MOLECULAR CHARACTERIZATION OF *ECHINOCOCCUS GRANULOSUS* ISOLATED FROM HUMAN AND DOMESTIC ANIMALS IN KIRKUK, IRAQ

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ABSTRACT

Total of fifty eight isolates of Echinococcus granulosus were collected from human, sheep, goat and cattle origins and characterized using PCR-RFLP methods. The results indicated that the cosmopolitan sheep strain of E. granulosus is the most prevalent strain in Kirkuk, Iraq. The study furthermore, demonstrated that the form of Echinococcus that infect human in Kirkuk, Iraq is a sheep strain.

Keywords: *Echinococcus granulosus*, Characterization, Hydatid cyst, Sheep strain, PCR-RFLP

INTRODUCTION

Echinococcosis or hydatidosis caused by the metacestode of *Echinococcus granulosus* is one of the most important worldwide zoonotic disease of human and domestic animals (Seres *et al.,* 2009; Ergin *et al.,* 2010; Hama *et al.,* 2012; Pestechian *et al.,* 2014). Molecular approaches have been extensively used and 10 distinct genotypes termed G1 – G9 were identified for *E. granulosus* (Sharbatkhori *et al.,* 2011). These include G1 and G2 as sheep strain, G3 and G5 as bovine strains, G4 and G6 as horse and camel strains, G7 as pig strain, G8 and G10 as cervidae strains and G9 as Polish patient strain (Grosso *et al.,* 2012). The objective of the present study was to molecularly characterize of *E. granulosus* strains from sheep, goat, cattle and human in Kirkuk Province, Iraq.

MATERIAL AND METHODS

Sample Collection: Fifty eight cysts of *E. granulosus*, including 22 samples isolates from sheep, 15 samples isolates from goat and 17 samples isolates from cattle slaughtered at Kirkuk slaughter house were collected.

Four human hydatid cysts were recovered from postoperatively patients admitted to General Kirkuk Hospital. Protoscoleces were aspirated from cysts, pooled and washed with sterile phosphate buffer saline (PBS), pH 7.2.

DNA Extraction: Total genomic DNA from protoscoleces was extracted using (Bioneer Accuprep Genomic DNA Extraction Kit, Korea) according to manufacturer's instructions. A mixture containing 200 µl of protoscoleces suspension, 20 µl proteinase K and 200 µl lysing buffer was incubated at 37 C° for 1 hour and then at 70 C° for 30 minutes. After incubation, 200 µl of absolute ethanol was added to the mixture and transferred to the QlAamp spin column and then centrifuged at 8000 rpm for 1 minute at room temperature. The column was washed twice using 500 µl of washing buffer by centrifugation at 8000 rpm for 1 minute. DNA was eluted from the column with 200 µl of deionized distilled water preheated at 70 C°. Maximum DNA yield was obtained by concentration and quality was assessed by both UV absorbance at 260 nm wave length and electrophoresis on the 1 % agarose gel.

Polymerase Chain Reaction Amplification: The PCR was initially performed using the oligonucleotide primers forward (Eg1f) (5′ CATTAATGTATTTTGTAAAGTTG 3′) and reverse (Eg1f) (5′ CACATCATCTTACAATAACACC 3′) which were previously reported to specifically amplify the fragment of nuclear ribosomal DNA (Bowles and McManus, 1999). The PCR amplification was conducted using a PTC-100 thermo cycler (Mj Research Inc., Waltham, MA) following the thermal profile previously used (Sarvi *et al.,* 2004). A stock buffered solution containing 250 µl 10X PCR buffer, 100 µl of $MgCl_2$, 12.5 µl of each dATP, dTTP, dGTP and dCTP was prepared in 1.5 ml eppendrof tube. The primers were used at a concentration of 20 pg/µl and deionized distilled water was added to bring the volume of the stock buffer solution to 1.5 ml. 2 µl of the primers, 5 µl of the target DNA and 42 µl of the stock solution were added on the 0.5 ml PCR tubes and mixed by vortexing. 1 µl of Taq DNA polymerase (Perkin Elmer) at a concentration of 5 u/µl were used. All PCR amplification reactions were carried out in a final volume of 50 µl. The thermal cycling profile were as follows: a 2 minutes initial incubation at 95 C°, followed by 40 cycles of 95 C° for 1 minute, 55 C° for 30 seconds and 72 C° for 45 seconds and final incubation at 72 C° for 10 minutes. The PCR products (8 µ) mixed with loading buffer (2 µl), were separated on a 1.5 % w/v Tris-borate/EDTA (TBE) agarose gel electrophoresis at 90 V for 30 minutes, and stained with ethidium bromide. Specific fragments were identified by size using 100 pb ladder under UV light transilluminator and photographed.

Restriction Fragment Length Polymorphism (RFLP): The PCR products were digested directly with 18 µl deionized water, 2 µl buffer and 1 µl of the restriction endonuclease RsaI at 37 °C, for 6 – 8 hour in a volume of 31 µl. Restriction fragments were separated on 1 % (w/v) agarose gel, stained with ethidium bromide and photographed. The 2000 DNA size marker (TaKaRa) was used to estimate the sizes of fragments.

RESULTS

Protoscoleces derived from hydatid cysts of different host origin were used to extract DNA. Protoscoleces of sheep hydatid cysts contain relatively higher amount of DNA than Protoscoleces of goat, cattle and human hydatid cysts (Table 1). The DNAs from all isolates were highly purified (1.83 – 1.84). The melting Tm and the base percentage composition (G + C) of the isolated DNA were found to vary from 85.2 – 86.6 C° and 38.7 – 42.2 % respectively. The isolated DNA was assessed by electrophoresis on the agarose gel (Figure 1). Then the isolates were characterized by amplification of the rDNA-ITS 1 region of the parasite. For sheep, goat and human isolates, two amplicons of about 1100 bp and 900 bp were recorded, while for cattle isolate, amplicon of 1000 pb for ITS fragment were produced by PCR (Figure 2). When the purified ITS 1 fragment of rDNA PCR products of sheep, goat and human isolates remained undigested (1100 bp and 900 bp in length), but that of cattle isolates were digested into two bands of approximately 650 pb and 350 bp respectively (Figure 3).

DISCUSSION

Although, the cystic hydatid and alveolar hydatid diseases give rise to important public health problems, current efforts to control disease are insufficient (McManus *et al.,* 2003) because of the wide diversity of cystic larval stages. In human and livestock and the possibility of interaction between cycles of transmission, in addition, to the epidemiological situation means that each focus requires specific control principles and methods (Moro and Schantz, 2009). Moreover, hydatidosis control is usually hampered by the ignorance of the true prevalence of the diseases and under estimation of the human suffering and invalidity they cause (Gauci *et al.,* 2005). The base compassion of DNA is considered to be characteristic of an organism and is usually expressed as the percentage of guanine + cytosine (G + C) bases.

Table 1: Characterization of DNA isolated from hydatid cysts of different origin

Hydatid cyst	DNA (µg/mg)	A260	A280	260/280	Tm (°C)	G + C (%)
Sheep	8.2	0.625	0.340	1.838	85.4	39.2
Goat	5.9	0.598	0.328	1.834	85.5	39.5
Cattle	7.8	0.510	0.277	1.841	86.6	42.2
Human	4.3	0.482	0.263	1.832	85.4	39.2

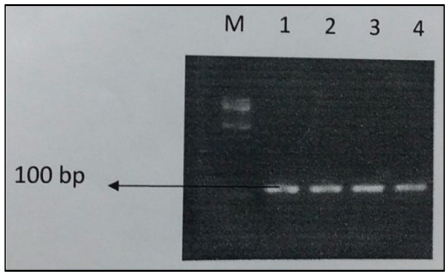

Figure 1: Agarose gel electrophoresis of protoscoleces DNA isolated from sheep (lane 1), goat (lane 2), cattle (lane 3) and human (lane 4)

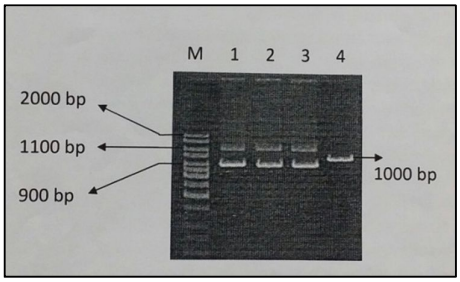

Figure 2: Agarose gel electrophoresis of ITS-PCR products from protoscoleces isolates of sheep (lane 1), goat (lane 2), human (lane 3) and cattle (lane 4)

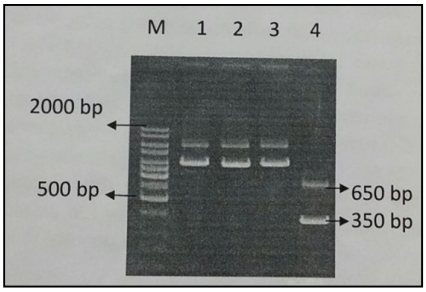

Figure 3: The PCR products of ITS from protoscoleces isolates of sheep (lane 1), goat (lane 2), human (lane 3) and cattle (lane 4) samples were analyzed by RsaI

The G + C value obtained for protoscoleces DNA isolated from different host origin was in the range (38 – 42 %), quite similar to that of other parasitic helminthes (Barrett, 1981). In the present investigation, analysis of melting temperature (Tm) of DNA was also used to differentiate between hydatid cyst protoscoleces of different host origin. This method is highly sensitive because Tm values did not overlap (Pestechian *et al.,* 2014). Thus, the sheep, goat and human DNA could be easily and rapidly distinguished from cattle DNA because of 1.2 °C difference in Tm. The accuracy of Tm analysis was confirmed by PCR. The RFLP - PCR amplification of ITS sequences of the nuclear ribosomal DNA (rDNA) have been shown to provide reliable alternatives to more traditional methods for the specific identification of *Echinococcus* strains from different intermediate host (Pestechian *et al.,* 2014). In this study, goat and human isolates examined gave very similar patterns to sheep strain after restriction enzyme digestion, whereas distinct ITS 1 RFLP pattern was obtained for cattle strain.

Therefore, the form of *Echinococcus that* infect human in Kirkuk, Iraq is a sheep strain. The results of this study were similar to previous studies in Iraq showing that the sheep strain is predominant in Kurdistan (Hama *et al.,* 2012) and in Nineveh province (Salih and Al-Jamain, 2002). In conclusion, a PCR-RFLP method established using the ITS sequences as the genetic markers provides a simple but yet powerful tool for accurate identification and differentiation of *Echinococcus strains*.

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