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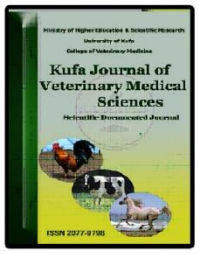

Direct detection of *Entamoeba bovis* in calves infected by diarrhea by using Polymerase chain reaction technique

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Abstract

This study carried out to direct molecular investigation of *Entamoeba bovis* from feces samples of calves which suffering from diarrhea that collected from different fields in Al-Diwanyia city by using polymerase chain reaction technique (PCR). This technique was dependent on used specific primers that amplification of small subunit ribosomal RNA gene in *Entamoeba* bovis. This primers were designed in this study by using NCBI-Genk data base (FN666248.1) and primer 3 plus for primers design. The PCR results were appeared that cattle infected with *Entamoeba bovis* in percentage of about (36%) 18 positive samples out of 50 diarrheic samples. We concluded that *Entamoeba bovis* is important causes of enteric infection in calf whereas, the polymerase chain reaction technique is very specific and rapid assay.

Key word: ***Entamoeba bovis***, **Calves** , **Polymerase chain reaction**

التشخيص المباشر لطفيلي *Entamoeba bovis* في العجول المصابة بالإسهال باستخدام تقنية تفاعل سلسلة البلمرة

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الخلاصة

تناولت الدراسة الحالية الفحص الجزيئي المباشر لطفيلي*Entamoeba bovis* لنماذج البراز للعجول المصابة بالاسهال التي جمعت من حقول مختلفة في مدينة الديوانية باستخدام تقنية تفاعل سلسلة البلمرة . تعتمد هذه التقنية على استخدام بادئات متخصصة التي تقوم بتضخيم الجين الرايبوسومي الصغير في *Entamoeba bovis* . صممت البرايمرات المستخدمة بالدراسة اعتمادا على موقع بنك الجينات العالمي (FN666248.1)و برنامج تصميم البادئات.

اظهرت نتائج تقنية تفاعل سلسلة البلمرة أن نسبة العجول المصابة بطفيلي *Entamoeba bovis* تشكل (36%) 18 عينة موجبة من أصل 50 عينة براز من عجول مصابة بالإسهال.

أوضحت الدراسة الحالية بان طفيلي *Entamoeba bovis* مسبب مهم للإصابات المعوية في العجول وان تقنية تفاعل سلسلة البلمرة ذات خصوصية وسريعة وتعتبر دراستنا الأولى في العراق.

Introduction

Entamoeba is protozoan parasite that can cause amoebiasis in various animal species and humans , Numerous species are found in humans and animals (1). Entamoeba detected in cattle was *Entamoeba bovis*

Entamoeba species are not pathogens in ruminants (2). *Entamoeba histolytica* is the parasite responsible

for invasive amoebiasis that includes amoebic dysentery and amoebic liver abscesses infection (3) Others such as *Entamoeba dispar* and *Entamoeba coli* are nonpathogenic species that frequently exists as a commensal parasite and harmless (4). Whereas, the Entamoeba gingivalis, which lives in the mouth, and *Entamoeba moshkovskii*, which is frequently isolated from river and lake sediments, *Entamoeba invadens* is a species that can cause a disease similar to E. histolytica but in reptiles (5,6). Some species of Entamoeba, such as *Entamoeba bovis*, inhabit the rumen of ruminant mammals; rarely, Entamoeba that are morphologically identical to *Entamoeba bovis* have been reported to cause serious invasive illness in some ruminants other than cattle (7). Diarrhea infection of neonatal and young calves is a common disease see in cattle. The other most common parasitic agents responsible for the diarrhea infection are parasites such as Giardia, Cryptosporidium, Eimeria and *Toxocara vitulorum*. However, bacterial and viral agents and nutritional factors also play role in the diarrhea (8). Light microscopy of fecal samples, the traditional diagnostic method, is unable to differentiate between cysts of pathogenic amoeba such as *Entamoeba histolytica* and *Entamoeba bovis* from other the non-pathogenic amoeba *Entamoeba dispar* (9,10). Therefore, newer methods, including serological antigen detection and polymerase chain reaction (PCR), are undergoing evaluation as diagnostic tools. The PCR assay is, sensitive and rapid method that can differentiates between Entamoeba species from stool specimens without the need for prior cultivation . In this study we aimed to develop PCR methods for use in the diagnostic laboratory for detection *Entamoeba bovis* in calves.

Materials and Methods

Stool sample collection. Fecal samples were collected from 50 cattle (calves) 1-10 month age old that infected by diarrhea from different filed in Al-Diwanyia province. The Fecal sample was transferred to a clean, dry plastic container and transported to the laboratory for analysis.

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 *Entamoeba bovis* by using specific primer for 18S small subunit (SSU) rRNA gene in Entamoeba bovis, the primers forward primer (ACGAGGAATTG GGGTTCGAC) and reverse primer (GCCTTGTGACCATACTCCCC) this primers were designed in this study using (NCBI- GenBank: FN666248.1) 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 58°C for 30 seconds, and extension 72°C for 1 minute and then final extension at 72°C for 5 minutes. The PCR products (782bp) were examined by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV illumination.

Results

PCR assay results for detection *Entamoeba bovis* were show in 18 positive out 50 fecal samples of calves at percent (38%).. The high prevalence of infection was show in calves have age from 1-2 month (7/16) positive samples at percent (36.84%). Whereas , less prevalence of infection was show in calves have age from 9-10 month (1/4) positive samples at percent (5.26%%) as show in the following (table 1 and figure 1):

Table (1): Positive samples results and percentage of *Entamoeba bovis*.

Age	No. of tested samples	PCR positive results	Percentage (100)
1-2 month	**16**	**7**	**36.84**
3-4 month	**13**	**5**	**26.32**
5-6 month	**10**	**4**	**21.05**
7-8 month	**7**	**2**	**10.53**
9-10 month	**4**	**1**	**5.26**
Total	**50**	**19**	**38.00**

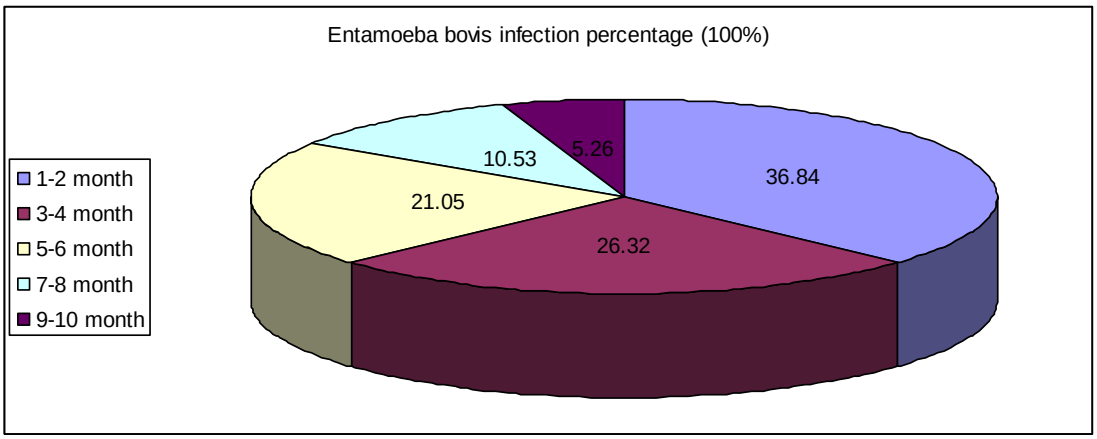

Figure (1): The percentage of *Entamoeba bovis* positive samples according to age.

PCR technique based 18S small subunit (SSU) rRNA gene for detection *Entamoeba bovis* were show good amplification of 18S small subunit (SSU) rRNA gene in extracted DNA from fecal samples as shown in the following figures:

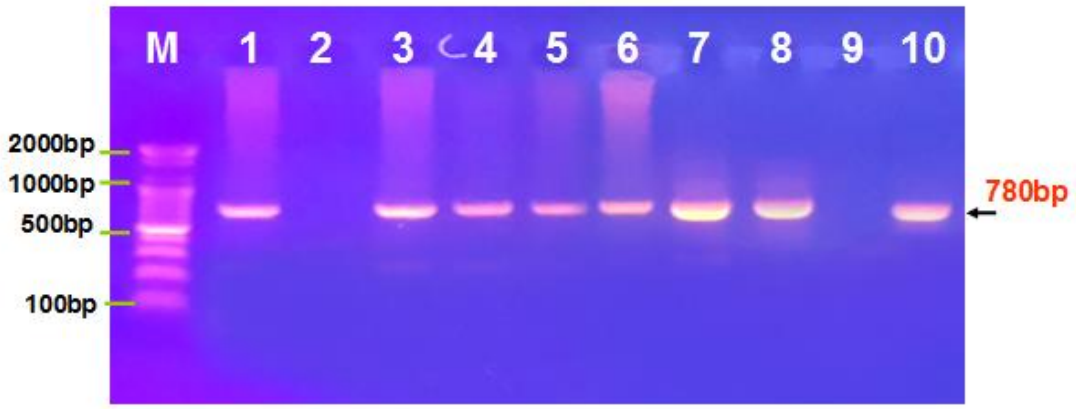

Figure1: Agarose gel electrophoresis image that shown the PCR product of 18S small subunit (SSU) rRNA gene that using in detection E. bovis. Where M: Marker (2000-100bp), lane (1,3-8, and 10) positive E. bovis at 782bp PCR product size.

Discussion

There are no adequate studies remember Entamoeba bovis infection in calf. In general, Entamoeba infections are asymptomatic, and some calf develop diarrhea or dysentery (11). The present study recorded *Endamoeba bovis* infection at (38%) 18 of 50 calves feces samples. The first study by Refaii (12) who recorded Entamoeba bovis infection in large ruminants in Egypt with percentage of about 85% in cattle, and 80% among buffaloes. Another study was observed a clinical infection of a 1.5 month old Jersey cross-breed calf with Entamoeba bovis (13). Other study recording the presence of *Endamoeba bovis* in calf samples and sheep by (28.5% ; 14.2%) also recorded the presence *Entamoeba histolytica* by Nested –PCR (85.7% ; 71.4%) and *E. dispar* (21.45; 35.7%) in cows and sheep samples respectively and empty human feces samples from *E. bovis* (14). (15) reported Entamoeba spp was least common parasite caused diarrhea in young calves, only 15 of 321 (6.4%) calves had entamoeba cyst and trophozoites. (16) was the first reported of *E. bovis* like organisms invading and causing pathological changes in the tissues of their host. (17) recorded infected in 45 of 64 sample which positive in microscopic examination of animal feces samples in rome garden and infection by 8% *E.dispar* and 9% *E. histolytica*.

In conclusion *Entamoeba bovis* is important causes of enteric infection in calf whereas, the polymerase chain reaction technique is very specific and rapid assay .

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