Original Article

Morphometrical and Molecular Identification of *Echinococcus granulosus* Genotypes in peri-urban wild dogs from an endemic focus in Northwest of Iran

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ABSTRACT

Echinococcus granulosus is a zoonotic parasite responsible for causing cystic echinococcosis in humans and animals. Cystic echinococcosis is recognized as a major public health problem in Iran, with numerous endemic areas spread throughout the country. Wild dogs (Canis familiaris) have been identified as the primary definitive hosts for *E.granulosus* and are known to play a vital role in the transmission and sustainability of the parasite's life cycle. Understanding the genetic diversity and distribution of *E. granulosus* genotypes in these wild dogs is important for effective control and prevention strategies. Between 2019 and 2022, a total of 68 peri-urban wild dogs, consisting of 47 males and 21 females, were captured, with unfortunate deaths due to car accidents or disease. Morphological and molecular investigation was performed to determine the presence *of E. granulosus*. The identification of *E. granulosus* genotypes was carried out by sequencing the COX1 and NADH1 genes. Of the 68 peri-urban wild dogs examined, 8 (11.7%) were positive for *E. granulosus* by morphological and molecular analysis. By performing PCR it was determined that the peri-urban wild dogs infected with *E. granulosus* carried the sheep strain (G1) genotype. This study successfully identified the presence of *E. granulosus* in peri-urban wild dogs, specifically with the G1 genotype. This finding highlights the potential risk that these dogs pose as carriers of this zoonotic parasite, which can be transmitted to humans and other animals. Further research and surveillance are essential to better understand the epidemiology of *E. granulosus* and to develop effective strategies for its control and eradication.

Keywords: *Echinococcus granulosus*, Genotypes, Iran, Molecular Identification, Wild Dogs

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1. Introduction

The occurrence of wildlife-transmitted zoonotic pathogens in peri-urban areas may be influenced by a variety of socio-ecological factors. Although *Echinococcus granulosus* has been widely reported to be prevalent among wild dog populations in peri-urban areas, our understanding of the specific factors influencing its occurrence remains limited (1). Echinococcosis, a zoonotic disease caused by the larval stage of the parasite *E. granulosus*, is a serious public health problem worldwide (2). It is the causative agent of cystic echinococcosis, commonly known as hydatid disease, which has significant economic and health implications(3). According to Grakh et al. (2020), there are several ways in which individuals can potentially contract *E. granulosus*, including through close contact with infected carnivores, consumption of eggs found in contaminated food, or even drinking contaminated water (4). Iran is recognized as one of the countries with a high prevalence of *E.granulosus*, and understanding the genotypic diversity of the parasite is crucial for effective control strategies. The parasite has a complex life cycle, with domestic and wild carnivores, particularly stray dogs, acting as definitive hosts (5). Wild dogs have been reported to contribute significantly to the maintenance and spread of *E. granulosus* in various regions worldwide, including northern Iran, which is considered an endemic focus of the disease. To fully undrrstand the dynamics, ecology, and potential public health risk associated with this parasite, it is crucial to identify and study its reservoirs in wildlife (6). The significance of *E.granulosus* has been highlighted by the invasion of wild dogs into peri-urban areas. Recently collected information has provided valuable insights into the behavior and territorial range of these wild dogs in peri-urban areas, contributing to our understanding of the potential risks to human well-being(7). The study combines both morphometric and molecular techniques to identify and characterize the different genotypes circulating in the local canine (dog) population. Morphometric analysis involves examining the morphological characteristics of the *E. granulosus* collected from infected dogs and comparing them to established criteria. This traditional method provides a first insight into the possible genotypes present in the region. However, due to its limitations, the molecular approach is essential to accurately distinguish and classify the genotypes. Recent molecular studies have shown that *E. granulosus* comprises a complex of genotypes, known as *E. granulosus s.l.* This complex includes *E. granulosus s.s.* (G1–G3), *E.equinus* (G4), *E.ortleppi* (G5), *E.canadensis* (G6, G7, G8 and G10) and *E.felidis* (8, 9). This research will contribute to the overall understanding of the genetic diversity and prevalence of *E. granulosus* genotypes in peri-urban wild dogs from the endemic focus in Northwestern Iran. The results of this study will provide valuable information for the design and implementation of effective control strategies and preventive measures, ultimately reducing public health and animal welfare burden of echinococcosis in the region.

2. Materials and Methods

2.1. Study Areas

Northwestern Iran (38 125′13″N 47 42′17″E) is located near the borders of Turkey and Armenia. It consists of several provinces, namely West Azerbaijan, East Azerbaijan, and Ardabil, located in the northwestern part of the country. The climate in the northwestern region of Iran can be classified as semi-arid to arid. Summers are generally hot and dry, with temperatures reaching highs of around 35-40°C (95-104°F). This area of Iran has a rich biodiversity, influenced by its diverse topography and climate. Wildlife enthusiasts can see various species, such as brown bears, mountain goats, wolves, and eagles in the mountainous areas.

2.2. Sample Collection

From 2019 to 2022, 68 carcasses of peri-urban wild dogs (47 males and 21 females) that died in road accidents were collected. Appropriate precautions and measures were taken to ensure the health and safety of the researchers during the study. The small intestines were examined for *Echinococcus* using a technique called IST (intestinal scraping technique) under safe conditions. The mucosa was carefully scraped with a laboratory spatula the intestine was opened longitudinally with scissors. The material was then washed onto a sieve, and *Echinococcus* was separated from the contents using a stereomicroscope. The collected samples were preserved in 70% ethanol until further testing. The intestines were isolated from each carcass, ligated, and preserved in 70% ethanol. All procedures were performed in accordance with the Code of Ethics (Code of Ethics: IR.UM.REC.1401.108).

2.3. Morphological and Morphometric Study

The collected *E. granulosus* specimens were subjected to morphometric analysis to determine various parameters.

Polyvinyl alcohol was applied to the glass slide containing adult scolexes for microscopic analysis. In this study, valid identification keys and the mentioned characteristics were used to identify adult worms (10). To identify the adult worms, the study relied on credible identification keys and the specified characteristics.

2.4. Molecular Analysis

2.4.1. DNA Extraction

Genomic DNA was extracted from all 68 wild dog samples using a DNA extraction kit (MBST, Tehran, Iran). Briefly, the *Echinococcus* samples were thoroughly homogenized using a pestle or vortex mixer to disrupt the tissue structure. The extracted DNA was stored at -20°C until further use to minimize DNA degradation.

2.4.2. PCR and Sequencing

The mitochondrial genes, COX1 and NADH1, were amplified according to the method described by Solgi et al.(11). The primer pair *JB11* (5'-AGA TTC GTA AGG GGC CTA ATA-3') and *JB12* (5'-ACC ACT AAC TAATTC ACT TTC-3') were used to amplify the 530 bp amplicon of the nadh1 gene. Jb3 and Jb4.5 primers were utilized to amplify the 444 bp amplicon of the cox1 gene. Amplification of the COX1 and NADH1 genes was performed using the thermal cycler instrument (Bio-Rad, USA) and a PCR program consisting of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 54°C for 30s, and extension at 72°C for 60s. A final extension step was performed at 72°C for 10min. The amplified fragments were visualized by staining with DNA safe stain (Cinaclone, Iran) and analyzed using a 1.5% agarose gel and a gel documentation system (Syngene, UK). Bidirectional sequencing of the COX1 and NADH1 genes from the selected PCR amplicons was performed by Cinnagen Company (Tehran, Iran) and subsequently analyzed by Chromas software ver: 2.6.6. The edited sequences were compared with each other using the BioEdit software ver: 7.2 (https://bioed it.software.infor mer.com/7.2/). Nucleotide queries from this study were submitted to the GenBank database.

3. Results

3.1. Prevalence

Of the 68 dogs with echinococcosis, 47 (69.1%) and 21 (30.8%) were male and female, respectively. molecular analysis. Morphological examinations were performed on these tapeworms. All tapeworms collected were recognized as *E. granulosus* based on their morphological characteristics. The mean total length of the adult tapeworms was 3.56 ± 0.2 mm, and the ratio of terminal proglottids as % of total length was 46.74 ± 0.95. The total number of rostellar hooks was 32.8 ± 1. Out of 68 peri-urban wild dogs examined, 8 (11.7%) were positive for *E. granulosus* by morphological and molecular analysis.

3.2. PCR cox1, NADH1 Gene, and Sequencing

Amplification of the COX1 and NADH1 genes was successful in all samples. However, only 40 samples were successfully sequenced for the COX1 and NADH1 amplicons (figure 1). The all of sequenced isolates were related to the G1genotypes. The final edited sequences of COX1 and NADH1 gene fragments were 360 bp and 371 bp, respectively. Regarding the COX1 gene sequence of different samples and their analysis, two haplotypes were identified in one polymorphic site (at position 95) in G1 strains. Meanwhile, there was no polymorphism in NADH1 gene in G1 isolates. The two amplified sequences derived from the COX1 gene of samples deposited in GeneBank as OR825007and OR825008. The two comprehensive sequences OR345478 and OR345479 showed 100% similarity to the sequences registered in the GenBank with accession numbers OR825007and OR825008, respectively. The sequences derived from the NADH1 gene of the samples were submitted as OR829749. The sequencing results of the NADH1 PCR products showed 100% similarity to the registered sequences in the GenBank with accession numbers MH686293.

4. Discussion

An investigation was performed in the northern regions of Iran to determine whether *E. granulosus* is prevalent among peri-urban wild dogs. In the current study, out of 68 wild dogs examined, 8 (11.7%) were positive for *E. granulosus*. According to a previous report, the infection rate of *E. granulosus* in stray dogs in the northeastern of Iran was 6.6%, which was lower than that of the present study (12). The prevalence of echonococcosis in the present study was also lower than the other studies from South America with a prevalence of 66.03(13), and Asia with a prevalence of 37.80%(14). Molecular results of the present study indicated that all isolated sample were related to *E. granulosus* genotype G1. There has been significantly less investigation of Echinococcosis genotypes among final hosts compared to intermediate

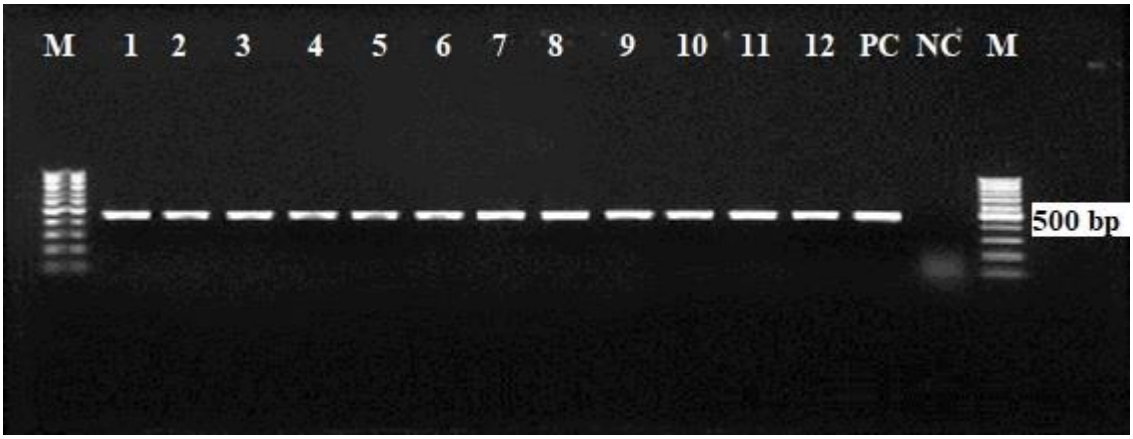

Figure 1: Agarose gel electrophoresis of Cox1–PCR products (1-12). M: DNA Ladder (100 bp), N: negative control, P: positive control.

hosts, both in Iran and worldwide. To date, there is limited information on the molecular characterization of genotype of E.granulosus in final host in Iran (15). In comparison , the *E. granulosus* G1 genotype was previously reported in dogs in northeastern Iran (16). Available molecular data from different livestock species, including sheep, goats, cattle, and camels, revealing the existence of G 1 genotypes in the north (17), northeast (18), and southwest (19), indicated that *E. granulosus* G1 genotype was circulating in these regions. It has been reported that *E. granulosus* genotype G1 is most commonly found in intermediate and final hosts throughout Iran (20, 21). These high similarities between the Iranian *E. granulosus* tapeworm sequences and those from different countries provide evidence of global genetic interconnection among populations of this species. It implies that *E. granulosus* may undergo international dispersion through various mechanisms, including animal migration, human movement, or the transportat of infected animals. The results of this study provide valuable insights into the prevalence of *E. granulosus* and the distribution of genotypes among peri-urban wild dog populations in Iran. The results confirm that echinococcosis is endemic in Iran. The identification of genotype G1 and the observed nucleotide variations highlight the importance of continuous monitoring and surveillance of this zoonotic parasite. In addition, the high similarity of genetic sequences with isolates from different countries underscores the need for international collaboration to address the challenges posed by *E. granulosus* and to develop effective strategies for control and prevention. The research highlights the need for enhanced surveillance, control measures, and public awareness campaigns.

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Authors' Contribution
Study concept and design: EE, HB
Data acquisition: MAD
Data analysis and interpretation: EE, HB
Manuscript preparation: MAD, EE
Critical revision of the manuscript: EE, HB
Statistical analysis: EE and HB

Ethics
It is declared that all ethical considerations were taken into account in the preparation of the submitted manuscript.

Conflict of Interest
The authors declare that they have no conflicts of interest.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

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