Comparison of Three DNA Extraction Methods for Detection *Echinococcus granulosus* Isolated from Sheep and Cows

Dunya N. Ahmed* **BSc**
Salwa S. Muhsin** **BSc ,PhD**
Abdulla L. Chyiad *** **BSc ,MSc**

Abstract-:

Background: Hydatosis caused by *Echinococcus granulosus* dog tap worm is zoonotic infection and economic important and to public health constitutes a major threat in certain regions of Middle East. There is establishment of preventive and control strategy for characterization of *E.granulosus* in all endemic area which is essential in all molecular studies, to check the DNA of the parasite.
Objective: Our study aimed to obtain the best from three extractions DNA methods from hydatid cyst protoscolecses isolated from sheep in Al-shawlla abattoir in Baghdad.
Subjects and Methods: Three methods were used to extract DNA samples (boiling, crushing and commercial) DNA samples were performed with electrophoreses on 1.3% agarose. Regarding DNA for methods were compared by time and facility and cost effectiveness.
Results: The most method were boiling and crushing DNA extraction because of their easy, quickness besides the commercial kit method which had good bands on gel electrophoresis but had most cost effectiveness and time.
Conclusion: The most method were boiling and crushing using for DNA extraction
Key words: DNA extraction methods, hydatid cysts. *Echinococcus granulosus*

Fac Med Baghdad 2018; Vol.60, No.1
Received: Dec,2017
Accepted: Feb., 2018

Introduction:

Hydatosis is worldwide Zoon sis caused by *Echinococcus granulosus* larval stage Hydatid cyst cause an economic loss and important health problem third world countries (1). It is high prevalence central aria and northern and eastern of Africa and in the Middle East (2. 3. 4). The difference between the strains of the one species of *E.granulosus* (5,6,7) may be affects the lifestyles and sensitivity of the strains to the chemical agents and the dynamics of the disease , epidemiology and control methods .The large difference in *E. granulosus* may be affect the life cycle of the pathogen , the specificity of the host , rate of development ,its sensitivity to the chemical treatments as well as the pathogenesis, accordingly, the development and production of an anti-inflammatory serum against *E. granulosus* (3).For this reason it is necessary to study the characterizing of exact etiological factor in different areas in order to assessment of disease transmission and control programs. The study of DNA technique plays a key role in many fields of parasitology and by this technique we can get a good diagnosis, epidemiology. Analysis of genetic structure of population and anti-inflammatory serum, the high sensitivity and specificity of polymerase chain reaction (PCR)technique in determine the small amount of parasite DNA we use this method in broad application and this is very importance reason to use this technique especially when we cannot isolate enough amounts of material from some parasites. Genomic DNA in total extraction o f *E. granulosus* molecular studies, using special kits preparing for this aim (8), besides in some countries are not always readily available. In addition to their high cost fins study, designed a assessment comparison of three of extracting genomic DNA from protoscolecses of hydated cyst methods to select the host positive method with facility and less cost

**Dept. of Nursing, Medical Technology, Institute of Baghdad/ University of Middle Technical.*
***Dept. of Community Health, Medical Technology, Institute of Baghdad/ University of Middle Technical*
Email: salwa_sbr@yahoo.com
****Dept. of Anesthesia, Medical Technology Institute of Baghdad/ University of Middle Technical*

Materials and Methods:

Several hydatid cysts overall 25 samples of sheep and cows were collected from Al-Shawlla abattoir in Baghdad Iraq. Protoscolecses were checked and aspirated from cysts and washed by normal saline several time in all samples till looked the supernatant clear. Then in 70% Ethanol the sediment will be preserved till time use Protoscolecses Sediments were selected for stage of DNA extract, Equal volumes of packed protoscolecses to each method to reject Ethyl alcohol by about 30-50µl washing twice with distilled water, add 300 µl lyses buffer (Nast 0 ,1M, EDTA 0,0M, Tris-Hcl 0,1M, SDS 1 %) to each tube containing sediment, the process of DNA subsequent performed in the following steps:
Wed for disruption of cell.

DIVA release, which was differs of the following method:
Method of Boiling: - The protoscolecses incubated at tool for ten minutes in each tube".
- Method of Crushing: - "from each tube the protoscolecses will be inserted on the glass slide by sample", "and in another glass slide crushed for about I minute and the product transported to the tube. In all methods "step two was similar by adding 30µl k (Fermentase ,Lithuania) to each tube which contain samples plus 300µl lyses buffer and incubated at 56c for one hour, Then, 300µl phenochloroform was added and centrifuged (5000 rpm / 5min) In a new tube put the supernatant with Chloroform by shaking at 50000 rpm for 5min, At Isopropanol (Merck, Germany) and 0.1 volume Sodium Acetate (Merck, Germany) (3M, PH=5.2) were added to the supernatant, and kept at-**20c^0** for 1 hour. Next, it was spun for 15 min in 14000 rpm and sediment was rinsed by 30µl 70% ethanol After spinning 5min at 5000rpm and" Eject ethyl alcohol , in 50µl in deionizer water the pellet was dissolved, and at-20c^0 stored.
- Method used Commercial kit:- Protoscoleces of hydatid cyst under sterile condition washed in using commercial DNA purification kit in accordance with the manufacturers' instruction (Bio life, Italina S.r.l, Italy), and stored at -20c^0 until DNA samples performed with electrophoresis on 1.3% agars get. DNA extracted: "of each method were loaded on 1 and 1.5% TBE (Tris 0.09M-Borate 0.09M-EDTA 0.02M) agarose gel respectively, Electrophoresis was carried out for I hour at 80V .Then the gels" "were stained in 100µl /L ethidium bromide0.5% solution for 25 min . In UV Tran illuminator the bands .visualized and photographed digitally

Result:
In this study we try to find quick, easy to use, inexpensive and effective method to extract DNA in any lab cannot get the special kits of DNA determination in easy way, the unexpensive kits have a stabile application when a large number of samples have low number of organisms are processed. Although the use of kits is fast and easy to obtain nucleic acid, many countries cannot use them because of the high price of these kits and sometime are not available. This eruption was accomplished by subjecting fifteen samples from twenty five to three different methods to compare and evaluate. These methods include mechanical boiling, crushing, and techniques. In mechanical crushing the result showed a high quality pure DNA, appeared as a sharp band in 2 to 3 samples in gel electrophoresis (Figure 1)

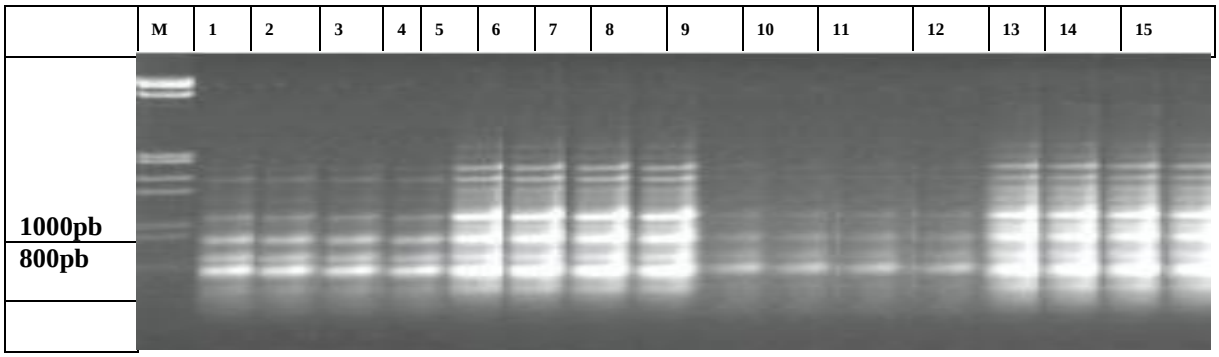

Fig.1 Extracted DNA from Protoscoleces in Agarose Gel Electrophoresis by Using 3 Preparations Methods.

Boiling lanes (1,2,3,4,5); Crushing lanes (6,7,8,9,10); Commercial kit lanes (11,12,13,14,15); and Agarose (1.5%) for I hour at 80 volt.

Discussion:-
Hydatosis is one of the most important zoon tic disease. There is many studies in Iraq indicated that cattle, sheep, human are commonly infected with hydatid disease (11) . "The main intermediate host for G1 genotype of *E.granulosus* is sheep", so it distribution is on worldwide, (12), has a significant impact on public health and economic (13). The molecular studies of DNA analysis and DNA-based methods are not enough for taxonomy of species and subspecies by using method requires care full attention to preparation of pure DNA in enough quantities (13.14). Technique using boiling water yielded pure DNA showing strong bands. The DNA extraction efficiency of each method. In all the samples the extract achieved a thick and sharp gel bands in electrophoresis (Figure 1). The result shows successful extraction of DNA from protoscoleces using physical treatment like boiling which is helpful in extraction of DNA and the thick band is may be upshot of a decrease the amount of inhibitory factors in along boiling method Heating the samples at 95oC is necessary for working the linkage between the lipids, Resulting in release of DNA isolate. This method need 15 minute without any additional work took just 15 min without any additional effort. This technique needs minimal equipments, take place 95ºC boiling water and this is

available in most laboratories. The big usefulness of the boiling technique is that the process is not need one use equipment, and has minimal risk. It is recommended as more proper to use routinely for isolation of DNA from protoscoleces. Because of expensive of liquid nitrogen to purchase and keep in suitable condition and fatigued, All these things limit its use, but it is simple. (15) There are some advantages and disadvantages of all PCR gel methods ,take into consideration the difference side of suitability for DNA extraction method such as PCR band quality , the most suitable methods considering the amp icon quality are , time required , cost effectiveness , work and simplicity ,boiling and crashing. The extraction of DNA of *E. granulosus* protoscoleces by simple, quick and low price methods can be used for future studies based on PCR amplification and may be other gens of *E.granulosus* or other organisms . In our study we found that boiling and crushing methods are the most convenient for *E.granulosus* DNA extraction considering simplicity, low cost and quickness, (9) Extract DNA of *E.granulosus* quality showed by another study by using modified Gnnagon extraction Kit and modify phenol -chloroform in PCR reaction is more typical with suitable products.(10).

Authors' contributions:
Salwa S. Muhsin ,Abudulla L. Chyiad: samples collecting (hydatid cysts and Protoscolecses aspirated from cysts , DNA extraction by boiling and crushing methods
Dunya N. Ahmed: DNA extraction by Commercial kit Dunya N. Ahmed , Salwa S. Muhsin, Abudulla L. Chyiad: Agarose Gel Electrophoresis

References:

1. Parse F, Haghpanah B, Pestechain N ,Salehi Molecular epidemiology of Echinococcus granulosus strains in domestic herbivores of Lorestan ,Iran. Jundishapur J Microbiol.2011; 4:123-30.
2. Sadjjadi SM. Present situation of echinococcsis in the Middle East and Arabic North Africa Parasitol in 2006; 55:197-202.
3. Dinkel A, Njoroge EM, Ximmerann A,walz M,Zeyhle E,Elmahdi. et al. PCR systems for detection of species and genoty pes of the Echinococcus granulosus complex w ith reference to the epidemiological situation in eastern Africa .Int Parasitol 2004; 34; 645-53.
4. Eckert J .Schantz PM Gaser RB,Torgcrson PR,Bessonov AS,\lovession SO. Geographic distribution and prevalence In:Eckert .l,Gcmmcll MA,Meslin Fx,Pawski zs(ed)\VII()/OIE Manual on Echinococcosis in Human and animals :A public health problem of global concern Paris: World organization for animal hcalth.2001; 100-42.
5. Bowies J, Blain I), and McManus D.P. A molecular phylogeny of the genus Echinococcus Parasitology, 1995; 110:317-328.
6. Thompson R.C.A, Lymbery A.J, and Constantine C.C .Variation in Echinococcus: towards a taxonomic revision of the genus. Adv. Paraasitol.1995; 35:145-176.
7. Thompson R.C.A. Intraspecific and epidemiology in Parasitology in focus (H. Mehlhorn ed). Springer, Berlin. 1988; 391-411
8. Gholami Sh , So sari M, Father M , Shanf M , DaryaniA , Hashemi MB and Vahadi \1. Molecular characterization of Echinococcus granulosus from hydatid cysts isolated from human and animals in Golestan Province, Nin th of Iran. Iran .1 parasitol; 2012;7(4);8-16.
9. Khersow II, Tappeh , Ilaleh . llanitian.Kamhi/. Diba .Comparison of four method for DNA extraction from Echinococcus granulosus protoscoleces .Turkey parazitol Dery.2012;36:100-4.
10. Rostami M.N, Kostani, M.Lahmi,F,Mojarad,E.N. Evaluation of four DNA extraction methods detection of Echinococcus granulosus genotype l.Gastrroenterology and Hepatology from bed to Bench.2011;4(2):91-94
11. Yehaia.K.IIossien,Salwa.S.Muhsin.Ban.N. Detection of polymorphism of hydatid cysts parasite isolated from different regions of Iraq wing PCR techniques' Al-Taqani. 2009; 22(1): 176-185.(In Arabic.(
12. Moro P, Schantz PM. Cystic echinococcosis in the Americas. Parasitol In. 2006; 55:S181- S186.
13. Mc\lanus DP, Thompson RCA. Molecular epidemiology of cystic echinococcosis parasitology 2003; 127:S37-S51.
14. R alii in i IIR,Kia EB,Mirhendi SII. Talebi A , Fasihi Harandi M,JablaU-zand N et a l . Anew primer pair in ITSI region for molecular studies on Echionococcus granulosus Iranian Journal of Public Health. 2007; 36:45-49.
15. Sharbatkhori M, Kia EB, Fasihi Harandi M, Jalalizand N, Zahabiun F, Mirhendi H. Comparison of Five Simple Methods for DNA Extraction from Echinococcus granulosus Protoscoleces for PCR Amplification of Ribosomal DNA. Iranian J Parasitol 2009; 4: 54-60

.