RESEARCH ARTICLE

Molecular Phylodiagnosis of *Taenia hydatigena* from Stray Dogs in Erbil Province-Kurdistan Region/Iraq

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ABSTRACT

Taenia hydatigena is one of the most prevalent taeniid species of livestock and dogs. A total of 52 fecal samples were collected from September 2019 to May 2020. The highest prevalence rate was observed in Rural area (21.15%, 11/52), as compared to Urban area (15.38%, 8/52). Nineteen (9 males and 10 females) out of 52 dogs were infected with *T. hydatigena*; there were no significant differences in prevalence based on molecular technique was observed between females (19.23%) and males (17.31%). The DNA's of taeniid eggs were extracted, amplified, and sequenced by targeting of mitochondrial cytochrome c oxidase subunit 1 (*cox1*). The partial nucleotide sequence was submitted to the GenBank and recorded under accession number (MT298194-MT298197). Molecular analysis indicates that the specimens characterized in this study belong to the same species and shared greater 98.71–100% identity with *cox1* sequences of *T. hydatigena*. This is the first molecular diagnosis of *T. hydatigena* in the stray dogs at Erbil Province. The data are a useful for health and educational authorities responsible for designing and implementing effective measures for disease control.

Keywords: Cytochrome c oxidase subunit 1; Phylogram; Stray dogs–Erbil; *Taenia hydatigena*

INTRODUCTION

Taenia hydatigena is a cosmopolitan and Member of the genus *Taenia*, which is the most important cyclophyllidean tapeworms (Gomez-Puerta et al., 2015). It is responsible for major medical and economic losses in animals (Ulziiargala et al., 2020), due to widespread parasite that can infect a wide range of herbivorous animals with its larval stage, *Cysticercus tenuicollis* (Myczkaa et al., 2019; AbouLaila et al., 2020). Furthermore, adult worms parasitize mostly present in small intestine of canids and produce eggs, which are infective to intermediate hosts (Mirbadie et al., 2019; Zhu et al., 2019). Taeniid tapeworms associated with the sheepdog life cycle. The disease in sheep-rearing countries is most prevalent; due to high number of stray dogs, and improper disposal of slaughtered home offal (Haddawee et al., 2018). Previous studies have indicated that *T. hydatigena* is one of the most prevalent *Taenia* species in dogs in Iraq (Faraj and Al-Amery, 2018), Iran (Emamapour et al., 2015), Turkey (Oguz et al., 2018), Russia (Moskvina and Ermolenko, 2016), and Italy (Liberato et al., 2018). The family *Taeniidae* (*Echinococcus* and *Taenia*) is the similarity of their eggs, in which they are microscopically indistinguishable (Mirbadie et al., 2019).

Taxonomy is a classical and essential system for organizing biodiversity (Boubaker et al., 2016). Recent advances in bioinformatics based on automated DNA sequencing and

statistical computer algorithms have radically altered how organisms are classified and how evolutionary relationships from higher taxa to species and populations can be reconstructed (Nakao et al., 2013; Zhu et al., 2019). In general, a few studies conducted mostly using partial mitochondrial cytochrome c oxidase subunit 1 (*cox1*) and *nad1* gene sequences have reported considerable levels of genetic variation among *T. hydatigena* populations from different geographical regions and hosts (Rostami et al., 2013; Omar et al., 2016; Muku et al., 2020). Polymerase chain reaction (PCR) assays have been widely used to detect and differentiate different species of parasites (Kilinc et al., 2019). The simplicity of this diagnostic tool and high-efficiency enabling for determination of parasite infection during the high patent period, as well as the pre- or late- patent infection period (Wang et al., 2018; Trasviña-Muñoz et al., 2020). This study was conducted to identify the strain of *T. hydatigena* from stray dogs of Erbil Province using the mitochondrial *cox1* gene sequence.

MATERIALS AND METHODS

Sample Collection and Egg Isolation

The study was carried out from September 2019 to May 2020, In this study, stray dogs were the street dogs is defined as any dog roaming in a public place without its owner or a person who is not responsible for it, was not treated for worms and usually fed on garbage. Fecal

samples were collected from both 18 male and 34 female dogs at different age groups. All animals were subjected to mandatory quarantine. During the first defecation of the animals, excrement was collected at the shelter. The whole stool was placed into a disposable plastic container, labeled, and transferred to the laboratory.

Detection of Taeniid-positive Egg Fecal Samples

Fifty-two fecal samples were collected and determined by the flotation and PCR technique from stray dogs, respectively. The fecal samples were filtered through double gauze in centrifuge tubes of 15 mL and were concentrated by centrifugation at $12,000\times g$ for 15 min. The eggs at the pellet were washed 3 times with PBS pH 7.4 by centrifugation at $1000\times g$ during 15 min. The precipitation was placed into a plastic tube and suspended in a Zinc sulfate solution (specific gravity 1.250), and Lugol's iodine was added to help in the identification of protozoan cysts and coccidial oocysts (Neves et al., 2014). A coverslip was then placed on the top of each tube and the tubes were left for 1 h to allow the eggs to float and attach. The eggs were visualized and characterized by microscope as shown in Figure 1 and sorted using a capillary (0.5 mm in diameter) to avoid proglottids or other impurities at -20°C until further analysis. The adult worms were obtained from nine naturally infected dogs after treatment with albendazole, at a dose of 0.3%g/kg body weight (Faraj and Al-Amery, 2018) as shown in Figure 2.

Genomic DNA eggs were extracted using the QIAamp DNA Stool Mini kit (Qiagen, Germany) according to the manufacturer's instructions. DNA elution was completed to 100 μL with Nuclease-Free water and stored at -20°C . The *cox1* gene was amplified by PCR using previously published primer, JB3 (forward): 5'TTTTGGGCATCCTGAGGTTTAT-3' and JB4.5 (reverse): 5'TAAAGAAAGAACATAATGAAAATG-3' to amplify 446bp for the *T. hydatigena* (Hama et al., 2018). The amplification reaction mixture (25 μL) consisted of 12.5 μL of (2 $\times$) Go-Tag master mix, 20 pmol (2 μL) of each primer (forward and reverse), 2 μL of template DNA, and 6.5 μL

nuclease-free water. The amplification reaction was carried out under the following conditions: a pre-amplification step at 94°C for 5 min, followed by 35 cycles with denaturing at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 45 s, and the final elongation of DNA strands, an ending-extension at 72°C for 10 min. Water instead of DNA was included in each set of PCR reactions as negative control. The PCR products were visualized by using 1.5% agarose gel electrophoresis (Promega, USA) at 100 volts for 55 min in 1 $\times$ TAE buffer (2M Tris-acetate, 50mM EDTA). The amplified products were commercially sequenced in both directions (Macrogen Inc. South Korea), and the generated sequences were examined using Accelrys Gene 2.5 program (Accelrys, Cambridge, UK) and compared against the NCBI database through the use of BLAST algorithm (<http://www.ncbi.nlm.nih.gov/BLAST/>).

Nucleotide Sequence and Phylogenetic Tree Analysis

The species of *Taenia* was confirmed by analyzing the nucleotide sequences of the *cox1* gene. The sequences were aligned through the ClustalW algorithm (Thompson et al., 1994), provided by BioEdit v7.2.5 (Hall, 1999), with sequences available in the GenBank (NCBI) database. Nucleotide sequences of the partial *cox1* gene of the *T. hydatigena* from stray dogs were deposited in the GenBank database under accession numbers MT298194- MT298197 through the use of BLAST algorithm (<http://www.ncbi.nlm.nih.gov/BLAST/>). Phylogenetic analyses were performed on individual partial gene sequences using MEGA software (Molecular Evolutionary Genetics Analysis 3.1; <http://www.megasoftware> and the neighbor-joining were used to build the tree.

RESULTS

Out of 52 fecal samples from stray dogs were analyzed for the presence of *T. hydatigena*, 14 (26.92%) and 19 (36.54%)

Figure 1: Show (a) Eggs of *Taenia* spp. (b) Adult worm of *Taenia hydatigena* without stain

Figure 2: Gel electrophoresis from polymerase chain reaction (PCR) amplification of *Taenia hydatigena*. DNA marker size (50-bp DNA ladder), (L₁₀₀), DNA marker size (100-bp DNA ladder), (L₅₀) Cn1=Control DNA extraction, Cn2=Control negative PCR, lanes 1, 4, 7, 8, 15, 17, 19, 28, 32, 38 and 45-specific product for *Taenia hydatigena* isolated from dog faces

were positive by centrifugation-flotation test and PCR technique, respectively. The highest prevalence rate was observed in the Rural area (21.15%, 11/52), while the lowest was in Urban (15.38%, 8/52), respectively. However, no significant differences (0.999) were found between the two different areas. Furthermore, the highest rate of infection was found in females (19.23%) as compared to male stray dogs (17.31%) as shown in Table 1.

The amplification of the fragment occurred in 100% of the taeniid egg samples. As well as, the expected patterns were obtained in the samples with eggs of *Taenia* spp. as shown in Figure 2. The result was corresponding with *T. hydatigena* as the multiple sequences alignment of the nucleotide with (Boufana et al., 2015) ID: KT372519 as shown in Figure 3. It revealed that, five isolates (dog 8, 9,

28, 38, and 49) under the accession number (MT298196) showed 100% identity to *T. hydatigena* (KT372519) and six isolates (dog 4, 6, 7, 11, 19 and 45) under the accession number (MT298195) showed 99.49% identity with *T. hydatigena* due to nucleotide changes (T → C) at position 41 and (T → A) at position 78. The similarity MT298194 (dog 1, 3, 15, 27 and 32) and KT372519 was 99.23% due to nucleotide changes (T → C) at position 41 and 216 and (T → A) at position 78, and finally, the difference between MT298197 (dog 10, 17, and 31) and KT372519 was 1.29% due to multiple nucleotide changes at position 36 and 41 (T → C), (T → A) at position 78, (G → A) at position 210, and (G → T) at position 300. Figure 4 shows the phylogenetic tree of the dog fecal samples with reference sequences KT372519, MN478491, MN175599, MT086496, MN216152, MN478491, MH638348, MN114527, JN831304, KP641176, JQ710588, and KF268023).

Table 1: Prevalence of *Taenia hydatigena* in stray dogs at Erbil Province

Variables	No. of tested dogs	Positive no. fecal centrifugal flotation test	Percentage	Positive no. by PCR	Positive no. by PCR
Address					
Urban	22	6	11.54	8	15.38
Rural	30	8	15.38	11	21.15
Gender					
Male	18	7	13.46	9	17.31
Female	34	7	13.46	10	19.23
Total	52	14	26.92	19	36.54

($P < 0.05$), PCR: Polymerase chain reaction

Figure 3: Alignment of a fragment of *Taenia hydatigena* (MT298194-MT298197) with GenBank accession number KT372519

Figure 4: Phylogenetic tree of representative sequences of *Taenia hydatigena* from Erbil province and reference sequences of other genotypes. The tree was obtained by neighbor-joining analysis using partial sequences from *cox1* gene

DISCUSSION

Epidemiological study of the zoonotic parasite is important for minimization of the risk to domestic animals (Liberato et al., 2018). The overall prevalence of *T. hydatigena* infections found in this study was 26.92% were positive by the flotation test. Previous studies conducted in Iran showed that the most prevalent rate (43%) observed parasite was *T. hydatigena* (Emamapour et al., 2015). These may be due to no health control measures and their habits exposed to natural infection more than owned dogs. The prevalence rate (32.54%) by molecular method (PCR), these agreed with (Zhu et al., 2019), who showed that no cross-reactivity was observed with other tapeworms inhabiting the small intestine as well as with each other. The minimum detected DNA ranged from 0.1 to 1 ng, low enough to produce results in case of low DNA yield (Mirbadie et al., 2019). Besides, the highest rate of infection was found in female (19.23%) as compared to male stray dogs (17.31%), these agreed with (Trasvina-Munoz et al., 2020) who showed that the highest rate was found in female 3% and the lowest rate was found in male stray dogs 2.6% at Northwestern Mexico by molecular method. As it does not seem to be any biological plausibility, we cannot exclude a selection bias due to the passive recruitment of the sampled dogs (Liberato et al., 2018).

To confirm infections of taeniasis, PCR has also been used successfully with reportedly high sensitivity and specificity to detect the presence of *T. hydatigena* in fecal samples (Zhu et al., 2019). Mitochondrial DNA (mtDNA) has been widely applied for molecular population genetics and systematics as an important genetic marker (Faraj and Al-Amery, 2018). This is because of its simple structure,

low level of reorganization, and rapid evolution (Hassan et al., 2017). Molecular analysis indicates that the specimens characterized in this study belong to the same species and shared a greater 98.71–100% identity with *cox1* sequences of *T. hydatigena* from GenBank (KT372519) as described by (Boufana et al., 2015). This represents the first molecular diagnosis of *T. hydatigena* in the stray dogs. Also, *T. hydatigena* in livestock is required for more extensive investigation of transmission. Dominant strains identification in the endemic regions and the determination of the prepatent periods of these strains is also vital for ensuring efficacy in control programs with the use of anti-parasitic drugs (Faraj and Al-Amery, 2018). The isolated parasite was phylogenetically compared to the reference parasite, based on the *cox1* gene coding sequence. The phylogenetic analysis demonstrated that the isolates share close homology with some reference strains isolated in Iraq (Hama et al., 2018); Iran (Rostami et al., 2013); Egypt (Omar et al., 2016); Sudan (Muku et al., 2020); Italy (Boufana et al., 2015) and Nigeria (Ohiolei et al., 2019). *T. hydatigena* is one of the most taeniid species among sheep and dogs. The parasite transmission among a range of intermediate host species could increase the chance of genetic variability within different populations of the parasite in the world (Gomez-Puerta et al., 2015; Federer et al., 2016; Wang et al., 2018; Hidalgo et al., 2019).

CONCLUSION

Dog feces contamination with *T. hydatigena* in Erbil Province is demonstrated at the molecular level for the first time. These feces also cause a potential risk to livestock health. For this reason, there is a need for centrally coordinated control and prevention methods against the infection.

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