Detection of *Cryptosporidium parvum* from feces samples of human and camels by using direct Polymerase Chain Reaction assay technique

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Abstract

The study was designed for molecular detected of *Cryptosporidium parvum* from human and camel by using direct Polymerase Chain Reaction assay technique. A total of 50 fecal samples from human and 50 samples from camel collected. The *Cryptosporidium parvum* positive isolates were identified by using specific primers for heat shock protein gene that designed in this study using NCBI-Genbank data base (Genbank code: GQ259151.1) and primer3 plus program for primer design. Results show that prevalence of infection with *Cryptosporidium parvum* was (24%) 12 positive out of 50 human fecal samples, whereas the prevalence of infection with *Cryptosporidium parvum* in camel was (14%) 7 positive out of 50 fecal samples. The study demonstrates that the direct Polymerase Chain Reaction (PCR) assay technique is a simple, rapid and valuable tool for the detection *Cryptosporidium parvum*.

Key words: *Cryptosporidium parvum*, PCR, human, camel.

تشخيص طفيلي *Cryptosporidium parvum* في عينات براز الإنسان والجمال باستخدام تقنية فحص تفاعل سلسلة البلمرة

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الخلاصة

تناولت الدراسة الحالية التحري الجيني لطفيلي *Cryptosporidium parvum* في الإنسان والجمال باستخدام تقنية فحص تفاعل سلسلة البلمرة. حيث تم جمع 50 عينة براز من الإنسان و50 أخرى من الجمال. شخصت *Cryptosporidium parvum* الموجبة باستخدام برايمرات خاصة للجين الذي يشفر بروتين الصدمة الكهربائية. صممت البرايمرات المستخدمة بالدراسة اعتمادا على موقع بنك الجينات العالمي (Genbank code: GQ259151.1) وبرنامج primer3 plus لتصميم البرايمرت. أظهرت نتائج تقنية فحص تفاعل سلسلة البلمرة أن نسبة الإصابة بطفيلي *Cryptosporidium parvum* تشكل (24%) 12 عينة موجبة من أصل 50 عينة براز من الإنسان ، بينما نسبة حدوث الإصابة بطفيلي *Cryptosporidium parvum* في الجمال (14%) 7 عينة موجبة من أصل 50 عينة براز. أوضحت الدراسة الحالية بان تقنية تفاعل سلسلة البلمرة ، بسيطة ، سريعة و ذات قيمة لتشخيص *Cryptosporidium parvum*.

الكلمات المفتاحية: الجمال ، الإنسان ، سلسلة تفاعل البلمرة ، *Cryptosporidium parvum*

Introduction

Cryptosporidium parvum is a coccidian intracellular protozoan pathogen that causes diarrhea and other severe diseases in humans and animals (1,2). Usually the immuno-compromised patient and the human immunodeficiency virus infected patients are more susceptible to infection with diarrhea due to *C. parvum* (3). *Cryptosporidium parvum* is affecting livestock worldwide. The dromedary camels also infected by C. *parvum* and other Cryptosporidium species such as *Cryptosporidium andersoni* and *Cryptosporidium muris* (4,5). Cryptosporidiosis is more severe infection in newborn animals and causes severe diarrhea that is sometimes accompanied with anorexia, stiffness, reduced milk intake, hyperpnoea, dehydration, growth retardation,

slow gait and depression (6,7). In adult animals are generally intractable to infection and infected animals can became as asymptomatic carriers that shed large numbers of Cryptosporidium oocysts into the environment and remain a main source of infection to other animals (8). Some of Cryptosporidium species such as *Cryptosporidium parvum*, *Cryptosporidium canis*, and *Cryptosporidium meleagridis* are of zoonotic important and their excreted oocysts might be the sources of human infection and of great public health concern (9). Many techniques have been used to detect Cryptosporidium infection in humans and animals. These include examination of stool for the presence of oocysts and detection of Cryptosporidium antigens. Moreover, histology and ultra-structural examination of biopsy materials for life-cycle stages (10). Modified Ziehl-Neelsen staining and fluorescein tagged monoclonal antibody immunofluorescence staining techniques are the most commonly used diagnostic for intestinal cryptosporidiosis (11). However the sensitivity and specifity of these tests for detecting *C. parvum* oocysts in stools has been reported to be 10,000 oocysts per gram of watery stool, while in formed stools 50,000 or 500,000 oocysts per gram are required for a positive IF or modified ZN staining test, respectively (12). Therefore, more sensitive and specific techniques such as molecular PCR assay are clearly needed to identify these oocysts in the stool specimens. This study aimed to used Polymerase Chain Reaction assay technique based heat shock protein gene for direct detection *Cryptosporidium parvum* in human and camel.

Materials and methods

Feces sample collection

50 Fecal samples were collected from human that suffered from diarrhea from Al-Diwanyiah hospital and another 50 fecal samples were collected from camel from different fields in Al-Diwanyiah province. The fecal sample was transferred to a clean, dry plastic container and transported to the laboratory for examination.

Genomic DNA Extraction

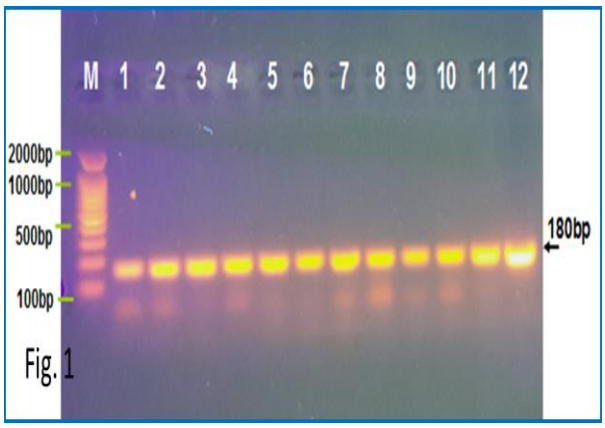
Genomic DNA was extracted from feces samples by using (Stool DNA extraction Kit, Bioneer. Korea). The extraction was done according to company instructions by using stool lysis protocol method with Proteinase K. After that, the extracted gDNA was checked by Nanodrop spectrophotometer, and then stored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

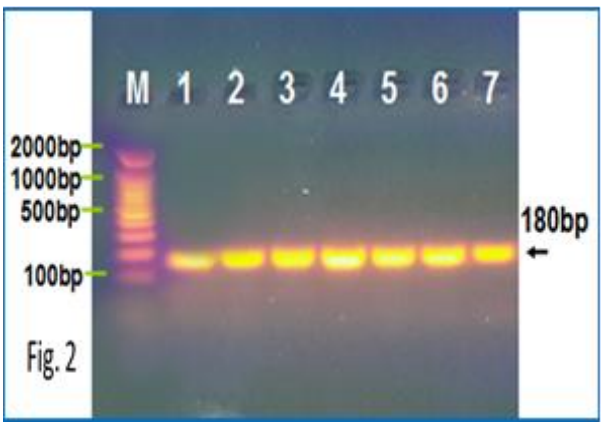
Polymerase chain reaction

PCR assay was performed for direct detection of *Cryptosporidium parvum* by using specific primer for heat shock protein gene in *Cryptosporidium parvum*, the forward primer (CGTGCAACT TTAGCTC CAGT) and reverse primer (AGCAACAGC TTCGTCTGGAT) this primers were designed by used (GenBank: GQ259151.1) and Primer3plus. The primers were provided by (Bioneer Company. Korea). Then PCR master mix was prepared by using (AccuPower® PCR PreMix kit. Bioneer. Korea). The PCR premix tube contains freeze-dried pellet of (Taq DNA polymerase 1U, dNTPs 250µM, Tris-HCl (pH 9.0) 10mM, KCl 30mM, MgCl2 1.5mM, stabilizer, and tracking dye) and the PCR master mix reaction was prepared according to kit instructions in 20µl total volume by added 5µl of purified genomic DNA and 1.5µl of 10pmole of forward primer and 1.5µl of 10pmole of reverse primer, then complete the PCR premix tube by deionizer PCR water into 20µl and briefly mixed by Exispin vortex centrifuge (Bioneer. Korea). The reaction was performed in a thermo-cycler (Mygene, Bioneer. Korea) by set up the following thermocycler conditions; initial denaturation temperature of 95 °C for 5 minutes followed by 30 cycles at denature-tion 95°C for 30 seconds, annealing 57.2°C for 30 seconds, and extension 72°C for 20 sec. minute and then final extension at 72°C for 5 minutes. The PCR products (180bp) were examined by electrophoresis in a 2% agarose gel, stained with ethidium bromide, and visualized under UV illumination.

Results

Results of PCR assay were indicate the prevalence of infection of *Cryptosporidium parvum* in human fecal samples was 24% (12/50), whereas, it was less prevalence (14%) (7/50) in camel fecal samples. The Polymerase Chain Reaction assay technique based heat shock protein gene for direct detection of *Cryptosporidium parvum* were show good PCR amplification in extracted DNA from fecal samples of human (Fig. 1), and of camel (Fig. 2).





Agarose gel electrophoresis images show the PCR product of heat shock protein gene using in detection of *Cryptosporidium parvum* in human fecal samples (Fig. 1), and in camel fecal samples (Fig. 2). Where M: Marker (2000-100bp), lane (1-12) in human, lane (1-7) in camel, at 180bp PCR product size.

Discussion

In this study we describe a rapid, sensitive, and specific method for the direct detection of *Cryptosporidium parvum* in stool specimens by Polymerase Chain Reaction technique. PCR-based assays have previously been used by others to detection of *Cryptosporidium parvum* DNA in human feces and from purified oocysts or paraffin-embedded tissues (13,14). PCR technology offers a good alternative to conventional diagnosis of *Cryptosporidium* from both clinical as well as environmental samples (15). The detection limits reported for PCR based methods by different authors have ranged from 100 to 2,000 oocysts per gram of human feces (16). The present study recorder 24% of infection in human. (17) Recoded the prevalence rate of cryptosporidium in children with diarrhea 18%. (18) recorded cryptosporidium oocysts were detected 14.9% of the tested samples by acid fast staining technique and 16.3% by using Eliza kit. (19) Higher rates of infection were reported in Mexican (26%). While recorded lower the prevalence rate for cryptosporidium was 1.5% in Jordan (20) and (21) recorded 6% by used PCR technique in Mexico. The other results of the present study revealed that 14% of the adult camels were infected with *C. parvum*. Other previous studies reported a higher prevalence rate of *Cryptosporidium* species 37.9% of the adult camels and demonstrated that the prevalence rate of infection in camel is high in both sexes and different age ranges and open areas may be associated with higher risk of infection through environmental contamination due to grazing other infected animals or to the spreading of manure (22). The common of the previous studies reported a higher prevalence rate of *Cryptosporidium* infection in younger animals (23).

In conclusion: *Cryptosporidium parvum* is important causes of diarrhea infection in human and camel. Whereas, the Polymerase Chain Reaction assay technique is a rapid, sensitive, and specific method for the direct detection of *Cryptosporidium parvum* in stool specimens.

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