Prevalence of Echinococcus granulosus sensu lato in stray Dogs in Wasit province/Iraq

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Abstract

There is a possibility that humans could contract Echinococcus granulosus sensu lato (s.l.) from infected canines. We identified the prevalence and factors associated for E. granulosus s.l. infection in stray dogs that were found in the Wasit province. In this investigation, taeniid eggs were detected in feces samples from 110 dogs using a sedimentation technique. Using a multiplex PCR, the taeniid eggs were identified at the molecular level.40 (36%) of the 110 dog feces samples contained taeniid eggs, and 22 (55%) of those were recognized as Echinococcus granulosus s.l. We discovered is Echinococcus granulosus infection present among stray dogs and of different localities of Wasit Province , namely AL- Kut center (35 samples ,9(23%) positive pcr), Jassan (30sample ,5(13%) positive pcr) , AL- Zubaydiya and AL- dubuni (25sample ,4(10%) positive pcr) ,AL- Numaniya (10sample ,3(8%) positive pcr) and AL-Hayy (10sample ,1(3%) positive pcr) from highest prevalence to lowest prevalence. Conclusion; The results of this study offer evidence-based information regarding the epidemiological and molecular features of CE in dog final hosts of Wasit Iraq.

Keyword: *Echinococcus granulosus; dogs; Wasit/Iraq.*

1. INTRODUCTION

Echinococcus granulosus sensu lato (s.l.) is one of five species of flatworms in the family Taeniidae class Cestoda (Echinococcus sensu stricto, Echinococcus ortleppi, Echinococcus canadensis, Echinococcus equinus, and Echinococcus felidis) [1, 2]. E. granulosus s.l. larvae are considered to be the causative agent of cystic echinococcosis (CE). The high zoonotic potential of E. granulosus s.l., particularly in relation to E. granulosus s.s. [3] infection, is a major source of concern for global public health. Except for Antarctica, CE is found on all continents, and in endemic areas (southern and eastern Europe, central Asia, southern America, China, and northern and eastern Africa), the prevalence of CE in humans may range from 5% to 10% [4].

Canids, such as dogs and foxes, are the definitive hosts for adult E. granulosus, with adult worms reproducing in the intestine and infective eggs being shed in the host's faeces [5;6]. A number of animals, including humans and livestock, serve as intermediate hosts for E. granulosus, by ingesting soil, water, or food contaminated with the parasites' eggs and developing parasitic larval stages in their viscera, most commonly in liver and lungs (5; 7].The cycle is completed when canids consume infected viscera from carcasses of infected intermediate hosts. In human cases, hydatidosis is characterized by asymptomatic incubation periods that can last many years

until the parasite-containing structures (hydatid cysts) grow, compressing viscera and triggering serious clinical signs that may ultimately cause death. In livestock, most often sheep and cattle, cases of hydatidosis are usually only detected at slaughter (7].

E. granulosus s.l. has an indirect life cycle that includes both definitive (wild and domestic carnivores) and intermediate hosts (wild and domestic ungulates), the adult parasite lives in the small intestine of the final host, where eggs are dispersed with feces, Intermediate hosts are infected through the ingestion of eggs that develop into larvae in the internal organs (metacestode), Humans can become infected by accident, acting as an abnormal host. When definitive hosts feed on infected organs of intermediate hosts, the life cycle continues. The aberrant hosts, on the other hand, represent an epidemiological dead end [8].

The prevalence of echinococcosis in dogs is higher in areas where: there are large numbers of free roaming dogs (FRD) and where owners feed offal or allow their dogs access to it; people have a low level of education; and there is no formal control Programme (9;10).

Diagnosis could be made in a cutting-edge research facility using PCR and sequencing techniques (11) .DNA taeniid cestodes for identification of has used specific sequences from both the nuclear genome, which was much larger and located on chromosomes in the nucleus, and the mitochondrial genome, which was small and circular,multi-copied in the cell (generally less than 20,000 bp in metazoans) (12).

We conducted this investigation to ascertain the incidence of the illness and its contributing factors among stray dogs due to the dearth of information on the epidemiology of E. granulosus s.l. in Iraq. Additionally, knowing the factors that increase a dog's risk of contracting E. granulosus can help with informed control and prevention efforts for dogs and provide guidance for lowering or preventing the risk of infection in humans.

2. Materials and Methods

Study regions of dog

Located at an elevation of 16.75 meters (54.95 feet) above sea level, Wasit has a Subtropical desert climate. The city's yearly temperature is 29.2ºC sometimes up to 47 degrees, Humidity 24.37%. Wasit typically receives about 20.6 millimeters (0.81 inches) of precipitation and has 34.09 rainy days (9.34% of the time) annually. The cold months from November to March. The study area to dog be an AL Kut center, AL - Numaniya , AL Hayy, Jassan, AL- Zubaydiya & AL- dubuni in Wasit province. Figure 1 shows the regions our study studied.

Figure: (1): Map of Wasit Government. (Directorate of the Environment for Wasit province).

- Faecal sampling collection.

Faecal sample collection. This research was carried out between the months of January and May of 2022. On the feces of 110 stray dogs collected from various parts of Wasit Province. The samples were collected at random in public places. On the same day, the feces were brought to the laboratory and kept at −80ºC to keep the eggs inactive until they

were used (13). The feces were macroscopic examined by observing the visible outer surface under sufficient light and inspecting the inner surface by crumbling the feces with a glass rod to determine the presence of cestodes. The method of formalin-ether concentration was used (14). Using a microscope, the presence of taeniid (Taenia spp., Echinococcus spp.) eggs was determined. All of these samples, including taeniid eggs, were chosen for further molecular research.

. Copromicroscopic and Molecular Analyses

The formalin-ether sedimentation method has been modified [15,16]. Was used to detect taeniid eggs in fecal samples. For proper emulsification, approximately 1 g of stool was collected with a spatula and emulsified in 3 mL of 10% formalin in a mortar using a pestle. The stool emulsion was poured into a 15 mL centrifuge tube through a fine mesh of 250 m. 2 mL of 10% formalin was used to wash the stool through the gauze, 3 mL of ethyl acetate, in place of the original method's diethyl ether [17], was added to the centrifuge tube's contents and mixed, For 5 minutes, the mixture was centrifuged at 448 g. Following centrifugation, four layers were formed in the tube: an ethyl acetate layer, a debris plug, a formalin layer, and the sediment. After removing the top three layers, the sediment was thoroughly mixed, on a glass slide, two droplets of sediment were added together with one drop of iodine solution, and a cover slip was then put on top, The coverslip was then thoroughly looked at using an optical microscope (4x, 10x and 40x). Molecular tests were used to further identify the discovered taeniid eggs, Molecular analyses were used to identify the taeniid eggs that were discovered. Presto™ Stool DNA Extraction Kit was used to extract genomic DNA, and a multiplex polymerase chain reaction protocol was followed as already described [18].The total reaction mix volume was 25 µL: 12.5 µL Master Mix; 2 uL of Primer Cest (20 pmol/µL) and of Primer (20pmol/µL) ; 7.5µL nuclease-free water and 3 µL of template DNA (5 ng DNA per tube). Primers amplify fragments of the mitochondrial cytochrome oxidase subunit 1 (cox1) gene specific for E. granulosus s.l. (PCR Cox1 gene F (GTTTAGGGGCTGGTGTTGGT) R (TGAGCCACCACAAACCAAGT) 772bp, Nested PCR Cox1 gene F (TCTCTGCATTTGGCTGGTGT) R CCGTAACTCCCCCAAACGTA) 619bp) . Agarose gel electrophoresis was performed on precast gels (E-Gel™ EX Agarose Gels, 1.5% Agarose, (iNtRON, Korea). using the E-Gel™ Power Snap Electrophoresis System . DNA ladder (iNtRON, Korea)) was loaded onto the agarose gel for size determination of the PCR products.

Statistical Analysis

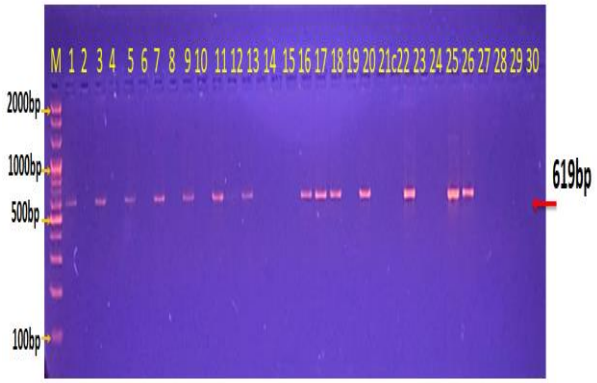
The Statistical Package for Social Sciences (SPSS) version 24 was used to examine the data and Microsoft Office Excel 2019, by using the Chi-squire, Level of significant was consider at P-value $\leqslant$ 0.05.

3. Results

The present data are the results of study carried out in Wasit province, Iraq, between periods from January 2022 to May 2022. The prevalence of E. granulosus s.l. was estimated in four administrative divisions' an stray dogs: The study area to dog be an AL Kut center, AL - Numaniya, AL Hayy, Jassan, AL-Zubaydiya & AL- dubuni. Fecal samples were collected from a random sample of 110 dogs to detect the presence of E. granulosus s.l. Extraction of parasite DNA from dog feces, directed on 40 samples, confirmed typical values for DNA concentration, and genomic DNA extracted from fecal samples was examined using a Nanodrop spectrophotometer (THERMO. USA), that check and measurement the purity of DNA through reading the absorbance in at (260 /280 nm)

total 40 of different E. granulosus DNA isolates were studied by using PCR amplification of partial mitochondrial cytochrome C oxidase subunit1 (Cox1) with PCR product of 619 bp, that by using the multiple Nested PCR Cox1 gene, Among the results were positive for 22(55%) them, 18 negative samples appeared, these negative samples were excluded from the following tests, Electrophoresis was used to visualize them using a 1.5% gel concentration for the best separation of large molecular weight DNA ,The PCR products of the isolates by using gel electrophoresis revealed major banding patterns ,figure (3-3)

Figure (3-1): Agarose gel electrophoresis image that showed the Nested PCR product analysis of Echinococcus granulosus mitochondrial cytochrome oxidase subunit 1 (COX1) gene from extracted DNA of dog's feces samples. Where M: marker (2000-100bp). The lane (1-30) showed some positive Echinococcus granulosus mitochondrial (COX1) gene from dog samples at (619bp) PCR product.



In the present work, we have successfully designed the primer of the nested pcr as an advantageous technique to amplify of a conserved region of the cox1 gene. This mitochondrial gene is a good molecular marker for separation E. granulosus species in dogs and it's metacestode in human in the region of cystic echinococcosis. This is crucial to develop a method that will be able to differentiate E. granulosus strains and is an important factor of the successful control of the disease Global, the cox1 gene give sharper phylogenetic information than other mitochondrial gene because of differences in its amino acid sequence appear more slowly than those in some other mitochondrial gene.

This agreement with Oguz et al(19)in Turkey, Lu et al., (20) in China , Mirbadie et al .,(21) The better effect of the COX1 gene in was the identification of Taenia in species and interspecies.

The Mt-cox1 and EgG1 Hae III genes have recently been used in the development of a copro-PCR for the identification of E. granulosus. No cross-amplification with Taenia hydatigena, Dipylidium caninum, Taenia ovisor, and E. multilocularis has been reported. (22).

The copro-DNA approach is based on the detection of DNA from the ring, eggs, and cells of the parasite and is a precise and sensitive method for the detection of Echinococcus infections in live animals and in the final host (23. (

The mPCR method can simultaneously detect different target pathogens species by using multiple specific primers in one tube, which is fast; reagent-, material- and labor-saving; and cost- effective . In addition, this method can also distinguish pathogens from mixed infections with accuracy and efficiency at the same time, Additionally, multiplex PCR, which is designed to concurrently identify many targets, is faster and less expensive than regular PCR (24 ; 25).

The prevalence seen in this study is very similar to that reported in Tunisia. Only six (55%) of the 11 dogs with E. granulosus infections discovered at necropsy had that tested positive for the parasite in the copro PCR (26).

Additionally, using the copro-PCR technique, the Turkish researchers demonstrated that 27% of the studied dogs' stool contained taeniid eggs, of which 51.85% were E. granulosus s. l. (27).

Despite the fact that numerous research have been conducted worldwide to identify the species of canine echinococcosis at the genetic level (28: 29: 22: 14) but in Iraq this is the first study that used copro-PCR technique and discovered infection with E. granulosus from dog feces samples in Wasit province.

The current study included examination of Faecal samples (n = 110) and the positive pcr was (n=22).The study reported also the infection in stray dogs of different localities of Wasit Province , namely AL- Kut center (35 samples ,9(23%) positive pcr), Jassan (30sample ,5(13%) positive pcr) , AL-Zubaydiya and AL- dubuni (25sample ,4(10%) positive pcr) ,AL- Numaniya (10sample ,3(8%) positive pcr) and AL- Hayy (10sample ,1(3%) positive pcr) from highest prevalence to lowest prevalence (table 3- 4).

Table (3-2): Prevalence of Echinococcus granulosus in stray dogs in Wasit

Location	No. samples	Positive samples In microscope	%	positive samples pcr	%
AL Kut center	35	19	17%	9	23%
Jassan	30	8	7%	5	13%
AL- Zubaydiya &AL- dubuni	25	5	5%	4	10%
AL- Numaniya	10	6	5%	3	8%
AL Hayy	10	2	2%	1	3%
Total	110	40	36%	22	55%

The highest prevalence rate in AL- Kut center (23%) stray dogs infected with E. granulosus were found in areas surrounding slaughterhouse, where offal of infected animals is improperly discarded due to non-stringent supervision; moreover it is possible that infected offal reaches city markets through informal channels (30: 31).

The reviews showed that there are differences in the prevalence rate of infection with E. granulosus in stray dogs of various regions and countries, and these are may be related to the methods of diagnosis (examination of fecal samples or performing postmortem), number of dogs examined, period of the study, numbers, and sexes of examined dogs, geographical distribution and climatic effect, also lack of dogs ownership registration, accessibility of stray dogs to intermediate hosts and the absence of periodic antihelminthic dosing, presence or absence of hygienic standard and presence of large number of stray dogs.

4. Conclusion

The results of this study offer evidence-based information about the molecular and epidemiological traits of CE in dogs as a final host in Wasit, Iraq. In this endemic location, new strategies and studies should be developed in this endemic region In order to effectively control CE .More research is needed to determine the prevalence and genotypes of parasites in dogs in Iraq.

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