EVALUATION OF COMMERCIAL TRIAGE MICRO PARASITE PANEL WITH PCR FOR DETECTION OF GIARDIA LAMBIA, ENTAMOEBA HISTOLYTICA AND CRYPTOSPORIDIUM PARVUM IN CHILDREN SUFFERING FROM DIARRHEA

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ABSTRACT

The Triage parasite panel (CerTestCrypto+Giardia+Entamoeba combo card tests ,Zaragoza(Spain), is a new qualitative enzyme immunoassay (EIA) panel for the detection of Giardia lamblia, Entamoeba histolytica/E. dispar, and Cryptosporidium parvum in fresh or fresh, frozen, unfixed human fecal specimens. A total of 320 diarrhea samples were collected from children under six years who was admitted to babylon teaching hospital ,the period of study was begining from December 2020 to May 2021,microscopic examination was also performed to examine the parasite,triageimmunocromatography assay was used to identify the three type of parasite that causes diarrhea in children and also determine single or mixed infection ,also multiplex PCR to evaluate rapid immunocromatography test .Our result found there is increase prevalence of total parasitic infection in the age group (4-6) years as(51.16%) rather than (30.23%) and (18.60%) in age group (2-4y,<2y), respectivilly. Also found the most common parasite prevalence was G.lamblia(55.82%) followed by E.histolytica (29.06% ) ,then C.parvum(15.12%) in direct microscopic examination, while foundin triage immunocromatography the high prevalence of G.lamblia as (68.33%) followed by C.parvum (18.33%) and E.histolytica (13.34%),and the result the result recorded mixed parasite infection, it was found the most mixed parasite infection in the children have giardia lamblia and cryptosporidium parvum in immunocromatography assay, the immunocromatography test have sensitivity (66.67%,93.18% and 100%),for detection of intestinal parasite (E.histolytica,G.lamblia and C.parvum) respectivilly, and the specificity of our result ,by immunocromatography test (94.55%,82.61% and 100%),for E.histolytica ,G.lamblia and C.parvumrespectivily,in conclusion the trigeimmunocromatography assay was rapid(10 minutes), sensitive and spesific method to determine single or mixed parasite infection.

Key words: diarrhea,children and Immunocromatography(IC)

I. INTRODUCTION

Diarrhea is described as the production of excessive stools water of abnormally free consistency, commonly associated with immoderate frequency of defecation and with excessive stool output (1).

Diarrhea is an infectious disease that kills infants and is one of the leading causes of infant morbidity and mortality worldwide, the diarrheal disease is the second most prevalent, accounting for around 9% of all deaths in children under the age of five, with the majority of cases occurring in Sub-Saharan Africa and southern Asia (2). Damage to the mucosal lining or brush boundary causes inflammatory diarrhea, which results in a passive loss of protein-rich fluids and a reduced ability to absorb these fluids. Bacterial infections, viral infections, and parasitic infections may all cause it (3).

The enteric protozoa E. histolytica, G. lamblia and Cryptosporidium spp. have been recognized as important causes of diarrhea among human beings(4).

Cryptosporidium is considered as a major diarrheal cause of diarrhea in children and immune-compromised people with life threatening for those children under 5 years of age (5),and Entamoeba histolytica usually causes asymptomatic infection but in a minority of cases causes symptoms ranging from a few loose stools to profuse
bloody diarrhea(6). Some of studies explained that *Giardia lamblia* (also known as G. intestinalis or G. duodenalis), (41-57) is the causative agent of the diarrheal disease, giardiasis, which is the leading cause of death in developing countries for children below five years of age(7).

These three parasites can lead to human infection via fecal–oral transmission of the cysts through contaminated food and water and person-to-person contact, they are common in both developed and developing countries, as well as they are increased risk in the latter due to poor sanitation standards(8).

Microscopy is time-consuming, labor-intensive, relies on the technician's experience and more repeat samples may be required to increase sensitivity(9). The diagnostic methods becoming more widely used therefore are based on either fecal antigen detection or parasite DNA, but both require considerable technical expertise. Polymerase chain reaction (PCR) methods for detecting intestinal parasites are increasingly available and exhibit excellent sensitivity and specificity compared to conventional methods such as microscopy and antigen detection assays. PCR is also expensive and requires skilled personnel, which limits its use(10). Rapid detection techniques of fecal antigen such as immuno-chromatography (IC), for detection of *C. parvum, G. lamblia* and *E. histolytica/dispar* were developed. The triple immunochromatographic tests proved to be a simple, fast and additive method for the simultaneous diagnosis of these parasites in stool samples. Also, it requires little exercise and can be used in individual base for timely screening(11). The aim of this study to evaluate Triage immunocromatography assay to identification parasite in children suffering from diarrhea under five years old.

### II. MATERIALS AND METHODS:

#### Samples Collection

From November 2020 to June 2021, a total of (320) stool samples were collected from children aged less than 6 years who were admitted with acute gastroenteritis to Babylon teaching hospital or outpatient wards. The stool samples were collected in a clean plastic cup with a unique number written on it. The study include three parts: 1- conventional method (direct smear and staining), 2- Rapid assay (IC), and 3- multiplex PCR and in the detection of protozoa diarrheal infection.

**Macroscopic examination:**

The feces were examined by eye investigating color, consistency, blood, mucus, smell and other substances(12).

**Microscopic examination:**

From each stool samples, smears with normal saline and lugol's iodine were examined for *Giardia lamblia* and Entameba histolytica. The smear was examined thoroughly under the low power and high power of the microscope(13). Modified Ziehl–Neelsen stain has been used in the current study for identification of cryptosporidium oocyst. slides were examined microscopically with a drop of oil under high power lens(14).

**CERTEST Crypto+Giardia +Entamoeba COMBO CARD TEST Immuno Chromatography (IC)**

The detection of *Giardia lamblia*, *Entameba histolytica* and Cryptosporidium parvum using a Rapid Chromatographic Immunoassay, According to the manufacturer company, (CertTest Crypto+Giardia+Entamoeba combo card tests , Zaragoza(Spain)).

**Molecular method**

Extraction of DNA was performed directly from (320) stool samples according to manufactured company (Presto™ Stool DNA Extraction Kit) protocol Geneaid/ USA.

The primers described by(15) were used in the present study in Table (1).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Target Gene</th>
<th>Primer Sequence 5’- 3’</th>
<th>Product Size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.histolytica</td>
<td>EH-F</td>
<td>ATTTGTAAAGTATTGTAATG</td>
<td>605</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EH-R</td>
<td>ATTGTAACCTTTTCATTGTAAC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Statistical Analysis

Data analysis were performed by chi square test using SPSS statistical software, the sensitivity and specificity of the results were also calculated by the following formula.

I : sensitivity : (true positive / true positive + false negative) × 100 , II: specificity : (true negative / true negative +false positive ) × 100(16).

III. RESULT AND DISCUSSION

A total of 320 diarrhea sample were collected and examinated in the children under six years old, precently at the pediatric hospital who admitted to babylon teaching hospitol with infection of diarrhea.

Table (2) The distribution causes of diarrhea according to age group

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Total</th>
<th>Bacteria (n)%</th>
<th>Viruses (n)%</th>
<th>Parasite (n)%</th>
<th>Other (n)%</th>
</tr>
</thead>
<tbody>
<tr>
<td>2y&lt;</td>
<td>116</td>
<td>39(36.44%)</td>
<td>59(49.16%)</td>
<td>16(18.60%)</td>
<td>2(28.57%)</td>
</tr>
<tr>
<td>2-4 y</td>
<td>109</td>
<td>35(32.71%)</td>
<td>45(37.5%)</td>
<td>26(30.23%)</td>
<td>3(42.85%)</td>
</tr>
<tr>
<td>4-6 y</td>
<td>95</td>
<td>33(30.84%)</td>
<td>16(13.33%)</td>
<td>44(51.16%)</td>
<td>2(28.57%)</td>
</tr>
<tr>
<td>Total</td>
<td>320</td>
<td>107(33.43%)</td>
<td>120(37.5%)</td>
<td>86(26.87%)</td>
<td>7(2.18%)</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>X²=0.16</td>
<td>P&gt;0.05</td>
<td>X²=26.8</td>
<td>X²=28.87</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

Our result found there is increase prevelance of parasite infection in the age group (4-6) years as(51.16%)rather than (30.23%) and (18.60%) in age group (2-4y,<2y), respectivilly, this result was agreement with (17) who was found that the highest rate of parasite infection (38.18%) and he observed the positiviy among children in the age group(4-6)years old, also the present study is compatible with (18) who was found a highest percent of infection (44.35 %) in (1-10) years, but disaggrement with(19) who was found the highest detection of parasite was made in children aged 7-12 months as (26.1%).

Figure(1) showed the distribution of parasite causes of diarrhea by using direct examination ,our result found the prevelance of Girdia lamblia as (55.82%) than (29.06% and 15.12%) for E.histolytica and C.parvumrespectivelly,this result disagreement with the study in Dohuk that found the prevelance of G.lamblia as (38.5%) (20). The infection rate of Giardiasis is higher than results of other studies conducted in Al-Karkh side of Baghdad ,where Giardiasisin AL-Mahmoudyia area in Baghdad ,which was 34%(21) ; in Wassit province 11%(22). our result found the prevelance of Entameba histolytica as (29.06%),this result agreement with (23) ,who was found the prevelance of E.histolytica as (25.95%),but disagreement with the study that found the prevelnce of E.histolytica as (7.4%) in Erbil(24).
Figure (1) The distribution of parasite causes of diarrhea according direct microscopic examination

Among the distribution of parasitic causes of diarrhea by using triage immunocromatography test, Figure (2) showed the distribution of intestinal parasite by using triage immunocromatography test, the result was found the high prevalence of G. lamblia as (68.33%) followed by C. parvum (18.33%) and E. histolytica (13.34%). This result agreement with (25) who was found the prevalence of G. lamblia, C. parvum and E. histolytica as (23%), (5%) and (2%), respectively. Also agreement with (11), who was confirmed the high prevalence of G. lamblia followed by C. parvum and E. histolytica as (30.07%), (19.8%) and (11%), respectively. But, (26) found that E. histolytica (25.63%) was the commonest followed by G. lamblia (19.38%) and then C. parvum (13.75%), which might be due to different in environmental conditions.

Figure (2) The distribution of parasite causes of diarrhea by using triage immunocromatography test
Table (3) showed the triage single and mixed intestinal parasite among children under six years, the result found the most common prevalence of intestinal parasite in the single infection, it was found as (88.48%), (62.5%), and (27.27%) in G. lamblia, E. histolytica, and C. parvum respectively, the result agreement with (27) who confirmed the most prevalence of parasite infection in G. lamblia followed by E. histolytica and C. parvum in Kenya. Also, some of study confirmed that the most prevalence parasite in G. lamblia followed by E. histolytica and C. parvum (28) (29), respectively as shown in table (2).

while the mixed infection involving two or three intestinal parasite are also observed, the most prevalence mixed infection was found in the G. lamblia and C. parvum, it was found two parasite (G. lamblia and C. parvum) in six children suffering from diarrhea followed by three parasite (G. lamblia, C. parvum, and E. histolytica), it was found in one children suffering from diarrhea followed by two parasite (C. parvum and E. histolytica), it was found in one children suffering from diarrhea followed by two parasite (G. lamblia and E. histolytica), it was found in one children suffering from diarrhea as shown in figure (3), the study was found the parasite single infection is more incidence than parasite mixed infection, this result agreement with (30), who was confirmed that immunocromatography assay was used to identify single and mixed infection, Atu et al found parasite infection of 13 (n=105, 12.38%) single infection and 10 (n=105, 9.52%) mixed infection by using Rida Quick test for three
parasite infection. (11) confirmed that the parasite single infection is more incidence than parasite mixed infection in Egypt.

Among the distribution of parasitic causes of diarrhea by using PCR for parasitie causes of diarrhea, Figure(4) The distribution of intestinal parasite by using PCR. our result found the high prevalence of G.lamblia as (67.16%) compared with other parasite ,this result agreement with many studies form developing and developed countries indicating that G. intestinalis is the most common protozoan parasite -causing diarrhea(31)(32)(33). In present study found the positivity rate for Cryptosporidium spp. was (16.42)% ,this result agreement with(34),who was found the higher rate of C.parvum as (11%) in Babylon.

PCR detection of enteric protozoa is largely dependent upon the method used for DNA extraction from the stool specimens. Most of the previously developed PCRnassays have reported high levels of sensitivity and specificity using pure genomic DNA samples (35). They are some studies used multiplex PCR assay developed for simultaneous detection of E.histolytica,G.lamblia and C.parvum in stool sample(36)(37).

Table (4) showed the PCR technique that revealed high rate of Giardia infections 67.16%, followed by 55.81 % for direct microscopy .this result agreement with (38) ,who was found high rate of Giardia infections 18.43% in PCR, followed by 15.20 % for direct microscopy in Kirkuk .While C.parvum found as (15.11%) in direct microscopic examination followed by (16.41) in PCR ,this result agreement with (39) ,who was found the high rate C.parvum as(21%) in PCR followed by (9.5%) in direct microscope examination in Egypt. In current study found the high prevalence of E.histolytica as (29.06%) in direct microscope examination and lower prevalence (13.33%),(16.41%) in immunocromatography and PCR respectively,this result agreement with many studies due to microscopy that exhibited many false positive results ,this may be due to misdiagnosis of other Entamoeba species such as Entamoeba coli, Entamoeba hartmanni, or the morphologically identical Entamoeba moshkovskii ,Also of trophozoites of several other nonpathogenic intestinal amebas or fecal macrophages being misdiagnosed as E. histolytica/E. dispar(10)(40).

Table (4) Comparison of the Triage Micro Parasite Panel, direct examination and PCR results for diagnosis parasite causes of diarrhea.

<table>
<thead>
<tr>
<th>Parasite/Tes</th>
<th>E.histolytica (n)%</th>
<th>G.lamblia (n)%</th>
<th>C.parvum (n)%</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal direct examination</td>
<td>25(29.06%)</td>
<td>48(55.81%)</td>
<td>13(15.11%)</td>
<td>86</td>
</tr>
</tbody>
</table>

Figure(4) The distribution of parasite causes of diarrhea by using PCR
Table (5) showed the immunocromatography test have sensitivity (66.67%, 93.18% and 100%), for detection of intestinal parasite (E. histolytica, G.lamblia and C.parvum) respectively, this result is agreement with (26), who was found the sensitivity for detection E. histolytica 62.5%, G.lamblia 97% and C.parvum 72%. Also disagreement with (27), who was found the sensitivity of G.L, E.H and C.P 100%, 100% and 73% respectively.

The specificity of our result, by immunocromatography test 94.55%, 82.61% and 100% for E. histolytica, G.lamblia and C.parvum respectively, our result is agreement with (10), who was found 91%, 85.5% and 100% for E.H, G.L and C.P respectively.

<table>
<thead>
<tr>
<th>Type of parasite</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.H</td>
<td>66.67%</td>
<td>94.55%</td>
</tr>
<tr>
<td>G.L</td>
<td>93.18%</td>
<td>82.61%</td>
</tr>
<tr>
<td>C.P</td>
<td>100.00%</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

Polymerase chain Reaction: gold standard

Figure (5): Agarose gel electrophoresis image showed multiplexPCR product analysis for GL, EH and CP gene M (Marker ladder 100bp). Lane 1: CP and EH, Lane 2: CP, EH and GL, lane3: CP and GL; lane 4; GL, lane 5; CP, lane 6; EH, lane 7; negative control.
REFERENCES


