animals in Egypt. J. Vet. Med. Sci. 76, 12:1569-75.

Marcellin, P, Kutala, BK, 2018: Liver diseases: A major, neglected global public health problem requiring urgent actions and large-scale screening. Liver Int. 38, 1:S2-6.

Massoud, AM, Hafez, AO, Abdel-Gawad, A G, El-Shazly AM, Morsy TA, 2008: Mirazid alone or combined with paromomycin in treating cryptosporidiosis *parvum* in immunocompetent hospitalized patients. J. Egypt Soc Parasitol. 38, 2:399-418.

Mathison, BA, Kohan, JL, Walker, JF, Smith, RB, Ardon, O, Couturier, MR, 2020: Detection of intestinal protozoa in trichrome stained stool specimens by use of a deep convolutional neural network. J. Clin. Microbiol. 58:e02053-19.

McHardy, IH, Wu, M, Shimizu-Cohen, R, Couturier, MR, Humphries, RM, 2014: Detection of intestinal protozoa in the clinical laboratory. J. Clin. Microbiol. 52:712-20.

Mousa, N, Abdel-Razik, A, El-Nahas, H, El-Shazly, A, Abdelaziz, M, *et al,* 2014: Cryptosporidiosis in patients with diarrhea and chronic liver diseases. J. Infect. Dev. Ctries 8:1584-90.

Peters, L, Burkert, S, Grüner, B, 2021: Parasites of the liver; epidemiology, diagnosis and clinical management in the European context. J. Hepatol. 75, 1: 202-18

Ramadan, ME, Ramadan, MEE, Yousef, M, 2015: *Cryptosporidium* infection in patients with acute on chronic liver failure (ACLF). Pluralidade 2, 4:102-15.

Rasmussen, HG, Lund, M, Enemark, HL, Erlandsen, M, Petersen, E, 2016: Comparison of sensitivity and specificity of 4 methods for detection of *Giardia duodenalis* in feces: Immunofluorescence and PCR are superior to microscopy of concentrated iodine-stained samples. Diag. Microbiol. Infect. Dis. 84:187-90.

Roberts-Thomson, IC, Anders, R, Bhathal, P, 1982: Granulomatous hepatitis and cholangitis associated with giardiasis. Gastroenterology 83, 2:480-3.

Roellig, DM, Yoder, JS, Madison-Antenucci, S, Robinson, TJ, Van, TT, *et al,* 2017: Community laboratory testing for *Cryptosporidium*: Multicenter study retesting public health surveillance stool samples positive for *cryptosporidium* by rapid cartridge assay with direct fluorescent antibody testing. PLoS ONE 12, 1:e0169915.

Ryan, U, Cacciò, SM, 2013: Zoonotic potential of *Giardia*. Int. J. Parasitol. 43, 12/13:943-56.

Ryan, U, Zahedi, A, Paparini, A, 2016: *Cryptosporidium* in humans and animals: One health approach to prophylaxis. Parasite Immunol. 38: 535-47

Santos, RB, Fonseca, Jr, Espinheira, L, Santana, A, Carolina AC, *et al,* 2011: Clinical, endoscopic and histopathological profiles of parasitic duodenitis cases diagnosed by upper digestive endoscopy. Arq. Gastroenterol. 48, 4:225-30.

Savioli, L, Smith, H, Thompson, A, 2006: *Giardia* and *Cryptosporidium* join the neglected diseases initiative. Trends Parasitol. 22, 5:203-8.

Shahat, S, El-Badry, A, El-Sheety, A, El Faramawy, M, Ismael, N, *et al,* 2020: Genotypic prevalence of *Cryptosporidium* in Egyptian Patients with liver cirrhosis. AIMJ 2:225-31.

Shrestha, S, Larsson, S, Serchand, J, 1993: Bacterial and cryptosporidial infection as the cause of chronic diarrhea in patients with liver disease in Nepal. Trop. Gastroenterol. 14:55-8.

Squire, SA, Ryan, U, 2017: *Cryptosporidium* and *Giardia* in Africa: Current and future challenges. Parasit. Vect. 10, 1:195-200.

Suzuki, HU, de Morais, MB, Medeiros, EH, Corral, JN, *et al,* 1994: Diagnostic limitations of trophozoites isolation of *Giardia lamblia* in duodenal aspirates. Arq. Gastroent. 31, 2:69-74.

Thompson, RCA, Ash, A, 2019: Molecular epidemiology of *Giardia* and *Cryptosporidium* infections: What's new? Infect. Genet. Evol. 75 103951. https://doi.org/10.1016/ j.meegid.103951

Wahnschaffe, U, Ignatius, C, Loddenkemper, O, Liesenfeld, M, Muehlen, T, *et al,* 2007: Diagnostic value of endoscopy for the diagnosis of giardiasis & other intestinal diseases in patients with persistent diarrhea from tropical or subtropical areas. Scand. J. Gastroenterol. 42, 3:39-6.

Weitzel, T, Dittrich, S, Mohl, I, Adusu, E, Jelinek, T, 2006: Evaluation of seven commercial antigen detection tests for *Giardia* and *Cryptosporidium* in stool samples. Clin. Microbiol. Infect. 12:656-9.

Yakoob, J, Abbas, Z, Beg, MA, Naz, S, Khan, R, M. Islam, M, Jafri, W, 2010: Prevalence of *Giardia lamblia* and *Cryptosporidium parvum* infection in adults presenting with chronic diarrhea. Ann. Trop. Med. Parasitol. 104, 6:505-10,

Youssef, FG, Adib, I, Riddle, MS, Schlett, C D, 2008: A review of cryptosporidiosis in Egypt. J. Egypt. Soc. Parasitol. 38, 1:9-28.

Yu, Z, Li, F, Zeng, Z, Huang, Z, *et al,* 2011: Prevalence & clinical significance of *Cryptosporidium* in patients with HBV-associated acute-on liver failure. Int. J. Infect. Dis. 15:e845-8.

Explanation of figures

Fig.1: Small intestinal mucosa showed pear-shaped or crescent-like *Giardia* (black arrows) in lumen with dense inflammatory cellular infiltrate in lamina propria (H &E stain, x400), and x100 within rounded circle (left side).

Fig.2: (Left): *Giardia* cyst and *Cryptosporidium* oocysts by DFA in stool sample (x1000). (Right): Histopathological examination of duodenal biopsies stained with modified ZN for cryptosporidiosis (blue arrow points to acid fast oocysts).