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Original Article

Efficacy of triage parasite panel in diagnosis of *Entamoeba histolytica, Giardia lamblia, and Cryptosporidium parvum* antigens in symptomatic children stool specimens

GAMAL A. ABU-SHEISHAA[1], Adel O.Hafez[1], HAYTHAM M. AHMED[2]

Department of Medical Parasitology[1], Department of Public Health Medicine[2], Faculty of Medicine, Al-Azhar University[1],Nasr City, Cairo, Egypt

(Correspondence : drgamalali912@gmail.com)

ABSTRACT

Background: Parasitosis are one of the most widespread and destructive infections all over the world, which lead to millions of annual morbidities and mortalities. Delay in diagnosis and treatment may lead to fatality. So, rapid and accurate diagnosis plays a critical role in patient's management.

Aim: Evaluation of immunochromatographic test (ICT) combi, for copro-antigen detection of amebiasis, giardiasis and cryptosporidiosis compared with microscopy and ELISA.

Methodology: A total of 95 stool samples. Group 1: Included 70 stool samples from symptomatic children complaining of gastrointestinal symptoms suggestive of intestinal amebiasis, giardiasis, and cryptosporidiosis. Group 2: Included 25 stool samples from healthy asymptomatic children. Samples examined by direct wet saline smear or stained with iodine, formol-ether sed. concentration technique, staining with modified Ziehl-Neelsen and antigen detection using ICT and ELISA. Microscopic examination was taken as standard reference and the sensitivity and specificity of the ICT (combi) was measured in comparison with copro- ELLISA.

Results: Our study revealed non-significant difference in age, sex, and residence between symptomatic children and controls p>0.5. Of 70 stool specimens, 25 were confirmed as true positives for *Entameba histolytica/dispar*, 30 for *Giardia*, and 11 for *Cryptosporidium* by wet mount microscopy directly or after using formol-ether concentration method and modified Ziehl-Neelsen staining for the detection of *C. parvum*. The sensitivities and specificities of ICT (combi) for, *Entamoeba histolytica, Giardia lamblia*, and *Cryptosporidium parvum* were 80.0%, 76.7%, 81.8%, and 88 % , 84%, 96% respectively. The sensitivities and specificities of Techlab copro-ELISA for *Entamoeba*, *Giardia*, and *Cryptosporidium* were 64.3%, 70%, 90.9%, and 100 % , 88%, 92% respectively.

Conclusion: ICTs (combi) are simple, fast, highly sensitive and specific can be used for rapid screening and diagnosis of amebiasis, giardiasis and cryptosporidiosis and able to recognize species in different parasite genera as *Cryptosporidium*, and *Entamoeba.* Also, differentiate pathogenic *E. histolytica* from nonpathogenic *E. dispar*. It can be used in combination with microscopy in symptomatic children having repeated negative results.

Keywords: ICT (combi), ELISA, *Entamoeba*, *Giardia*, and *Cryptosporidium* and copro-antigen

Introduction

Entamoeba histolytica, *Giardia lamblia*, and *Cryptosporidium parvum* are of the most common protozoan enteric pathogens in humans associated with diarrhea all over the world **(Fletcher et al., 2012).**

Nearly 10% of the world population are infected with *E. histolytica*, 1% of which develop the invasive form of the disease with up to 100,000 annual deaths in the tropical areas and developing countries **(Morf and Singh, 2012).** Prevalence of *G. intestinalis* range from 20% - 30% in developing countries and 2% - 5% in developed countries that influence about 200 million individuals allover the world **(Kurdova et al., 2007).** The Global Burden of Disease Study (GBDS) rating that, cryptosporidiosis was associated with more than 99,000 deaths and 8.3 million disability adjusted life years **(Murray et al., 2012),** mostly happened in developing countries.

Their transmition occurs by feco-oral routes, ingestion of contaminated food or water, person to person, and zoonotic transmission **(Thompson and Smith, 2011).**

In amebiasis, 90% of infected persons are asymptomatic, the others have symptoms of intestinal amebiasis ranges from colitis to dysentery and extraintestinal amebiasis **(Fotedar et al., 2007),** the common is amoebic liver and/or lung abscess and a delay in management may be fatal. *G. lamblia* infection leads to diarrhea and malabsorption, infections in children have a negative influence on growth and development **(Lane and Lloyd, 2002).** Cryptosporidiosis in human is usually presented by abdominal pain or cramps, anorexia, low grade fever, vomiting, malabsorption, diarrhea, and weight loss. Diarrhea could be sometimes profuse and prolonged or even intractable and fetal especially in immunocompromised patients **(Bouzid et al., 2013).**

The diagnosis depends mainly on microscopic detection of the parasite oocysts, cysts and/or trophozoites. However, it is labor-intensive, time-exhaustion, needs technician's experience, and sensitivity is low **(Garcia et al., 2000 ; Vanathy et al., 2017),** and demand the examination of at least three independently collected samples to minimize parasite-induced variability due to difference in parasite daily shedding **(Riddle et al., 2016).** Besides, traditional microscopic techniques are incapable to recognize species in different parasite genera as *Cryptosporidium* **(Amar et al., 2004),** and *Entamoeba*, and differentiate pathogenic *E. histolytica* from nonpathogenic *E. dispar* **(Fotedar et al., 2007).**

Antigen detection assays such as ELISA and rapid immunochromatographic tests for *E. histolytica*, *G. lamblia*, and *C. parvum* have confirmed to be profitable in diagnosis of these infections **(Goñi et al., 2012; Van den Bossche et al., 2015; Saidin et al., 2017).** Yet, their diagnostic sensitivity and specificity varied among studies, and several tests indiscriminate between the species **(Saidin et al., 2019).**

This study aims to evaluate the validity of the diagnostic implementation of ICT (combi), for copro-antigen detection of amebiasis, giardiasis and cryptosporidiosis compared with microscopy and ELISA.

Subjects, Materials and Methodology

The present study was carried out on a total of 95 stool samples from 2 groups. Group 1 included 70 stool samples from symptomatic children whose ages ranged from 2 to 16 years (9.3 ± 1.94) of both sexes complaining of gastrointestinal symptoms suggestive of intestinal amebiasis, giardiasis, and cryptosporidiosis. While group 2 included 25 stool samples from healthy asymptomatic children whose ages ranged from 2 to 18 years (8.4 ± 4.82).

The studied groups were subjected to complete history taking including age, sex, residence, complaints including diarrheal history: type, consistency, color, odor, number of motions, volume and containing blood or mucus and complete general and local abdominal examination.

Inclusion criteria: Group 1 : Symptomatic children with one of the following symptoms **(as Stated by CDC, 2019)** Dysentery, intermittent or continuous profuse voluminous watery diarrhea, flatulence, greasy stool, abdominal pain or cramps, nausea, vomiting, weight loss, anorexia, malaise and fever. Group 2: Non-symptomatic apparent healthy children.

Exclusion criteria: for both groups children received anti-parasitic drugs or other medications as Laxatives and Antibiotics two months before.

The study was achieved in the parasitology department from August 2020 to June 2021, and approved by the Ethics Committee of the faculty of medicine, Al-Azhar University. All the study participants and their parents were informed about the aim and the procedures, and written consents were gained from them or thier parents.

Stool samples were collected in clean, dry, leak proof containers and send immediately to lab., separated into three parts. The first part was examined fresh macroscopically by naked eye for consistency, color, odor, blood and mucus, the presence of adult worms or segments and microscopic examination by:

a- Direct wet mount saline smear to detect cysts and/or trophozoites and Lugol's iodine smear for detecting glycogen and nuclei of protozoan cysts **(Garcia and Bruckner, 1997).**

b- Wet mount microscopy using Formol-ether sedimentation concentration method **(Cheesbrough, 2009),** examined using a low (10×) and high-power objectives (40×) respectively.

c- Stained mounted smears with modified Zeihl-Neelsen stain to reveal *Cryptosporidium parvum* oocysts **(Garcia and Bruckner, 1997).**

The second part for rapid ICT RIDA®QUICK *Entamoeba* /*Giardia*/ *Cryptosporidium* Combi (R-BioPharm, Darmstadt, Germany) The test was done according to the manufacturer's instructions. It is an enzyme immunoassay for the detection of *E. histolytica*, *G. lamblia*, and *C. parvum* antigens in fresh or fresh frozen, unperserved fecal samples. The presence of the specific antigens (positivity) was detected by the color of bands which were for *Entamoeba* green, *Giardia* red-pink , and *Cryptosporidium* blue.

Immediately, third part was frozen and stored at -20°C for detection of *Entameba, Giardia*, and *Cryptosporidium* antigens using TechLab *E. histolytica* II (T5017), *Giardia* II (PT5012), and *Cryptosporidium* II (PT5014) (TechLab, Blacksburg, VA, USA) ELISA according to the manufacturer's instructions.

Statistical analysis:

Data were collected, and statistically analyzed by using statistical package for Social Sciences Program, version 18 (SPSS Inc. Chicago, Illinois, USA). Descriptive statistics in the form of frequencies, percentages, means, and standard deviations were performed. The differences between the studied variables were analyzed using a T-test for quantitative variables and chi-square tests for qualitative variables. P-value < 0.05 was considered a sign of significance.

Results

Our study demonstrated non-significant difference in age, sex, and residence between symptomatic children and controls p>0.5. The rate of symptoms in males was higher than in females living in rural areas **(table 1).**

Of 70 stool specimens, 25 were confirmed as true positives for *Entameba histolytica/dispar*, 30 for *Giardia*, and 11 for *Cryptosporidium* by wet mount microscopy directly or after using formol-ether sed. concentration method and modified Zeihl-Neelsen staining for the detection of *C. parvum*. used as reference standard test **(table 2).**

The sensitivities and specificities of ICT (combi) for, *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum* were 80.0%, 76.7%, 81.8%, and 88 % , 84%, 96% respectively. The sensitivities and specificities of Techlab copro ELISA assays for *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum* were 64.3%, 70%, 90.9%, and 100 % , 88%, 92% respectively **(table 3).**

Table 1: Demographic data of studied groups:

		Group (1) No. = 70	Group (2) No. = 25	P. value
Age(year)	Range	2-16	2-18	0.37
	Mean± SD	9.3 ± 1.94	8.4 ± 4.82	
Gender	Male	42(60%)	14(56%)	0.73
	Female	28(40%)	11(44%)	
Residence	Urban	22((31.4%)	10(40%)	0.44
	Rural	48(68.6%)	15(60%)	

Table 2: Results of different diagnostic assays for *E. histolytica*, *G. lamblia* and *C. parvum* in studied groups:

	Group (1) No. = 70						Group (2) No. = 25					
	E. histolytica		*G. lamblia*		*C. parvum*		*E. histolytica*		*G. lamblia*		*C. parvum*	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Microscopic examination	25 (35.7%)	45 (64.3%)	30 (42.8%)	40 (57.2%)	11 (15.7%)	59 (84.3%)	2 (8%)	23 (92%)	3 (6%)	22 (94%)	0 (0%)	25 (100%)
ICTs(combi)	20 (28.6%)	50 (71.4%)	23 (32.9%)	47 (67.1%)	9 (12.9%)	61 (87.1%)	4 (16%)	21 (84%)	4 (16%)	21 (84%)	1 (4%)	24 (96%)
TechlabELISA	18 (25.7%)	52 (74.3%)	21 (30%)	49 (70%)	10 (14.3%)	60 (85.7)	0 (0%)	25 (100%)	3 (6%)	22 (94%)	2 (8%)	23 (92%)

Table 3: Performance of different diagnostic assays in diagnosis of *E. histolytica*, *G. lamblia* and *C. parvum*:

Parasite/Assay	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	DA (%)
E. histolytica					
Formol- ether Conc. Method	84.0%	92.0%	91.3%	85.2%	88.0%
Rapid ICT	80.0%	88.0%	83.3%	80.8%	84.0%
Entameobea II ELISA	64.3%	100.0%	100.0%	75.9%	86.0%
G. lamblia					
Formol -ether Conc. Method	76.7	88.0	88.5	75.9	81.8
Rapid ICT	76.7%	84.0%	88.5%	75.0%	81.8%
Giardia II ELISA	70.0%	88.0%	87.5%	71.0%	78.2%
C. parvum					
Modified ZN stain	100.0%	100.0%	100.0%	100.0%	100.0%
Rapid ICT	81.8%	96.0%	90.0%	92.3%	91.7%
Cryptosporidium ELISA	90.9%	92.0%	83.3%	95.8%	91.7%

Discussion

Parasitosis are one of the most destructive and widespread infections all over the world, leading to millions of annual morbidities and mortalities **(WHO, 2010)**. Rapid and accurate diagnosis represents a critical weapon in the fight against parasitic infections **(Momcilovic et al., 2019).**

Our study revealed non-significant difference in age, sex, and residence between symptomatic children and controls ($p > 0.5$). The rate of symptoms in males was higher than in females living in rural areas. There were variable findings between Egyptian studies concerning gender differences where **El-Nadi et al, (2017)** revealed similar findings with non-significant gender differences while **Yones et al, (2019)** revealed significant gender differences. This could be explained by their markable outdoor activity with more exposure to parasitic transmission.

Concerning residence this in accordance with **Mathew et al, (2014)** who found that, intestinal protozoal prevalence was higher among rural children. On the other hand, **Ahmed, (2013)** in Gharbia Governorate found high prevalence of *E. histolytica* and *G. lamblia* in urban than rural communities. This may be due to poverty, poor living and hygienic conditions, drinking of underground water which may be contaminated with sewage, also the extensive utilization of human and animal excreta as fertilizer in agriculture, in addition to the close contact with animals.

In our study, stool samples were examined by direct wet smear or stained with iodine, formol-ether concentration technique, staining with modified Ziehl-Neelsen and antigen detection using ICT (combi) and ELISA. Microscopic examination was taken as standard referrence and the sensitivity and specificity of the ICT was calculated compared to copro-ELLISA. The results of microscopic examination were 25 (35.7%), 30(42.8%) and 11(15.7%) proven as true positives for *Entameba histolytica/dispar*, *Giardia lamblia*, and *Cryptosporidium spp.* respectively. Our results were in agree with Van den Bossche et al (2015) study in which 60 stool samples were examined microscopicaly and reveal positivity for *E. histolytica/dispar* (24), *G. lamblia* (29), *Cryptosporidium* spp. (4) and *G. lamblia* + *E. histolytica*/dispar (3). Also **Saad et al, (2015)** in study conducted on 115 cases, microscopic stool examination showed that 14 cases (12.1%) were positive for *Entamoeba histolytica*, 19 cases (16.5%) were positive for *Giardia lamblia*, and 7 cases (6%) were positive for *Cryptosporidium parvum*.

In the present study the outcome of ICT (combi) for *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium parvum* copro-antigen in stool samples, showed that 20 cases (28.6 %) were positive for *Entamoeba histolytica*, 23 cases (32.9%) were positive for *Giardia lamblia* and 9 cases (12.9 %) were positive for *Cryptosporidium parvum*.. The sensitivities and specificities of ICT for, *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum* were 80.0%, 76.7%, 81.8%, and 88 % , 84%, 96% respectively. These results were in agreed with **Van Lint et al, (2013); Van den Bossche et al, (2015); Selim et al, (2015).** Conversally, it were higher than reported by **Goñi et al, (2012)** who detecteded 62% sensitivity and 96% specificity for *E. histolytica*. Also, **Ibrahim et al, (2015)** in study carried out on diarrheic/dysenteric stool samples from clinically suspected individuals from Beni-Suef, Egypt, detected 28.6% sensitivity and 86.1% specificity. The lower specificity in our study can interpreted by the high *E. dispar* samples, which impacts specificity because the ICT kit is able to differentiate *Entmoeba* species.

As for *G. lamblia*, it agreed with **Weitzel et al, (2006)** who found that, the sensitivity obtained by Rida Quick Combi was 80% and specificity was 98**%. Goni et al, (2012)** detected the sensitivity and specificity of the triple ICT were 96.8% and 99.5% respectively for *G. lamblia* detection. Also, **Swierczewski et al, (2012)** used triage parasite panel on 266 samples in Kenya and found that the sensitivity 100% and specificity 100% in detection of *G. lamblia*.

Regarding *C. parvum*, **Gutiérrez-Cisneros et al, (2011)**, with triage parasite panel, got 92% sensitivity in diagnosis of C. *parvum*. Also, **Goni et al, (2012)** detecteded lower results in detection of *C. parvum* by the triage where the sensitivity was 72.7%. Also

Swierczewski et al, (2012) found 73% sensitivity in *C. parvum* detection, which might be due to difference in the monoclonal antibodies used.

The sensitivities and specificities of Techlab copro ELISA assays for *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum* were 64.3%, 70%, 90.9%, and 100 % , 88%, 92% respectively. This is coincided with results of **El-Hamshary et al, (2008)** where the sensitivity and specificity reached 88.24% and 90.48%, respectively in comparison to microscopy for *E. histolytica*/*E.dispar*. Others, in India and Iraq, the sensitivity and specificity of Techlab copro ELISA assays for *Entamoeba histolytica* was reported to be (20%), (60%) and (86.7%), (93.4%), respectively, in comparison with microscopic ex. **(Al-Basheer et al., 2014; Mohanty et al., 2014).**

Oreby et al, (2019) detected that, by copro-antigen ELISA 15 cases were positive including the 11 microscopically positive cases (37.5%) and 25 case were negative (62.5%), Sensitivity 73.3%, specificity 100% PPV 100% and NPV 86.2%. So, copro-antigen ELISA test was more sensitive with higher NPV in patients with *Giardia* infection than microscopic examination. **Ghallab et al, (2016)** in study for detection of cryptosporidiosis copro-antigen ELISA had 43.9% sensitivity and 100% specificity and PPV.

The modified ZN staining method used in our study had 100% sensitivity and specificity. These results revealed that modified acid-fast stain proved to be good positive and good negative test for detection of cryptosporidiosis. It is similar to **Salman, (2014)** who said that, the modified ZN staining method was sensitive, simple, rapid, and had 78.8% sensitivity and 98.3% specificity. In contrast, **Weber et al, (1991)** who said the modified acid-fast stain exhibit a relatively low sensitivity.

False-negative and positive copro-antigen test results for, *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum* were detected in our study. **Garcia et al, (2003)** and **Selim et al, (2015)** stated that, false negative results for *Giardia* with ELISA were obtained when small numbers of parasites are present in stool or due to non-homogenized samples. TheTechLab *E. histolytica* II kit detects *E. histolytica* Galactose/N-acetylgalactoseamine (Gal/GalNAc) lectin protein in stool samples, it is highly immunogenic and conserved, and due to the antigenic differences between the lectins of *E. histolytica* and *dispar* it can be used to detect *E. histolytica* **(Haque et al., 1997).** Also, microscopy may gave false-negative with a low parasite density, or when intact life-cycle stages are absent **(Ali and Hill, 2003).**

Conclusion

ICTs (combi) are simple, fast; highly sensitive and specific can be used for rapid screening and diagnosis of amebiasis, giardiasis and cryptosporidiosis and able to recognize species in different parasite genera as *Cryptosporidium*, and *Entamoeba*. Also, differentiate pathogenic *E. histolytica* from nonpathogenic *E. dispar*. It can be used in combination with microscopy in symptomatic children having repeatedly negative stool samples. The modified ZN staining method confirmed to be highly sensitive, specific, and good positive and negative test for detection of cryptosporidiosis. ELISA is sensitive, specific, easy to perform and accurate method could be used in epidemiological studies and diagnostic purposes.

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الملخص العربى

فعالية اختبار لوحة الفصل الكروماتوجرافى المناعى الطفيلى فى الكشف عن مُستضّدات الإنتاميبا هيستوليتيكا والجيارديا لامبليا و خفيات الأبواغ فى عينات براز الأطفال ذوي الأعراض المرضية.

جمال على أبو شعيشع[1]، عادل عمر حافظ[1]، هيثم محمود أحمد[2]

قسم الطفيليات الطبية[1] ،قسم الصحة العامة[2]، كلية الطب – جامعة الأزهر

خلفية البحث: الأمراض الطفيلية واحدة من أكثر الإصابات تدميراً وانتشاراً في العالم ، حيث تتسبب في ملايين الاعتلالات والوفيات سنوياً. التأخر في التشخيص والعلاج قد يسبب الوفاة، والتشخيص السريع والدقيق يلعب دوراً حاسماً في التدابير الوقائيه للمرضى.

هدف الدراسة: تقييم اختبار الترحيل الكروماتيجرافى (ICT combi)، لتشخيص داء الأميبيا (amebiasis) ، وداء الجارديا (giardiasis) ، وداء خفيات الأبواغ (cryptosporidisis) من خلال مقارنته بالفحص المجهرى والاليزا (ELISA copro-antigen assay).

منهجية البحث: أجريت الدراسة على 95 عينة براز من الأطفال، المجموعة الأولى: شملت 70 عينة براز من الأطفال الذين يعانون من أعراض دالة على التهابات الجهاز الهضمى كنتيجة للاصابة بالأميبا والجارديا أو خفيات الأبواغ والمجموعة الثانية: شملت 25 عينة براز من أطفال أصحاء لا يعانون من أعراض. وفُحصت عينات البراز عن طريق اللطخة المباشره بمحلول الملح أو تم صبغها باليود، وطريقة التركيز بالفورمول- إيثر، وصبغة زيل - نيلسن وتحديد الأنتيجينات فى البراز باستخدام (ICT combi) والـ ELISA. تم أخذ الفحص المجهري كمعيارمرجعى وتم حساب الحساسية والخصوصية للاختبار (ICT combi) بالمقارنة مع ELLISA.

النتائج: أظهرت دراستنا أنه لا توجد فروق دات دلالة إحصائية في العمر، والجنس، والإقامة بين الأطفال الذين يعانون من الأعراض المرضيه والمجموعة الضابطه $p>0.5$. ومن بين 70 عينة من البراز، تم تأكيد 25 منها على أنها إيجابية حقيقية للـ*Entameba histolytica/dispar* ، و 30 للـ *Giardia* ، و 11 للـ *Cryptosporidium* بواسطة الفحص المجهرى وعلى نتائج صبغة زيل - نيلسن للكشف عن *C. parvum*. الذي أستَخدَم كاختبار معيارى مرجعى. كانت الحساسيات والخصوصيات الخاصة (ICT combi) للإنتاميبا هيستوليتيكا، والجيارديا لامبليا، وكريبتوسبوريديوم بارفوم 80%، 76.7%، 81.8%، و88%، 84%، 96% على التوالى. وكانت الحساسيات والخصوصيات الخاصة ب Techlab copro ELISA بالنسبة للإنتاميبا هيستوليتيكا والجيارديا لامبليا وكريبتوسبورديوم بارفوم 64.3% ، 70% ، 90.9% ، و 100% ، 88% ، 92% على التوالى.

الاستنتاج: اختبار(ICT combi) بسيط وسريع وذات حساسية عالية وخصوصية عالية يمكن استخدامه في الفحص والتشخيص السريع للإنتاميبا هيستوليتيكا والجيارديا لامبليا وكريبتوسبورديوم بارفوم وقادرعلى تمييز أنواع العديد من الطفيليات مثل الكريبتوسبوريديوم والإنتاميبا وتمييز أنواع الطفيليات المتماثلة المسببه للمرض مثل إنتاميبا هيستوليتيكا من الغير مسببه للمرض مثل إنتاميبا ديسبر. ويمكن استخدامه بالاقتران مع الفحص المجهري في الأطفال ذوي الأعراض الذين لديهم عينات براز سلبية بشكل متكرر.