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MOLECULAR CHARACTERIZATION AND INCIDENCE RATE OF HYDATID CYST ISOLATED FROM CATTLE IN AL-DIWANYAH PROVINCE, IRAQ

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ABSTRACT : The paper describes a field survey performed to determine the prevalence of hydatid cyst disease in cattle at slaughters in Al-Diwanyah abattoir. The study lasted for five months from November 2017 to April 2018. During the study, 200 cattle were examined. The study included 15 samples were collected from fluids and the germinal layer. The isolation and identification of *E. granulosus* were done using microscopic visualization followed by confirmation using a polymerase chain reaction (PCR) technique targeting the antigen B (AgB2) gene. The results indicate that(7.5%) of cattle were infected. The livers of cattle demonstrated a higher incidence of hydatid cysts than the lungs, with 6.66% oflivers and 20% of lung being infected, respectively. Also, the results of the study showed that the sex of the slaughtered animals has non-significant effect on the distribution of HC, as the rate of HC in males and females were close. The microscopic results revealed the presence of characteristic cysts. All *E. granulosus* isolates in cows appeared to be contained this gene show one distinct band (MW400 bp) when electrophoresed on agarose gel. The results of this study indicated that the PCR technique had a high specify in the detection of *E. granulosus* especially this species that encoded to AgB2 gene isolated from cowsin comparison to other routine diagnostic tests.

Key words : *Echinococcus granulosus*, incidence rate, *AgB*2 gene, cattle, PCR.

INTRODUCTION

Cystic echinococcosis (CE or hydatid sickness) is a zoonotic contamination brought about by the larval phase of the taeniid tapeworm *Echinococcus granulosus*. The parasite's life push is kept up through mutts (which harbor the grown-up worm in their small digestive tract) and a scope of residential domesticated animals that fill in as middle of the road has. *E. granulosus* eggs are discharged in the excrement of tainted mutts and may subsequently sully soil, grass and water. Ungulates (hoofed creatures) can get tainted by touching on field polluted with hound excrement. Ingested eggs bring forth inside the digestive system, infiltrate the gut divider and are conveyed by the circulatory system to various organs and tissues (primarily the liver and lungs) where they form into sores (metacestodes) that can in the end cause extreme neurotic harm. People can get tainted by ingesting eggs through devouring defiled nourishment or water or from dealing with the dung of contaminated canines. (Romig et al.,2006 ; Seimenis, 2003) The larval stage grows for the most part in the liver and lungs in warm blooded creatures, Complete hosts become tainted after utilization of offal from contaminated middle of the road has. (Siracusano *et al*, 2007). CE causes a lot of monetary harm due to in-wrinkled mortality, constrained butcher, diminished profitability, loss of body weight, decreased rearing worth and significant expenses of sterile measures, also, the malady has extraordinary social significance,be-cause contaminated creatures are the key component that keep up the existence cycle of the parasite in endemic territories (Bessonov, 2007). Hydatidosis in Iraq is brought about by *Echinococcus granulosus* which is hyper endemic (Molan, 1993; Molan *et al*, 1990). In domesticated animals its impact is by pulverization of some creature viscera or the entire remains, when intensely tainted, the evil creature will deliver less milk, fleece and meat. It is generally basic in region where sheep/hound cycle works and is advised in every single Iraqi area and domains (Saeed *et al*,2000; Eckert *et al*, 2001). Hydatidosis is analyzed by various ways as X-beam, CT examine, other immunological and serological tests including present day system Polymerase Chain Response (PCR), which have high affectability and explicitness in identification of hydatidosis disease added to that utilized in genotyping of *E. granulosus* to

encourage treatment and inoculation (Leder and Weller, 2003).

MATERIALS AND METHODS

Samples

Totally 200 cows were examined at slaughters in Al-diwanyah abattoir. Caprinehydatid cyst were removed from infectedliver and lung after visceral inspection of these organs and then transported by ice boxes with normal saline to Parasitology Laboratory, College of Veterinary Medicine, University of Al-Qadisiyah for examination.

Microscopic visualization

Microscopic examination was made for cysts fluid to determine the cyst fertility through investigation of live protoscolices, the isolation and identification of *E. granulosus* were done using microscopic visualization.

PCR

The DNA was extracted using Genomic DNA extraction kit (Geneaid, China) and following the kit instructions. The DNA was estimated for quantity and quality using a Nanodrop. A characterizing and confirming step was done using a PCR technique targeting the antigen B (*AgB*2) gene. The piece targeted was at 400bp of length. The primers used were from (Tawfeek *et al*, 2009) and they are F: GGATCCTTCGTGGCCG-TCGTTCAAGC and R: TCGACAAATCATGTG-TCCCGACGCA. The kit employed for the PCR mastermix Bioneer (South Korea) and following the instructions accompanied with the kit using 10pmol from each primer. For the PCR conditions, the denaturation was at 95! for 3min, 35 cycles were for the (main denaturing at 95! for 1min, annealing at 55p C for 1min, and extension at 72^{0}C for 1min), and the ending extension at 72^{0}C for 10min. PCR products were run on 1.5% agarose gel pre-treated with ethidium bromide. The product separation was visualized using a UV imager.

RESULTS

A totalof 15 hydatid cyst were isolated from 200 examined cattle, the prevalence rate of infection (7.5%). The livers of cattle demonstrated a higher incidence of hydatid cysts than the lungs with 6.66% of livers and 20% of lung, respectively.

The results of the present study showed that the sex of the slaughtered animals has non-significant effect on the distribution of HC, as the rate of HC in males and females were close (Table 2).

All studied isolates gave a PCR product representing a fragment of the AgB2 gene (approximately 400 bp) (Fig. 1).

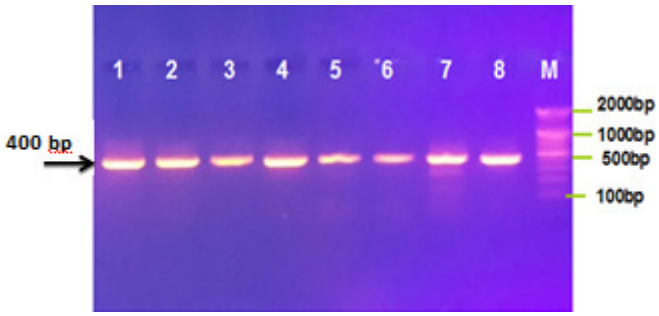

Fig. 1 : Image of agarose gel electrophoresis of the *E. granulosus* PCR products for the *AgB*2 gene from cattle samples.

Table 1 : Prevalence of hydatid cystic disease in livers, lungs and bothin cattle.

No. of examined	No. of infected	Percentage of infection	Infected organs (%)		
			Liver	Lung	Liver & Lung
200	15	7.5	10(66.6%)	3(20%)	2(13.3%)

Table 2 : Total rate of intestinal hydatid cysts with sex in cattle.

Sex	No. of examined cattle	No. of infected cattle	Percantage (%)
Male	172	13	7.5
Female	28	2	7.1

DISCUSSION

Hydatidosis causes impressive financial misfortune in animals because of the judgment of organs. Consequently, it is legitimate to discover dependable information for checking epidemiologic parts of infection and plan gauge information. It is recommended that a productive meat review administration should work as a significant screen of creature sickness, being especially important in the field of constant and not well characterized conditions, which are not obvious to either the stockowner or his veterinary specialist, yet which must be of extensive monetary and creature wellbeing criticalness (Blamire *et al*,1980). In this investigation, a general 200 butchered creatures (cows) were inspected for cystic hydatidosis in Al-Diwanyah region. The outcomes indicated that 15 (7.5%) of dairy animals were contaminated with hydatid cystic malady, Table 1 our outcome compares with Louis *et al* (2011), which demonstrated that the frequency was 7.77% in cows in Erbil territory, Kurdistan Territorial Iraq. While these outcomes couldn't help contradicting after effects of Wijdan (2013), which recorded predominance of Hydatidosis 4.3% in Slemani Territory. Additionally,

disconformity with the discoveries from Rana *et al* (2018) which detailed pervasiveness rate 1.84% in the Blessed City of Karbala, yet in AL-Najaf AL-Ashraf Area recorded low proportion of Hydatidosis in dairy animals about 0.93% (Al-Shabbani, 2014). Every one of these reports recorded in Iraq, just as in steers of different nations. The commonness of hydatid cystic sickness was checked in 586 bovines in the Isfahan, focal piece of Iran,The general predominance of Hydatidosis was seen as 6.5% (Mehdi *et al*, 2013). Ecological and occasional conditions, the executives and farming frameworks and number of tests gathered can be the most significant factors behind the fluctuation in results. Among 15 bovines tainted 10(66.6%) of which were in livers, 3 (20%) in lungs and 2 (13.3%) were in the two livers and lungs. Numerous investigations have assessed the predominance of CE in livers or lungs of domesticated animals, Rana *et al* (2018) found that the contamination was for the most part in the lungs of bovines, yet our outcome relates with Louis *et al* (2011), Wijdan (2013), Mehdi *et al* (2013) which indicated that the disease rate in livers was higher than that in lungs. The perception that the liver is the inclination site in domesticated animals might be clarified by the liver was filling in as an essential obstruction in the body after the entrance of the intestinal divider.

The consequences of the present examination uncovered that the sex of the butchered creatures has no impact on the commonness pace of HCs, as the pace of HC in guys and females were close. This discovering is in concurrence with Wijdan (2013), it expressed that both sex has a similar opportunity to get contamination which for the most part rely upon the contact with the wellspring of disease and propensity for brushing. Interestingly, Mehdi *et al* (2013) revealed that females of steers were bound to have HC disease than guys as guys were butchered in more youthful age while female cows were generally kept up for longer periods than guys to give posterity a few times before butchering. Since quite a long while broad writing on the use of sub-atomic organic techniques has been distributed so as to segregation *Echinococcus* species. PCR is one of the techniques utilized for atomic portrayal of *Echinococcus* confines (Nakao *et al*, 2007). This examination was directed to distinguish and decide *E. granulosus* larval stage in steers of Aldewanyia abattoir through AgB2 quality disengaged from dairy animals was intensified by PCR and PCR items were electrophoresed, for example, in (10).

CONCLUSION

The PCR technique had a high specify in the detection of *E. granulosus* especially this speciesthat encoded to AgB2 gene isolated from cowsin comparison to other routine diagnostic tests.

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