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

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Abstract

This 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. The results show that prevalence of infection with *Cryptosporidium parvum* was (24%) of 12 positive samples out of 50 human fecal samples, whereas the prevalence of infection with *Cryptosporidium parvum* in camel was (14%) of 7 positive samples out of 50 fecal samples. these study demonstrate that the direct Polymerase Chain Reaction (PCR) assay techniques is a simple, rapid and valuable tool for the detection *Cryptosporidium parvum*.

Key Word: *Cryptosporidium parvum*, PCR, Human, camel

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

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 و 50 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) و برنامج 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%) 7عينة 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 immunocompromised 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 and ersoni* and *Cryptosporidium muris* (4,5). Cryptosporidiosis is acute infection in newborn animals and causes severe diarrhea that is sometimes escort with anorexia, hardness , lowering milk intake, hyperpnoea , dehydration, growth retardation, , lazy walk and depression

(6,7). Infected animals can became as asymptomatic transporter that shed many of Cryptosporidium oocysts into the environment and remain a main source of infection to other animals and the infection is mostly rebellious in adult animals (8). The excreted oocysts of some Cryptosporidium species such as Cryptosporidium parvum,Cryptosporidium canis, and Cryptosporidium meleagridis might be the sources of human infection , therefore this parasite has zoonotic important and great public health worry (9).

Many techniques have been used to detect Cryptosporidium infection in humans and animals. These include screening of stool samples that contian oocysts and detection of Cryptosporidium antigens. furthermore , histology and ultra-structural examination of biopsy materials for life-cycle stages (10). The fluorescein tagged monoclonal antibody immunofluorescence staining method and Modified Ziehl-Neelsen staining method 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 6oocysts per gram of aqueous 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-Diwanyia hospital and another 50 Fecal samples were collected from camel from different

filed in Al-Diwanyia 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 TTAGCTCCAGT) and reverse primer (AGCAACAGCTTCGTCT GGAT) 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

thermocycler (Mygene, Bioneer. Korea) by set up the following thermocycler conditions; initial denaturation temperature of 95 °C for 5 minutes followed by 30 cycles at denaturation 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

The results of Polymerase Chain Reaction assay technique were for direct detection of *Cryptosporidium parvum* were show in 12 positive out 50 fecal samples of human stool samples at percent (24%). Whereas , less prevalence of infection was detect in camel in 7 positive out 50 fecal samples of stool samples at percent (14%). 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 as shown in the following figures:

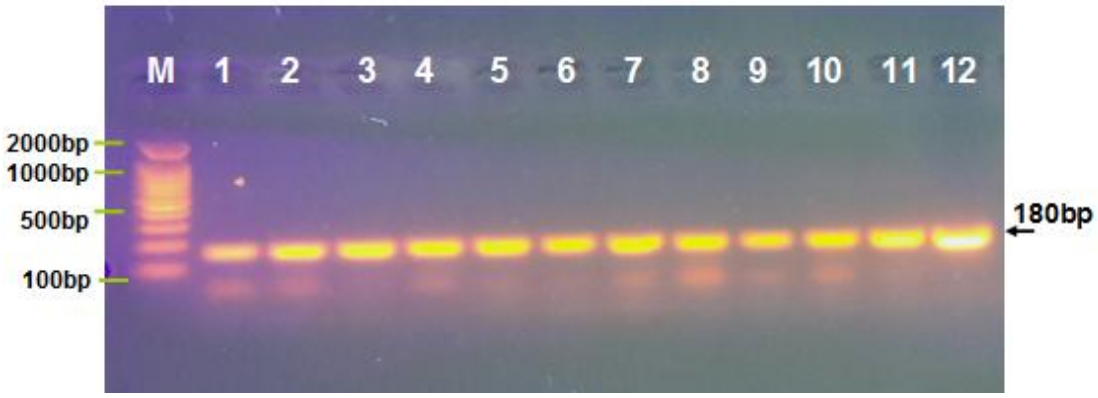

Figure 1:Agarose gel electrophoresis picture that offer the PCR product of heat shock protein gene that using in detection Cryptosporidium parvum in human fecal samples. 9Where M: Marker (2000 -100bp), lane (1- 12) positive C. parvum at 180bp PCR product size.

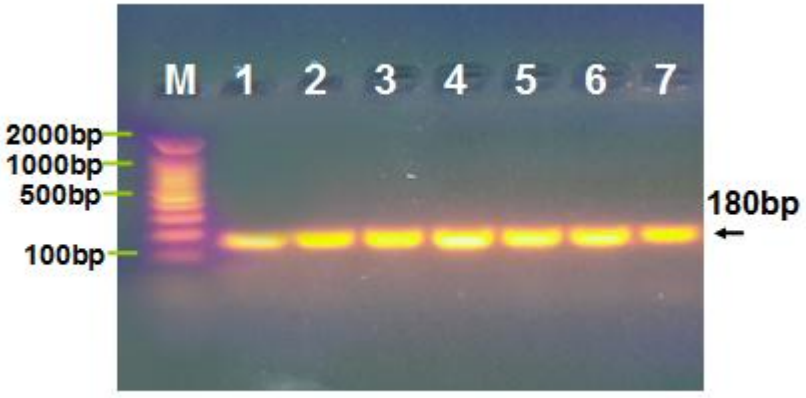

Figure 2: Agarose gel electrophoresis picture that display the PCR product of heat shock protein gene that using in detection Cryptosporidium parvum in camel fecal samples. Where M: Marker (2000-100bp), lane (1-7) positive C. parvum at 180bp PCR product size.

Discussion

In this study we describe a, fast specific, and sensitive method for the direct detection of Cryptosporidium parvum in stool samples by Polymerase Chain Reaction technique. PCR-based assays have previously been used by others to diagnosis of Cryptosporidium parvum DNA in human stool 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 different authors have reported detection limits for PCR based methods by ranged from 100 to 2,000 oocysts per gram of human stool specimens (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 showed that 14% of the adult camels were infected with C. parvum. Other preceding studies notified a higher prevalence rate of Cryptosporidium species 37.9% of the adult camels and elucidated that the higher prevalence ratio of infection in both sexes in camel and different age ranges and open areas may be is related with higher risk of infection through environmental contamination due to grazing with other infected animals or to the spreading of manure (22) . The prevalence rate of Cryptosporidium infection was high in younger animals that reported by previous studies (23).

conclusion.

Cryptosporidium parvum is important causes of diarrhea infection in human and camel. whereas, the Polymerase Chain Reaction assay technique a fast specific, and sensitive method for the direct detection of Cryptosporidium parvum in stool specimens .

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