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Molecular detection of *Taenia hydatigena* cysts from the visceral organs of sheep slaughtered at -AL-Qadissyia governorate abattoir

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Abstract

Detection of Taenia *hydatigena* infection in animals using ordinary tests is a time-consuming. Therefore, this study was designed to develop and choose the optimal PCR technique with specifically designed primer for cystic fluid to detect *Taenia hydatigena* infection in sheep in Iraq. The isolated nucleotide sequences of *T. hydatigena* from Iraqi sheep was reported to Gen Bank with other researchers previously. Various visceral organs (livers, spleens, and lungs) suspected to have cysticercus were collected from 40 sheep and sent to a parasitological laboratory for identification. The DNA was extracted from all cystic fluid and amplified for molecular identification using conventional PCR technique. *T. hydatigena* was detected using PCR, and the percentage of the highest positive result was 19 (47.5%). Moreover, the percentages of the positive internal organ descending were 12 (63.2%), 5 (26.3%) and 2(10.5%) for Liver, lung, and spleen, respectively. In conclusion, this study approved the ability of the designed primer to detect *T. hydatigena* infection in different internal organs in sheep.

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Key words: *Taenia hydatigena* , cysticercus in sheep and PCR

Introduction

Taenia hydatigena is a zoonotic cestode. A carnivore is the definitive hosts, while herbivore acts as intermediate hosts, where they cause hydatidosis and cysticercosis (Brunetti & White, 2012). Sheep represent the high proportion of the livestock population in Iraq. Worldwide, as with all species of Taeniasis, the transmission of diseases between host occurs via ingestion. However, Taenia species are unique amongst the Cestoda in demanding two obligate mammalian hosts for life cycle transmission; the cestode matures in the carnivores with the liberation of fertilized

eggs, egg to herbivore, then herbivore to carnivore (Hoberg, 2006). It is also considered that cysticercosis was usually gained by eating diets or drinking water that contaminated by tapeworm eggs from human feces (Bobes *et al.,* 2014). In contrast with cysticercosis, the taeniasis is comparatively unhurt. The mature stages of this cestodes are infecting the small and large intestine of humans and causing a few nonspecific symptoms, like nausea and abdominal pain (Pawlowski & Schultz, 1972). Recently, molecular techniques have developed for diagnosing the diseases in human and animals. It is considered as a very helpful for the accurate recognition of human taeniid cestode isolates. To overcome the restriction of identification of taeniasis that depends on the morphology, a numerous molecular approaches have been developed, inclusive the use of DNA probes (González *et al.,* 2002). Molecular tools are progressively used to develop these areas of facts, and to use a proportional mito-genomic approach to evaluate mitochondrial (mt) DNAs as a source of the new molecular score (Omar *et al.,* 2016). Mitochondrial genes are the most genetic markers for molecular-based access to evolutionary biology and population genetics (Hajibabaei *et al.,* 2007; Will *et al.,* 2005). Taeniasis is chronic zoonotic diseases that present the variable clinical manifestations. Currently, the diagnosis of these diseases depended on the antigen detection from the cyst containing fluid. This tool is seldom done and most specific, due to the complications involved. Detecting the presence of *Taenia hydatigena* deoxyribonucleic acid (DNA) by the polymerase chain reaction (PCR) could provide a definitive diagnosis of Taeniasis. Review of literature revealed very limited information on the genetic nature of *Taenia hydatigena* in sheep in Iraq. Therefore, this study intended to choose the optimal PCR technique with specific primer design for cystic fluid to detect Taenia hydatigena in the internal visceral organs of sheep at AL-Qadissyia governorate abattoirs.

Material and methods

The internal organs including Liver, spleen, and lung were collected from 40, three months old sheep. These samples were sent to a parasitological laboratory in the Al-Qadissyia Veterinary medicine for parasite investigation. The determination of parasite was done according to the method described previously by Utuk & Piskin, (2012). All organs were cut into pieces about one-centimeter and kept in warm water for 20 minutes. Then the tissue was removed by compression, and the sediments were examined under light microscope. Thirty-Three cystic samples were collected from the obtained sediment. Twenty cysts were examined under light microscopy. While, the remaining parasites were taken in phosphate buffered saline (PS) (Dermauw *et al.,* 2016). DNA extraction was done for all cystic fluid by Tissue/Blood DNA Mini Kit (Geneaid, Korea), according to the manufacturer's instructions. The primer pair was used to amplify the partial mitochondrial cytochrome c oxidase subunit 1 gene (cox1) of *Taenia hydatigena.* The sequence of the primer was designed according to National Center for Biotechnology (http://www.ncbi.nlm.nih.gov/). The websites accession numbers were (JN831297.1, FJ518620.1, GQ228819.1, and JN831305.1). The primers were designed from Primer3 plus (http://primer3.wi.mit.edu/) website. The sequence of the designed primers are presented in Table. 1.

Table 1: shows designed primer used in this study

Name of primers	sequences	Base pair	Melting temperature	G-C contents
Cox1F	5′-AGGAGCTGGTATTGGGTGAA –3	20 bp	59.5C°	50.0%
Cox1R	5′-TCCAAAAGCATCAGGACTCA–3′	20 bp	59.4C°	45.0%

PCR amplification was completed using conventional PCR conditions in a final volume of 25 µl: 5 µl of DNA template (20 ng/µl), 12µlMilliQ water and 1.5µl for both primer forward and reverse primer (10 µM). All mixer were collected on each PCR tubes containing 5µl of AccuPower® ProFiTaq PCR PreMix (Bioneer, Korea). The PCR reactivity was held out in a Techne TC-512 thermocycler. The following cycling conditions were including the following: initial denaturation(95 °C, 15 min), succeed by 35 cycles of denaturation (95 °C, for thirty seconds), annealing (56 °C, 40 s) and sixty seconds for extension have (72 °C), and a last extension step (72 °C, 10 min). All the amplification of a single fragment of the expected size established by gel electrophoresis on a 1.5 % agarose gel (TBE, 1 %) stained with ethidium bromide.

Results

The overall incidence of *Taenia hydatigena* in sheep was 19 (47.5%). Moreover, its incidence in the various internal organs was 12 (63.2%), 2(10.5%) and 5 (26.3%) for Liver, spleen, and lung respectively. (Table. 2).

Table 2: shows the percentage of *T. hydatigena* cysts in visceral organs

Visceral Organ	Number of visceral organs	percentage
liver	12	63.2%
lung	5	26.3%
spleen	2	10.5%
Total of Taeiniasis	19	47.5%
Statistical analysis	X^2= 6.012, P value= 0.049, significant differences	

Both male and female sheep revealed the incidence of *Taenia hydatigena* infection. The percentages of infection in female and male were 63.2% and 26.3% respectively (Table. 3). However, statistical analysis *Taenia hydatigena* revealed non-significant differences (P > 0.05) in infection rates among the infected male and female.

Table 3: showed the percentage of *T. hydatigena* from suspected cysticercus in both sexes.

Sex	Suspected cysticercus	Positive results	percentage
Male	20	8	63.2%
Female	20	11	26.3%
Total	40	19	47.5%
Statistical analysis	X^2= 0.322, P value= 0.57, non-significant differences		

The PCR techniques were proper to all visceral organs for the partial cytochrome oxidase subunit I (cox1) gene. The amplified fragments size were approximately 446bp (Figure 1)

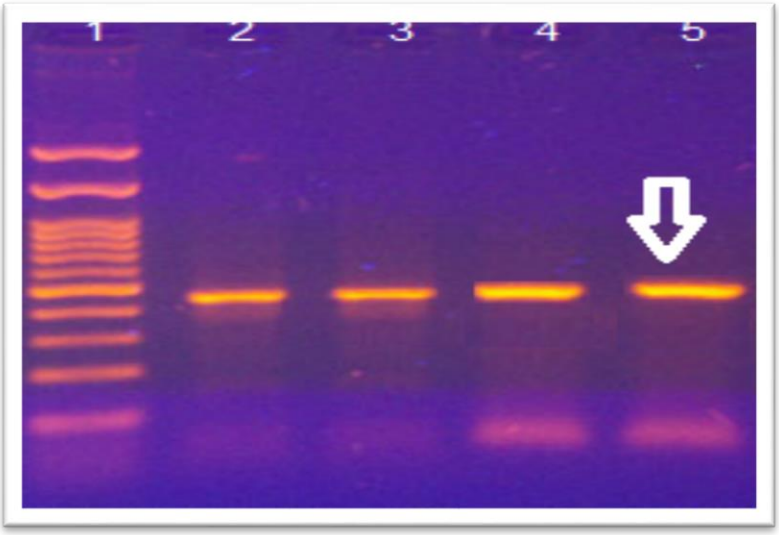

Figure 1: lane 1 represented a size of DNA ladder 1500 bp, lane 2,3,4 and 5 are a single amplified product, the arrow(⇩) showed the desired product 420 bp from PCR amplification.

Discussion

Taenia hydatigena is a global tapeworm found in domestic animals worldwide, having been recorded with the prevalence of 16.7% in sheep from Germany (Hasslinger & Weber-Werringhen, 1988), and 33.3% in sheep and 30.2% in goats from Nigeria (Fakae, 1990; Nwosu *et al.*,1996). Precise identification of the causative agents of various Taenia infections is crucial for understanding the epidemiology and transmission mechanism, vaccine development, exact diagnosis, treatment, and effective prevention and control of the taeniid species.

The present study revealed the incidence of *Taenia hydatigena* infection in sheep at Al-Qadissyia province abattoir. Out of 40 samples, 19 (74.55%) were positive. For the author's knowledge, this is the first study that determined the incidence of *Taenia hydatigena* using polymerase chine reaction with a designed specific primers in Al-Qadissyia province/ Iraq and according to the search of the surveillance and statistical data (Biu & Murtala, 2012). These results are compatible with a previously reported mean infection rate of cysticercus of *T. hydatigena* in goats (71.9%) in Nigeria, even though it was higher than that found in the current study. However, in India, the reported prevalence rate was 4.83% and 2.33% in sheep and goat respectively (Singh *et al.,* 2015). The current study revealed that the incidence of infection occurred just in sheep because these animals are the most slaughtered in abattoirs in Iraq. The investigation of disease distribution was depended previously on the morphological studies (Ghaffar, 2011; Yildirim *et al* ., 2006). Accordingly, high incidences of Taenia hydatigena has been reported from sheep in Ethiopia (Sissay *et al.,* 2008), where, the overall incidence was 79 %, 26% and 68% for *Taenia hydatigena, Cysticercus ovis*, and *hydatid cysts*. The results of the current study also revealed higher incidence percentage of *T. hydatigena* in female than male. However, non-significant differences ($P>0.05$) was observed. Besides, the results of the visceral organ studies were descending 63.2%, 26.3 and 10.3 for liver, lung, and spleen respectively.

It is worth to mention that DNA sequence analysis of mitochondrial genes is a reliable and sensitive tool for determining the genetic relatedness between various helminth species. The PCR technique is the more used method in the genetic studies of Taeniasis (González *et al.,* 2002; Mayta *et al.,* 2008).

Therefore, the development of direct PCR techniques has allowed developing a specific region of partial mitochondrial cytochrome c oxidase subunit 1 gene (cox1). This gene was used previously, by several researchers (Tsukihara *et al.,* 1996) because it is the gene that was often applied universally as a DNA barcode to identify multiple eukaryotic cells. Moreover, this gene was sequence conserved among inter- and intra-specific level. (Hebert *et al.,* 2003). The primer pair designed in this study approved its ability to differentiate *C. tenuicollis* from another cystic structure. Moreover, partial sequencing of the 446 bp amplicon of JB3 and JB4.5 primer pair enabled us to detect *T. hydatigena* at the species level. In conclusion, this study provides molecular data on the high incidence of *T. hydatigena* cysts in sheep Al-Qadissyia province abattoir. So attention should be taken because this species of the genus *Taenia* are responsible for significant medical and economic losses in humans and animals. The authors recommend another future study in different provinces in Iraq to understand the epidemiology of this parasite.

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