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DETECTION OF *GIARDIA INTESTINALIS* COPROANTIGENS IN DIARRHEIC SAMPLES BY IMMUNOCHROMATOGRAPHIC AND ELISATECHNIQUES

By

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Abstract

Giardia intestinalis is one of the most common diarrhea-causing protozoa. The present study aimed to search for specific and sensitive diagnostic tests to avoid loss of infected cases with *Giardia intestinalis* by detection of *G. intestinalis* coproantigens in diarrheic samples through comparison between direct parasitological method, an enzyme linked immunosorbent assay (ELISA) and immunochromatographic test (ICT). A comparative cross-sectional study including 75 cases suffering from diarrhea and other gastrointestinal symptoms suggestive of intestinal giardiasis as abdominal distention, abdominal pain, anorexia, nausea, vomiting and weight loss, and 25 cases were without any clinical manifestations enrolled in this study. For every case, complete history taking and full clinical examination were done. Stool samples were collected from all cases and investigated by direct parasitological method, ELISA, and immunochromatographic techniques.

The results showed that the sensitivity of immunochromatographic technique was 96% and specificity was 96% while sensitivity of ELISA was 98% and specificity was 96% on comparing their results to the microscopic examination of stool samples for *Giardia intestinalis.*

Keywords: *Giardia intestinalis*, coproantigens, diagnosis, ELISA, immunochromatographic test.

Introduction

Diarrheal disease is considered one of the leading causes of the morbidity and mortality worldwide (Gaafar, 2011). The diarrhea is defined by the World Health Organization (WHO) as having three or more loose or liquid stool per day or as having more stool than normal for that person (WHO, 2005). *Giardia intestinalis* is one of the most common diarrhea causing parasitic protozoa (Geurden *et al,* 2010). *Giardia* is a genus of intestinal flagellates that infects a wide range of vertebrate hosts (Soliman *et al,* 2011).

However, *Giardia intestinalis* (synonyms *Giardia duodenalis, Giardia lamblia*) is the only species found in humans (Pestechian *et al,* 2014). The *Giardia* is transmitted by the feco-oral route. The cyst is the infective stage of the organism and can be transmitted by ingesting the contaminated food, by person to-person contact or by drinking of contaminated water (Barbosa *et al,* 2008). Giardiasis may lead to a wide spectrum of clinical manifestations, from the asymptomic to severe illness. The symptoms of giardiasis are largely non-specific and vary from intestinal symptoms as diarrhea, abdominal discomfort, nausea and mild weight loss to extraintestinal symptoms like fever, lymphadenopathy, urticaria, maculopapular rash, polyarthritis, and pulmonary infiltrate (Grazioli *et al,* 2006).

Chronic infections occur in endemic regions, which may be related to re-infection with different strains (Jenikovaa *et al,* 2011). The symptoms could be similar to those caused by other gastrointestinal pathogens, such as those of the bacterial and viral gastroenteritis (Lewthwaite *et al,* 2005). The non-specific symptoms make diagnosis of giardia-

sis difficult. The diagnosis of giardia- sis is frequently based on microscopical de- tection of the organisms in stool samples. Yet, the method is time consuming and depends on the skill of an experienced micro- scopist (Weitzel *et al,* 2006).

Diagnosis via microscopic examination of a single stool specimen has a low sensitivity and may therefore miss up to 50% of *Giardia* infections. Because of the intermittent shedding of the parasites, microscopic ex- amination of three consecutive stool specimens were required to reach the sensitivity of over 90% (Wahnschaffe *et al,* 2007). So the development of sensitive, cost-effective and rapid diagnostic methods is of importance (Jelinek and Neifer, 2013).

ELISA proved highly sensitive and specific for the diagnosis of giardiasis (Garcia, 2007). It is cost effective diagnostic method which can detect small quantities of copro-antigens of parasites even in mild infection. It can detect different soluble antigens dispersed in fecal matter rather than detecting cysts, trophozoites, or antigens on the surfaces of these morphologic forms (Kamel *et al,* 2013). Immunochromatographic (ICT) devices are promising tools in the diagnosis of giardiasis and are reasonably reliable in identifying the positive and negative individuals. Moreover, they are cost-effective, easy to interpret by the less experienced personnel. They could be used in large-scale surveys, they are also ideal for use under field conditions because the results can be read visually and laboratory equipment was not required (El-Moamly, 2014). Thus, the different immunological methods for coproantigenic identification of *G. intestinalis* as ELISA & immunochromatographic test have been developed as alternative methods for laboratories in diagnosis of giardiasis (Chacarova, 2010).

The present study aimed to evaluate the specific and sensitive diagnostic tests to avoid loss of infected cases with *G. intestinalis* by detection of *Giardia intestinalis* copro-antigens in diarrheic samples through comparison between direct parasitological method, ELISA and immunochromatographc techniques.

Subjects, Materials and Methods

Study type: A comparative cross -sectional study. The study was conducted in the period from June, 2013 to September, 2014 in Pediatrics, and Internal Medicine Outpatient Clinics, Zagazig University Hospitals, and Department of Parasitology. The present study was conducted on hundred subjects aged 1- 60 years; they were selected from Pediatrics and Internal Medicine Outpatient Clinics of Zagazig University Hospitals. For every one complete history taking and the full clinical examination were done. Seventy-five cases were complaining of diarrhea and other gatro-intestinal symptoms suggestive of intestinal giardiasis as abdominal pain, distention, anorexia, nausea, vomiting and weight loss, and twenty five cases without any clinical manifestation were considered as a control group. From all cases, stool samples were obtained.

Ethical aspects: An informed written consent was obtained from each subject shared in the study (young age subject consent was obtained from his parents). The study purpose and procedures were explained to the adults and parents of young children according to the ethics committee of the Faculty of Medicine Zagazig University.

Parasitological study: From all cases, stool samples were obtained and investigated by the direct parasitological examination. Successive stool samples were collected from each case for macroscopic and microscopic examination. Direct smear: unstained smear, Eosin stained smear and the Lugol's iodine stained smear, Formol-ether sedimentation concentration techniques (Cheesb-rough, 1987).

Study design: After direct parasitological study of fecal samples of all studied cases, the cases were classified into three groups according to the results of parasitological examination and clinical manifestations as shown: GI (50 cases): they were subgrouped into (GIa: 25 cases of giardiasis only and GIb: 25 cases of giardiasis with other parasites). GII (25 cases): were negative for *Giardia* but showed other parasites. GIII (25 cases) were

negative for *Giardia* and other parasites, without any clinical manifestation and considered the healthy control group.

Detection of *G. intestinalis a*ntigen in fecal specimens by ELISA using the commercially available Ridascreen [R-Biopharm AG, Anderneuen Bergstraße 17, D-64297 Darmstadt, Germany] Kits. The method was done according to manufacturer's instructions. ELISA readings were at 450 nm. The cut-off value was determined by addition of 0.15 absorbance units to the measured absorption of the negative control. As the absorbance negative control value was (0.043) the cut-off value was (0. 193). Samples were considered positive if the absorbance value was higher than (10%) over the determined cut-off value. The samples were considered negative if the absorbance value was lower than (10%) under the determined cut-off value.

The detection of *Giardia intestinalis* antigen in fecal specimens by a quick immuno-chrmatographic test using commercially available RIDA-Quick (R-BiopharmGmb H, Darmstadt, Germany) kit. The method was done according to manufacturer's instructions. Samples were considered positive if red and blue bands were seen in the strips and were considered negative if only the blue band was visible in the test strips.

Statistical analysis: Data were subjected to statistical analysis using SPSS (version 20). Differences between frequencies (qualitative variables) and percentages in groups were compared by Chi-square test (X^2). Differences between means (quantitative variables) in parametric two groups by t test. P value was set at < 0.05 for significant results and < 0.001 for high significant results.

Results

One hundred cases were divided into three groups according to the results of microscopic examination of stool samples and clinical manifestations of individuals included in the study. These groups were classified into GI: 50 cases (GIa: 25 cases of giardiasis only and GIb: 25 cases of giardiasis with other parasites), GII: 25 cases (parasites oth- er than *Giardia*) and GIII: 25 cases (healthy control group without any of the clinical manifestation).

Twenty-five cases out of 50 *Giardia* infected cases (GI) were associated with other parasitic infections and remaining 25 cases were isolated (pure) *Giardia* infection. *Entamoeba histolytica* parasites were found to be the most associated parasitic infection with *G. intestinalis* (15%), *Hymenolepis nana* and *Giardia* (9%) and with *E. vermicularis* (1%).

The age, sex and residence distribution in *G. intestinalis* cases showed the commonest affected age group was 6-18 years (50%) with significant difference ($P<0.05$) when compared to GII and GIII. Males were more affected than females (62% and 38% respectively) without significant difference ($P>0.05$). The infection was more common in rural than urban areas (60% & 40% respectively) without significant difference ($P>0.05$).

All *Giardia* infected cases came to the Outpatient Clinics by diarrhea. Diarrhea alone (46%) followed by abdominal distention (14%), abdominal pain (12%), weight loss (12%), anorexia (10%) and finally nausea and vomiting (6%) without significant difference ($P>0.05$) between cases infected with isolated *Giardia* parasite (GIa) and those infected with *Giardia* and other parasites (GIb). Using ELISA for detection of the *Giardia* copro-antigens, the test was positive in 49 out of 50 cases (98%). The mean optical density readings of giardiasis cases showed a highly significant difference ($P<0.001$) when compared to GII & GIII. The highest copro-antigens positive cases were aged 6-18 years (48%) with highly significant difference ($P<0.001$) when compared to other age groups. Regarding the residence, most copro-antigens positive cases were from rural area (60%) with significant difference ($P<0.05$) compared to urban ones (38%). Copro-antigens positive cases were males (62%) without significant difference ($P>0.05$) when compared to females (36%). The sensitivity and specificity of ELISA test for detection of *Giardia* copro-antigens recorded 98% and 96% respectively.

Immunochromatographic test was positive in

48/50 cases (96%), with a highly significant difference (P<0.001) as compared with others. The most positive cases were aged 6-18 years (48%) with highly significant difference (P< 0.001) as compared to other ages.

Most positive cases were from rural ones (58%) with significant difference (P<0.05) as compared to urban ones (38%).

Most positive cases were males (60%) without significant difference (P>0.05) compared to female (36%) Sensitivity and specificity of immunochromatographic test for *Giardia* coproantigens showed 96% & 96% respectively. Details are given in tables (1 to9).

Table 1: *Giardia intestinalis* infection and other parasites among different groups.

Parasite	No.	%
GI= GIa+ GIb	50	50%
GIa (*Giardia* alone)	25	25%
GIb (*Giardia intestinalis* + other parasites)	25	25%
Giardia + *Entameba histolytica*	15	15%
Giardia + *Hymenolepis nana Giardia* + *Entrobius* vermicularis	9 1	9% 1%
GII (Parasites other than *Giardia*)	25	25%
Entameba histolytica	17	5%
Entrobius vermicularis	5	17%
Hymenolepis nana	3	3%

Table 2: Prevalence of *G. Intestinalis* infection regarding age, sex, and residence between GI & GII.

Items	GI (GIa+GIb)= 50 cases		G II =25cases		X^2 (Chi square test)	P
	No.	%	No.	%		
Age:1-6	15	30	12	48		
6-18	25	50	6	24		
18-30	4	8	5	20	11.2	0.01*
30-60	6	12	2	8		
Male	31	62	14	56		
Female	19	38	11	44	0.4	0.5
Residence: Rural	30	60	18	72	1.6	
Urban	20	40	7	28		0.2

P > 0.05 = insignificant difference, (*)= significant as P < 0 .05

Table 3: Prevalence of *Giardia intestinalis* infection regarding clinical presentations in GI.

Groups	GIa		GIb		total		X^2(Chi square test)	P
	No.	%	No.	%	No.	%		
Diarrhea alone	12	48	11	44	23	46	0.17	0.67
Diarrhea + abdominal distension	4	16	3	12	7	14	0.57	0.44
Diarrhea + abdominal pain	3	12	3	12	6	12	0	1
Diarrhea +weight loss	2	8	4	16	6	12	2.6	0.1
Diarrhea + anorexia	2	8	3	12	5	10	0.8	0.37
Diarrhea + nausea and vomiting	2	8	1	4	3	6	1.3	0.24

P > 0.05 = insignificant difference

Table 4: Mean optical density of ELISA coproantigens among different groups.

	No.	Coproantigens +ve		mean ±SD	T test (P)		
		No.	%		G1a & G1b	G1 & G2	G1 & G3
G1a	25	24	96%	0.95±0.24			
G1b	25	25	100%	1.02±0.31			
G1	50	49	98%	0.98±0.28	0.85	0.0002**	0.00005**
GII	25	1	4%	0.08±0.02			
GIII	25	1	4%	0.06±0.01			
Total	100	51	51%	0.42±0.28			

P > 0.05 = insignificant difference, (**)= highly significant as P < 0 .001

Table 5: Prevalence of giardiasis by detection of ELISA coproantigens regarding age, residence and sex in GI.

Groups		Copro antigen +VE		P (Chi square test)
		N	%	
Age	1-6	15	30	0.00068**
	6-18	24	48	
	18-30	4	8	
	30-60	6	12	
Residence	Rural	30	60%	0.02*
	Urban	19	38%	
Sex	male	31	62%	0.062
	female	18	36%	
total		49	98%	

P > 0.05 = insignificant difference, (*)= significant as P < 0 .05, (**)= highly significant as P < 0 .001

Table 6: ELISA sensitivity & specificity for *Giardia* coproantigens versus microscopic stool examination.

No. of +ve cases by stool examination (True +ve =GIa+GIb)	No. of +ve cases by ELISA test	Sensitivity	No. of -ve cases by stool examination (True-ve = GII+GIII)	No. of -ve cases by ELISA test	Specificity
50	49	98%	50	48	96%

Table 7: Immunochromatographic test among different groups.

	No.	ICT +VE		Chi square test		
		No.	%	P G1a & G1b	P G1 & G2	P G1 & G3
G1a	25	24	96%	0.1	0.00074**	0.00021**
G1b	25	24	96%			
G1	50	48	96%			
GII	25	1	4%			
GIII	25	1	4%			
Total	100	50	50%			

(**)= highly significant as P < 0 .001

Table 8: Prevalence of giardiasis by immunochromatographic test regarding age, residence and sex in GI.

Groups		Item		P (Chi square test)
		No.	%	
Age	1-6	15	3	0.00029**
	6-18	24	0	
	18-30	4	4	
	30-60	5	8	
Residence	Rural	29	5	0.024*
	Urban	19	8	
Sex	male	30	6	0.07
	female	18	0	
total		48	9	

P > 0.05 = insignificant difference, (*)= significant as P < 0 .05, (**)= highly significant as P < 0 .001

Table 9: Sensitivity and specificity of immunochromatographic test to detect *Giardia* coproantigens versus microscopic stool examination.

No. of +ve cases by stool examination (True +ve =GIa+GIb)	No. of +ve cases by (ICT) test	Sensitivity	No. of -ve cases by stool examination (True-ve = GII+GIII)	No. of -ve cases by ICT	Specificity
50	48	96%	50	48	96%

Discussion

Diarrheal disease is one of the leading causes of morbidity and mortality worldwide (Hartog *et al,* 2013). It is one of the primary causes of mortality in children less than five years of age in developing countries, where it accounts for 1.8 million deaths annually (Mathers *et al,* 2008). Diarrheal illness in early childhood also contributes to future physical stunting and cognitive impairment (Dillingham and Guer-

rant, 2004).

In the present study, we tried to evaluate specific diagnostic methods of *G. intestinalis*, as it is one of the commonest intestinal protozoa causing gastroenteritis. Cases complained of diarrhea and other gastrointestinal symptoms suggestive of intestinal giardiasis as abdominal distention, abdominal pain, anorexia, nausea, vomiting and weight loss as a consequence.

In the present study, GIb *Giardia* infected cases were associated with other parasitic infections. *Entamoeba histolytica*, *Hymenolepis nana* and *E. vermicularis* were the most associated parasitic infection with *Giardia intestinalis*. Blackwell *et al.* (2013) reported that 50% of those infected with *G. intestinalis* infection mostly were infected with any helminth at least one helminth. Also, Al-Mekhlafi *et al.* (2013) recorded that *Trichuris trichiura, Ascaris lumbricoides*, hookworm infections and *Entamoeba histolytica/ dispar* were detected in 71.6%, 40.2%, 10.1% & 9.2% of samples, respectively. Regarding co-infections, about 60% of children had *Giardia* with *Ascaris* and/or *Trichuris.*

Stool samples of cases of GII were negative for *Giardia* parasites but other parasites were detected. These parasites included *Entamoeba histolytica* parasites, *Enterobius vermecularis* eggs and *Hymenolepis nana* eggs, they were included to detect cross re- activity of used kits between other parasites antigen and *Giardia* antigen in stool. Stool samples of GIII were negative for *Giardia* and other parasites, they were included in the study as a healthy control group to detect the sensitivity of the ELISA and immuno-chromatographic test in the detection of *Giardia* antigen in stool.

As for the age, this study was done on the age group (1-60) years old, it was found that in GIa (giardiasis) prevalence of infection at age group 6-18 (school age) years old was the highest. Also, in GIb (*Giardia* + other parasites) infection prevalence at age group 6-18 years old was the highest. This can be explained by increased activity and the wide range of contact and playing out- doors with other children in their school. These results agreed with that of Helmy *et al.* (2009) who found that the highest percentage of infection was in the 10 to 20- years old age group (56.3%) after examination of 41 Egyptian patients infected with giardiasis aged between 0-65 years old. Al-Mekhlafi *et al.* (2013) in rural Malaysia on children infected with *Giardia* (7 months to 12 years in age) found that the highest levels of infection with *Giardia* were in children between 7 & 10 years. This could be attributed to poverty, in general, as children of poor families being forced to work outdoors to help their parents. Faubert (2000) stated that specific age prevalence of giardiasis continues to rise through infancy and childhood and begins to decline in adolescence.

On the other hand, Mateo *et al*. (2014) stated that giardiasis was commonest in children aged 1-2 years in a study conducted in Majadahonda (Northwest of Madrid, Central Spain) daycare centers. In this study infants and toddlers were particularly susceptible to oral-fecal transmitted infectious diseases, presumably because of their immature and inexperienced immune systems, high hand-to-mouth activity, and undeveloped hygienic habits. Childcare facilities provided adequate environments for the fast spread of enteric infections with children confined within limited spaces, particularly if appropriate sanitation & hygiene standards not fulfilled (Lee and Greig, 2008).

Also, Ibrahim (2012) stated that giardiasis was commonly seen in children aged one month-two years, followed by two-four years, and this high prevalence might be attributed to the low immunity against various pathogens, as these age groups were comparatively less resistant to diseases (Haq *et al,* 2006). The other reason could be related to a number of factors such as poor health hygiene and toilet training, overcrowding, low socioeconomic status and climatic conditions (Ulukanligil and Seyrek, 2004). Additionally, the children feel free to play anywhere irrespective of the cleanliness or dustiness due to the absence of separate play grounds. The playing areas were main sources of diseases with waste materials of homes and industries (Munazza *et al*, 2011).

As regards the sex, prevalence of giardiasis in the present study was 62% in males and

38% in females. The variation in sex distribution was insignificant. Apparently higher giardiasis prevalence in the present males probably because they have a wide range of movement in society and more contact with animals. This result agreed with Helmy *et al.* (2009) who recorded higher prevalence in males (58%) than females (24.4%) in patients infected with giardiasis in a study conducted in Egypt. Al-Mekhlafi *et al*. (2013) reported that giardiasis was more common in males than females in rural Malaysia. Also, Minvielle *et al.* (2004) recorded that giardiasis was commonest in males than females in Argentinian rural community. On the other hand, Mateo *et al.* (2014) stated that the prevalence of giardiasis is equal among male and female. Zaglool *et al.* (2011) stated that the incidence of giardiasis was equal among males and females.

In the present study, patients (Gla & GIb) were from rural areas (60%) appeared to be more susceptible to infection than those of urban areas (40%). Such difference was statistically insignificant. This could be explained due to presence of human to human transmission which represent an important role with bad personal hygiene and spread of insects especially flies in different localities of the community. Also, Al-Mekhlafi *et al.* (2013) found a high prevalence of infection (50%) in a rural area of Karachi, Pakistan, Malaysia. This high percentage of *G. intestinalis* infection in rural areas may be due to drinking of under- ground water which is contaminated with sewage, the use of human feces as manure (Monis and Andrews, 1998) and increased exposure to animal contact as a zoonotic giardiasis (Sprong *et al,* 2009).

In the present study; diarrhea was the presenting symptom among the 50 cases. It occurred alone in 46% of cases followed by abdominal distension that occurred in 14% of cases, abdominal pain 12% and weight loss 12%, anorexia 10% finally nausea and vomiting 6%. These results agreed with Helmy *et al.* (2009) who found that the main gastrointestinal manifestation was diarrhea with giardiasis. Abdominal pain was in 95% of patients. Other manifestations, such as flatulence (26.8%), weight loss (9.8%), anorexia and nausea (22%), and fatigue (9.8%) were reported; approximately 25% of the patients indicated more than one complaint. Also, agreed with Muhsen and Levine (2012) who stated that diarrhea is the presenting symptom in giardiasis patients and that giardiasis is the most common cause of persistent diarrhea and growth retardation among children.

On the other hand, many studies recorded symptoms other than diarrhea to be the most frequent clinical manifestation among giardiasis patients. Almirall *et al.* (2013) reported that the most prominent clinical signs of giardiasis are abdominal pain and asthenia especially in hospitalized children, abdominal pain and bloating, (Taherkhani *et al,* 2009), weight loss was found to be 100% in all *Giardia* infected children (Shatla *et al,* 2004), vomiting (Taherkhani, 2002), and dyspepsia (Zalipaeva, 2002). Hanson and Cartwright (2001) recorded giardiasis mostly in asymptomatic cases.

In the present study and the above previous studies indicate that the spectrum of symptoms of *G. intestinalis* infection is extremely broad, ranging from asymptomatic infection to a variety of GIT manifestations which were found to be non-specific as they are equally present in patients with pure giardiasis and those with mixed *Giardia* infections. So, it could not depend on clinical presentations as a guide for diagnosis of *G. intestinalis* infection, as approved by Gendrel *et al.* (2003). Microscopic examination of the stool is the most established diagnostic method and remains a valuable technique in assessment of patients with diarrhea or suspected giardiasis, its advantage include simplicity, low cost, high specificity at the genus level, the non-invasive nature and the ability to detect other parasitic infections associated with *Giardia* (Lebwhol *et al,* 2003).

However, Barazesh *et al.* (2010) mentioned thatmicroscopic examination has some limitations first *G. intestinalis* cysts may be misdiagnosed as it is small and similar in appearance to many pseudo-parasites such as yeast. Also, trophozoites break up rapidly in the

stool, so cannot be used to measure the severity of infection.

In the current study, microscopic examination was taken as the gold standard in the diagnosis of giardiasis and the sensitivity and specificity of the other two tests were calculated in comparison with the results of microscopic examination. As for ELISA, *Giardia* coproantigens was detected in 49 cases of GI (98%), one case in GΠ (2%) also, in GШ one case was positive. The sensitivity of the RIDA-SCREEN kit used in the present study was 98% while specificity was 96%.

Regarding GI, onlyone microscopic detected *Giardia* positive case was recorded as negative by ELISA coproantigens. This sample was considered as false negative sample due to basis that antigen concentration might be less the assay detection limit. Besides, ELISA assay for the *Giardia intestinalis* coproantigens proved highly sensitive. Howewver, the difference in the sensitivity of ELISA in different studies could be attributed to different groups of populations studied, and the difference in the strains of *Giardia* with subsequent variations in the antigenic characters. Garcia *et al.* (2003) stated that false negative results with ELISA were obtained when small numbers of parasites are present in stool.

Regarding GII which was negative for *Giardia* infection but positive for other parasites, one case was positive by coproantigens ELISA. This might be due to cross-reaction with other parasites. Twenty-four negative cases were considered as true negative as their readings were 10% below the cut off value.

Regarding the control cases negative for giardiasis and other parasitic infections by the direct microscopy, no antigen was detected in any specimen and its level was 10% below the cut off value except for one false positive result occurred in a *G. intestinalis* control specimen (either due to low infection intensity missed by microscopic examination or false positive case).

In the present study, the specificity of the coproantigens detection ELISA was 96%; as two *Giardia* negative specimens were ELISA positive. These two false positive samples might be explained on the basis that these cases may be asymptomatic carrier where cysts could not be detected in their stool sample which is particularly prevalent in highly endemic areas as Egypt, or either the patients are in the prepatent period. Barazesh *et al.* (2010) found that ELISA de- tected small quantity of coproantigens of parasite even in mild infections and if the live parasite was absent in the fecal samples. It can detect different soluble antigens dispersed in fecal matter rather than detecting cysts, trophozoites, or antigens on the sur- faces of these morphologic forms. Hawash (2014) found a positive reaction in three samples when used the Tech-Lab ELISA Kit for the negative samples and recorded the sensitivity and specificity of ELISA for *Giardia* compared to reference microscopy as 100% and 95.7% respectively. Kamel *et al.* (2013) detected two out of 41 *G. intestinalis* infected samples false negative results and the ELISA sensitivity was 95.12% while the specificity was 92.85% in comparison with the ordinary microscope.

In the present study, the rapid immunochromatographic techniques (ICT) (Strip test) in GI revealed that 48 samples were positive (96%) and two samples were negative (4%), in GII one sample was positive showing cross reactivity of used kit with other parasites tested in the study, while in GΠI, one case was positive 2% not detected by microscopic examination (either due to low intensity of infection that was missed by microscopic examination or false positive cases) and 24 samples were negative 98%. Sensitivity and specificity of immuno-chromatographic techniques were 96% and 96% respectively. Furthermore, Mateo *et al.* (2014) reported 100% and 95% for sensitivity and specificity respectively for immuno-chromatographic assay named (Stick Crypto- Giardia; Operon, Zaragoza, Spain) among children (0–3 years old) attending three public day care centers in Majadahonda, Madrid and Central Spain. Chakarova (2010) reported 89.9% and 93.8% for sensitivity and specificity respectively in patients infected with giardiasis by using the RIDAQUICK kit immunochromatographic

assay of *Giardia*. So, the immunochromatographic assay has the advantage of being rapid, easy, doesn't require the medical experience, sensitive, specific and allow simultaneous diagnosis of giardiasis in one step. On the other hand, direct microscopic examination is consider- ed to be time consuming procedure, requires subjective experience in identification of fecal samples, and needs three successive samples and the patient compliance for the effective diagnosis.

Generally speaking, in Egypt the zoonotic giardiasis is one of the commonest protozoan infections in human especially the children causing diarrhea (Mahmoud *et al*, 2014). This common flagellated protozoan parasite infects the small intestine of a wide range of vertebrate hosts with the neglected risk of zoonotic infection emanating from ruminants even in high prevalence areas (Helmy *et al*, 2014). El-Mohammady *et al*. (2012) stated that the acute giardiasis diarrhea continued to be a major cause of morbidity and mortality in children from developing countries including Egypt. They added that the determination of the frequency of diarrhea in an area, along with the proportion of disease caused by specific enteric agents of different origins proved to be considered the first step in controlling diarrheal diseases. Control measures to prevent or reduce giardiasis will depend on certain measures which prevent faeco-oral transmission. So health promotion and environmental sanitation is required and treatment of infected communities reduces the opportunity for water supply contamination and person to person spread (Lane and Lloyd, 2002). Parents and day care staff should be aware of the need to exclude children with diarrhea from day care centers, to seek medical diagnosis and treatment (Sykora *et al*, 1988).

Conclusion

No doubt, giardiasis is a real zoonotic protozoan disease of worldwide problem particularly among children can be diagnosed by the detection of its cysts in the different environmental specimens by using microscopic or immunological or the molecular based examination also by using different concentration techniques. The proper treatment must be based on accurate diagnosis.

Thus, ELISA and immunochromatographic assays are rapid, easy, sensitive and specific. They proved useful in epidemiological studies as they enable examination of large number of cases in short time. They should be used after repeated negative results of microscopic examination and before shifting to other invasive techniques to confirm *G. intestinalis* infection and for follow-up of treatment.

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