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Original Research Article

Genotyping of *Hydatid Cyst Isolated* from Human and Domestic Animal in Babylon Province

Hager Hassan Karеam[1*], Qasim Jawad Amir[1]

[1]Department of Parasitology, Collage of Veterinary Medicine, Al-Qasim Green University, 51013 Babylon, Iraq

***Corresponding Author:** Hager Hassan Karеam
Department of Parasitology, Collage of Veterinary Medicine, Al-Qasim Green University, 51013 Babylon, Iraq

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Abstract: The study involved 200 feces samples of cattle and sheep, in Babylon Governorate in Iraq. Used for diagnosis of *hydatid cyst*, polymerase chain reaction (PCR), using specific primers and the method was the conventional PCR. Cattle and sheep was classified into subgroups according to age, area and sex in this study association of these risk factors with rate of infection. The diagnosis of hydatid cyst in cattle and sheep was determined by (pcr). study which are collected from human, sheep, cattle The prevalence of *Hydatid Cysts* infections was highest in Al-Qassim and western hamza districts which were 36.36b % (40) of cattle, 22.5a% (9) of sheep and 17.5a % (7) of cattle , 22.72b% (25) of sheep respectively , while the prevalence of Hydatid Cysts in Al-Hilla District was 18.18ab% (20) of cattle, 37.5c% (15) of sheep and the lower prevalence of was in Al-Musayyab and Al-mahaweel district which was 9.09a % (10) of cattle, 12.5ab % (5) of sheep and 13.63ab % (15) of cattle , 10b % (4) of sheep respectively. The results of present study were exhibited on high prevalence of in cattle and sheep of some districts in Babylon province. The statistical analysis recorded that there was Significant difference (at P<0.05) difference in the prevalence of parasite among geographical areas. In the present study the incidence of hydatid cysts was different according to age of the host. In sheep the incidence of *hydatid cysts* has a positive proportion to age of sheep; a high prevalence rate (45b%) was observed in age of sheep (1-2 years); while less prevalence rate observed in (>3) years ages (10a%),In cattle a higher rate (58.18b%) was recorded at ages (2-5 years) while a lower rate (3.63a%) was recorded in (>8) years with Highly significant difference at P<0.05 difference in the prevalence of parasite between age intervals. The result of infection with hydatidosis according to sex revealed that Females were exposed to infection more than males, with no significant difference at P<0.05 in sheep and cattle.

Keywords: *Hydatid Cyst*, PCR, Cattle and Sheep, Iraq.

1. INTRODUCTION

Hydatidosis caused by *Echinococcus granulosus* larval stage is a zoonotic disease and one of the most important for human and domestic animals and this disease leads to many medical, veterinary and economic problems. Iraq is regarded as one of the countries plagued by the endemic sickness of hydatidosis (ALhadidi, Al-Hamairy *et al*., 2022). Hydatid disease is a widespread disease in all countries of the world, and due to the presence of large numbers of stray dogs infected with adult wormsThe disease is known by many names, including Hydatidosis, Echinococcosis, or Echinococcosis Cystic. Carnivorous are a definitive host to the parasite. The canine family includes Canine (dogs, hyenas, wolves, leopards, and some other ferocious animals), while animals are herbivores. Herbivorous such as (sheep, cows, camels, buffaloes, horses, etc.) as an intermediate host of the parasite The disease affects the organs in which it resides in humans and the intermediate host alike primarily the liver and lung, spleen, brain, and other muscles except for hair and nail areas (Al-Nuaimi and Al-Hassani 2023) Humans acquired infection by being in contact with infected dogs harboring adult *E. granulosus* in their intestine result in excretion of eggs in the feces. Thus, one way that humans and other intermediate hosts can be infected is by swallowing eggs that contaminate food, water or the environment generally the disease is usually asymptomatic. However, it can clinically manifest as a

complicated cyst. The most frequent complication is compression or rupture of pericystic structures Another important complication is a hypersensitivity reaction caused by circulating immune complexes or by the activation of an alternative complement pathway, which give rise to glomerulonephritis or an anaphylactoid reaction, respectively, finally, the cyst can become superinfected by other microorganisms bacterial and fungal infections(Baraak 2014)According to the WHO (2021), the incidence rates of human hydatidosis in endemic regions can reach more than 50 per 100 000 inhabitants per year, and the prevalence can reach between 5% and 10% in some areas of Peru, Argentina, China, Central Asia, and East Africa(Peralta, Cuntó *et al*., 2023). The worldwide prevalence of echinococcosis is between 9% and 66%(Shams, Khazaei *et al*., 2022) The cysts are slow growing and contain clear fluid and many protoscolices.Each year, they grow approximately 1 cm and can reach a diameter of up to 20 cm (Peralta, Cuntó *et al*., 2023). They develop mainly in the liver (70%) and lungs (20%), and 10% can occur in any part of the body (brain, body muscles, kidneys, bones, heart wall, and eye socket) (Sanei, Esfahani *et al*., 2021).

2. MATERIALS AND METHOD

The study included collecting samples from 110 cattle and 40 sheep in Babylon province. These samples were subjected to molecular detection by PCR assay Rate of hydatid cyst infection in cattle and sheep determined by PCR assay.

2.1. Collection of Samples

Samples were collected from 110 cattle and 40 sheep, of both sex and different ages, were examined in different areas of the Babylon province, including: Al-Musayyab, Western hamza, Al-Qassim, Al-Mahaweli, Al-Hilla from September 2023 to January 2024.

2.2. Primers That Used in the Present Study

The primers and sequences with product size (mitochondrial subunit 1 of the cytochrome c oxidase 1 (cox1) gene).

No	(COX1) Primer sequence 5´ to 3´	Amplicon size	Annealing TM	Ref
F	TTTTTTGGGCATCCTGAGGTTTAT	444 bp	54	Elyasi, H., & Golmohammadi, R. (2020).
R	TAAAGAAAGAACATAATGAAAATG			

2.3. Statistical Analysis

Data were summarized, presented and analyzed using statistical package for social science (SPSS version 16) and Microsoft Office Excel 2007. For the determination of the significant difference among one way analysis ANOVA was used. A p-value.

3. RESULTS AND DISCUSSION

Molecular Study

Conventional PCR

For confirmation, DNA extraction was performed for samples, including microscopically positive samples. The results showed PCR amplification of of cytochrome c oxidase subunit I (COX1) gene of *Echinococcus granulosus*. In cattle and sheep samples, as shown in Figuare 1. The PCR product size was 444bp.

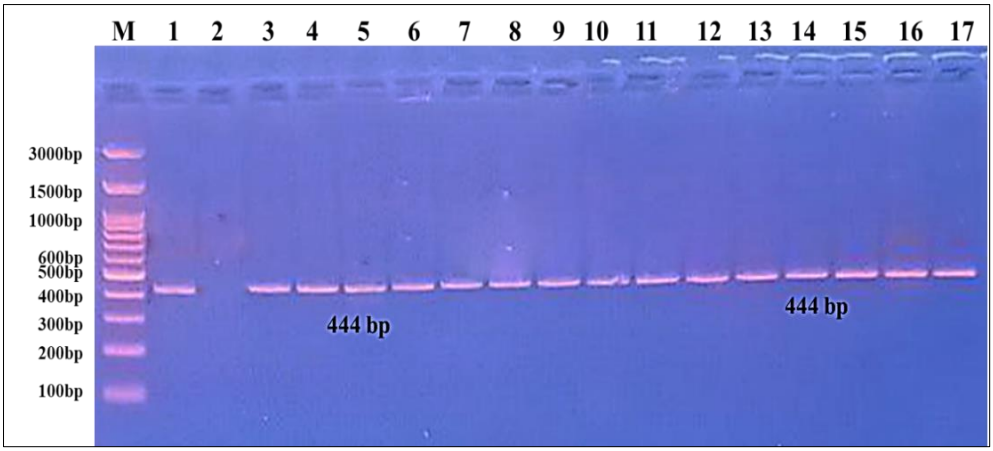

Figure 1: It shows the PCR amplification results of cytochrome c oxidase subunit I (COX1) gene of Echinococcus granulosus. Samples isolated from animals. Agarose gel picture appears the PCR product bands with molecular weight of 444 bp. (M) refers to (3000 bp) DNA ladder, (1) positive control. (2) Negative control

1) Rate of Infection of Hydatid Cyst in Animal According to the Geographical Area in Cattle and Sheep

Two hundred samples included in this study which are collected from human, sheep, cattle The prevalence of *Hydatid Cysts* infections was highest in Al-Qassim and western hamza districts which were 36.36b % (40) of cattle, 22.5a% (9) of sheep and 17.5a % (7) of cattle, 22.72b% (25) of sheep respectively , while the prevalence of Hydatid Cysts in Al-Hilla District was

18.18ab% (20) of cattle, 37.5c% (15) of sheep and the lower prevalence of was in Al-Musayyab and Al-mahaweel district which was 9.09a % (10) of cattle, 12.5ab % (5) of sheep and 13.63ab % (15) of cattle, 10b % (4) of sheep respectively. The results of present study were exhibited on high prevalence of in cattle and sheep of some districts in Babylon province. The statistical analysis recorded that there was Significant difference (at P<0.05) difference in the prevalence of parasite among geographical areas in (Table 1). The current study agreement with study in Iran that founded the infection rate of CE is estimated to be 5.9% in sheep, 8.8% in cattle (Khalkhali *et al*., 2018). And nearly similar to results were reported by Ethiopia 20.5% (Abebe *et al*., 2014), and Libya 15% (Kassem *et al*., 2013). The curreent study disagreement with Al-Diwaniyah study that recorded rate of hydatidosis infection in cattle and sheep with rates of infection 50% and 73.33% (Agha and F. Al-Mohammad 2015), and Al-Khafaji, (2006), Al-Ghezi, (2008), Taher, (2009) and Baraak, (2014) in Iraq which recorded rates 61.8%, 55.6%, 76.67% and 50% respectivel. The world: in Moldova 59.3% (Chihai *et al*., 2016). The hydatid cyst infection was highly prevalent in buffaloes (12%), followed by sheep (10%), cows (9%), and goats (5.1%) in Pakistan (Khan, Shahid Niaz *et al*., 2021). This difference in the prevalence might be due to the difference in sample size, geographic variations, and different climatic conditions.

Table 1: Rate of infection of of *hydatid cyst* in animal according to the geographical area in cattle and sheep

Geographical area	Cattle		Sheep	
	No.	%	No.	%
Al-Musayyab	10	9.09a	5	12.5ab
Western hamza	25	22.72b	7	17.5ab
Al-Gassim	40	36.36b	9	22.5a
Al-mahaweel	15	13.63ab	4	10b
Al-Hilla	20	18.18ab	15	37.5c
Total	**110**	**100**	**40**	**100**
X^2	30.11		11.87	
P value	<0.0001(HS)		0.018(S)	

S: Significant difference at P<0.05

HS: Highly significant difference at P<0.05

Different letters between any two percentage denote to the significant difference.

2) Rate of Infection of Hydatid Cyst in Animal According to the Age in Cattle and Sheep

In the present study the incidence of hydatid cysts was different according to age of the host. In sheep the incidence of *hydatid cysts* has a positive proportion to age of sheep; a high prevalence rate (45b%) was observed in age of sheep (1-2 years); while less prevalence rate observed in (>3)years ages (10a%), in cattle a higher rate (58.18b%) was recorded at ages (2-5 years) while a lower rate (3.63a%) was recorded in (> 8) years with Highly significant difference at P<0.05 difference in the prevalence of parasite between age intervals (Table). These results were agreement with results Hansh *et al*., (2023) whose determined the prevalence of cystic echinococcosis occurred at a significantly higher rate in the age group above >4 years (27.34%) compared to the infection within other age groups. These result is disagreement with studies prevalence was 0.047% 1.5–3 years and 0.92% 5–10 years cattle, 0.016% 1.5–3 years and 0.031% 1.5–3 years and 0.047% > 3 yearssheep Shawish *et al*., (2023), and Smallest age cohort, <1-≤2 years had lowest prevalence (2.3%) while the highest prevalence (23.8%) was observed in age group of >5 years Mehmood *et al*., (2020). Also Khan *et al*., (2021) was found that the disease was relatively high prevalent in animals of age > 5 years followed by 1–5 years and < 1 year.

Table 2: Rate of infection of hydatid cyst in animal according to the age in cattle and sheep

Age interval	Cattle		Age interval	Sheep	
	No.	%		No.	%
<2 years	13	11.81a	<1 years	5	12.5a
2-5 years	64	58.18b	1-2 years	18	45b
5-8 years	29	26.36c	2-3 years	13	32.5b
> 8 years	4	3.63a	>3 years	4	10a
Total	**110**	**100**		**40**	**100**
X^2	101.67			17.86	
P value	<0.0001(HS)			<0.0001(HS)	

HS: Highly significant difference at P<0.05,

Different letters between any two percentage denote to the significant difference.

3) Rate of Infection of Hydatid Cyst in Animal According to the Sex in Cattle and Sheep

The result of infection with hydatidosis according to sex revealed that Females were exposed to infection more than males, with no significant difference at P<0.05 in sheep and cattle. These results were compatible with results which recorded from Jasim *et al*., (2024) which he founded that the infection rate females of sheep and cattle, were statistically higher than males. Similar results had been observed by Lazim, (2019) and Hajimohammadi (2022). These differences between the sex may be due to the males are raised in special sheds, as well as different types of nutrition for the purposes of fattening and benefiting from meat, unlike females raised in fields and pastures and slaughtered at late ages. Also the Females kept longer near house than males, which are typically utilized for milk production and reproductive functions, thereby increasing their exposure to infected dogs, as well as the stress of pregnancy and lactation so they are more affected than males (Haleem *et al*., 2018). These study current contradict findings Aziz *et al*., (2022) gender, male sheep, and cattle both had a higher prevalence (66.7%, 76.2%, respectively) than females. The resonse for these difference in infection rates between females and males may be due Males had the highest prevalence of infection which could beexplained by the high number of slaughter males.

Table 3: Rate of infection of hydatid cyst in animal according to the sex in cattle and sheep

Sex	Cattle		Sheep	
	No.	**%**	**No.**	**%**
Male	18	36a	13	43.33a
Female	32	64b	17	56.66a
Total	**50**	**100**	**30**	**100**
X^2	7.84		1.06	
P value	0.005(HS)		0.302(NS)	

NS: No significant difference at P<0.05

HS: Highly significant difference at P<0.05

Different letters between any two percentage denote to the significant difference.

CONCLUSION

Iraq is endemic with different genotypes of *E. granulosus*. Mitochondrial DNA extracted from the germinal layer, yielded larger amounts than that extracted from protoscolices.

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