Detecting of virulence factors COWP gene and CP15 gene for  
Cryptosporidium parvum by polymerase chain reaction (PCR)

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Abstract

The present study aims to diagnose the parasite Cryptosporidium parvum isolated stool samples of patients to reviewers Diwaniya Teaching Hospital and Maternity Hospital and Children in the province of Diwaniya. The infected ranged from the age less than one year to 12 years at the period from beginning of May to the end of November 2016.

110 samples stool were collected and the number of infected samples is (30) sample. After examining samples in direct examination of the sample wet method.

The study included verification of virulence factors Cryptosporidium Oocyst Wall Protein (COWP), Surface Protein of Sporozoite (CP15) in parasite using conventional PCR technique.

The study showed that age groups less than one year were the highest infection rate (43.3%) while the age group of (9-12) years showed lowest infection rate (10%) with a significant difference between them, infection rate in females (56.6%) was higher than males (43.3%) but did not reach the level of significant.

These ratios also appeared in the rural areas are the highest (63.3%) compare with urban areas (36.6%) with a significant difference between them.

DNA was extracted from positive stool samples and then after amplified using virulence factors of parasite COWP gene (281bp), CP15 gene (230bp) and the results appeared present these factors in all positive samples of C.parvum.

Key words: Cryptosporidium parvum, COWP, CP15, PCR

Physiology Classification QR1-502-71
Introduction

Protozoan parasites of the genus Cryptosporidium infect the gastrointestinal tract of many animal species and cause cryptosporidiosis (1). This parasite causing mild to severe diarrhea depending on the host's immune status, the infection can spread to extraintestinal, hepatobiliary, pancreatic and pulmonary regions of the body leading to chronic disease and wasting (2). The infection can be a cute and self-limiting illness in immunocompetent patients. Cryptosporidiosis can become a chronic and life-threatening disease in immunocompromised patients (3,4). The infection of cryptosporidiosis occurs by the faecal oral route and contamination water with infective oocysts, oocysts release sporozoite which invade the intestinal epithelium cells predominantly localized to the jejunum and ileum (5,6).

Virulence factors for Cryptosporidium have been identified as genes involved in the initial interaction processes of cryptosporidium oocysts and sporozoites with host epithelial cells including excystation, gliding motility, attachment, invasion, parasitophorous vacuole formation, intracellular maintenance and host cell damage (7,8). Surface protein of sporozoite (CP15) is expressed by the infective sporozoite and merozoite stages (9) involved in the invasion and the host immune response to infection (10,11,12) and its apparent role in the invasion of mammalian cells by C.parvum sporozoites (13).

Cryptosporidium Oocyst Wall Protein (COWP) gene has been localised in the wall forming bodies of early and late macrogametes and the inner layer of the oocyst wall, there are at least two alleles of the COWP gene in the C.parvum population, one associated only with the human host, the other with both animals and humans(14).

Materials and Methods

Sample collection

110 human stool samples were collected from patients microbiology laboratory to Diwaniya teaching Hospital and Maternity Hospital and Children, and then transport to laboratory and stored in freeze.

Microscopic examination

Stool samples examined by preparation of direct wet smear mix a drop of saline solution on a glass slide with a small sample of feces using wooden sticks, then put the slide cover and examine using microscope under power zoom 10X and 40X.

Genomic DNA Extraction

Genomic DNA was extracted from feces samples by using (Stool DNA extraction Kit, Bioneer, Korea). The extraction was done according to company instructions by using stool lysis protocol method with Proteinase K. After that, the extracted gDNA was checked by Nanodrop spectrophotometer, and then stored at -20C at refrigerator until used in PCR amplification.

Polymerase chain reaction

PCR assay was performed for detection of virulence factors genes (Cryptosporidium Oocyst Wall Protein (COWP) gene and Surface Proteins of Sporozoite (CP15) of Cryptosporidium parvum. The primers were designed in this study using NCBI-Genbank
The primers were provided by (Bioneer company, Korea). As following table (1):

Table (1): prefixes of *C. parvum* used in the study with the nucleotide sequence (PCR reaction).

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence</th>
<th>Amplicon</th>
</tr>
</thead>
<tbody>
<tr>
<td>COWP gene</td>
<td>CCGAATGTCTCCAGGCAC</td>
<td>281bp</td>
</tr>
<tr>
<td></td>
<td>GTATATCTGGTGGGCAGACC</td>
<td></td>
</tr>
<tr>
<td>CP15 gene</td>
<td>CACTCGATTGTGTCTCCCC</td>
<td>230bp</td>
</tr>
<tr>
<td></td>
<td>TTCTTGGGGGTGGTGGGAAG</td>
<td></td>
</tr>
</tbody>
</table>

Results

1. Prevalence of *Cryptosporidium spp.* according to a microscopic examination

The results of the current study, depending on the method of direct wet smear that there are 30 samples out of 110 samples were positive to infection of the parasite as the shown in the figure (1):
2. prevalence of *C. parvum* according to the sex

Results of the study showed that the percentage of female infection with parasite was 56.6% compared to males where the percentage of infection was 43.3% but it didn't reach the level of significance at \( p > 0.05 \). table (2):

<table>
<thead>
<tr>
<th>Sex</th>
<th>The number of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>13</td>
<td>43.3 a</td>
</tr>
<tr>
<td>Females</td>
<td>17</td>
<td>56.6 a</td>
</tr>
</tbody>
</table>

Chi-square value (\( X^2 \)) = 1.771

3. prevalence of *C. parvum* according to the age groups

Results of the current study showed that the rate of infection at the age of less than one year (43.3%) was the highest and lowest infection at the age of (9-12) years with a significant difference at \( p > 0.05 \). table (3):

<table>
<thead>
<tr>
<th>Age groups</th>
<th>The number of infected patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than one year</td>
<td>13</td>
<td>43.3 a</td>
</tr>
<tr>
<td>1-4</td>
<td>9</td>
<td>30 a</td>
</tr>
<tr>
<td>5-8</td>
<td>5</td>
<td>16.6 b</td>
</tr>
<tr>
<td>9-12</td>
<td>3</td>
<td>10 b</td>
</tr>
</tbody>
</table>

Chi-square value (\( X^2 \)) = 26.244
4. prevalence of C.parvum according to the nature of residence

The results of the current study showed that the infection rate in rural areas is the highest (63.3%) compared to the urban areas (36.6%) the lowest rate of infection with a significant difference at p>0.05 table (4):

Table (4): prevalence of C.parvum according to the nature residence

<table>
<thead>
<tr>
<th>Nature of residence</th>
<th>The number of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban areas</td>
<td>11</td>
<td>36.6 a</td>
</tr>
<tr>
<td>Rural areas</td>
<td>19</td>
<td>63.3 b</td>
</tr>
</tbody>
</table>

Chi-square value ($X^2$) =7.136

*similar letters means non-significant differences at the level of probability of 0.05 using test $X^2$.
*Different letters means significant differences at the level of probability of 0.05 using test $X^2$.

5. Molecular study

Results of molecular test PCR showed that all positive samples for the parasite C.parvum (100%) contain virulence factors studied which were Cryptosporidium Oocyst Wall Protein and Surface Protein of sporozoite as the figure show (2 and 3) the molecular weight of the COWP factor (281bp) percentage (100%) and the molecular factor of the CP15 (230bp) percentage (100%) and were examined by electrophoresis in a 1.5% agarose gel and stained with ethidium bromide and examined using UV transilluminator.

![Agarose gel electrophoresis image](image)

. Figure (2): Agarose gel electrophoresis image that show the PCR product of Cryptosporidium oocyst wall protein (COWP) gene in Cryptosporidium parvum positive isolates. Where M: Marker (1500-100bp), lane (1-10 ) some positive C. parvum at 281bp PCR product size.
Discussion

*Cryptosporidium parvum* is an intracellular protozoan parasite of the family Cryptospidiiidae and phylum Apicomplexa (15). This disease occurs worldwide and is ubiquitous in the environment (16). Cryptosporidiosis is in the top five most common causes of infections diarrhea around the globe (17).

Results of the current study showed that the parasite *Cryptosporidium parvum* affected all age groups but the infection rate in less than one year old (43.3%) was the highest and recorded less injured rate in the age group (9-12) years and the reason for this due to the immune system is incomplete at the age of less than one year as well as the left breast feeding to artificial feeding and ignorance many mothers things sterilization and cleanliness of the water and milk bottles this is consistent with (18).

The relationship between spread of disease in both sexes is non significant relationship, the percentage of infection in females reached (56.6%) while in males (43.3%) this is consistent with (19).

The results of this study that the parasite is most prevalent in rural areas (63.3%) and the lowest rate in urban areas (36.6%) and this is consistent with (20) and the reason due to presence of grazing such as cows and sheep in rural areas compared with urban areas at least interested in raising cattle and sheep.

Results proved presence COWP factor in all positive samples for infection percentage 100% , its higher than of infection rate that recorded (14) reached 98% that showed existence of least two alleles of the COWP gene in the *C.parvum* one associated only with the human host and the other with both animals and humans, and supports that cryptosporidiosis in humans is not necessarily a zoonosis(21).

The study proved presence CP15 factor in all positive samples for infection percentage 100% and this consistent with (22), this factor it's apparent role in the invasion of mammalian cells by *C.parvum* sporozoites (13).
References


تحديد عوامل الضراوة CP15 gene و COWP gene لطفيلي الابواغ الخبيئة Cryptosporidium parvum

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الخلاصة
تهدف الدراسة الحالية إلى تشخيص طفيلي الابواغ الخبيئة Cryptosporidium parvum المعزول من عينات براز المرضى المراجعين إلى مستشفى الديوانية التعليمي ومستشفى الولادة والأطفال في محافظة الديوانية.

تراوحت أعمار المصابين ( أقل من سنة – 12 سنة) للفترة الواقعة من الأول من شهر آيار وغاية نهاية شهر تشرين الثاني للعام 2016. تم جمع 110 عينة براز وكان عدد العينات المصابة 30 عينة بعد فحص العينات بطريقة الفحص المباشر للعينة الرطبة .

تأتي الدراسة متمكئ عن عوامل الضراوة Cryptosporidium Oocyst Wall Protein (COWP) و Surface Protein of sporozoite (CP15) في الطفيلي Cryptosporidium parvum

أظهرت الدراسة ان الفئات العمرية أقل من سنة كانت أعلى نسبة إصابة حيث بلغت (43.3%) بينما الفئات العمرية من (9-12) سنة أظهرت أقل نسبة إصابة بلغت (10%) مع وجود فروقات معنوية بينها وكانت نسبة إصابة الإثاث (56.6%) وهي أعلى من نسبة إصابة التكبر (43.3%) إلا أنها لم تصل إلى مستوى المعنوية كما ظهرت هذه النسب في المناطق الريفية في الأعلى (63.3%) بالمقارنة مع المناطق الحضرية (36.6%) مع وجود فروقات معنوية بينها.

تم مضاعفة الحمض النووي المستخلص من العينات الموجبة باستخدام عوامل الضراوة لطفيلي Cryptosporidium parvum (COWP (281bp) و CP15 (230bp) و(CWOP) و(CP15)

الكلمات المفتاحية: Cryptosporidium parvum ,COWP ,CP15 ,PCR