Estimation of humural immune response on the rabbits that immunizing with Hydatid cyst antigens by using IHAT and ElISA.

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Summary:

Background: Hydatid disease also known as echinococcosis or hydatidosis , is caused by infection with larva (metacestoide) of tape worms of the genus Echinococcus.

Materials and methods: Twelve Rabbits were immunized with three types of antigens (Hydatid cyst fluid antigen; Protoscoleces antigen and Germinal and Laminated layer antigen) according to a specific immunization program and we used indirect hemagglutination test(IHAT) and Enzyme Linked Immuno Sorbent Assay(ELISA) for detecting the anti-Echinococcus antigens in the serum of these animals.

Results: The highest titter of antibodies were recorded in the Rabbit serum when use the first antigen (Hydatid cyst fluid) by using IHA method (1:64)after the end of immunization program while in ELISA method reached (1:128), The serum level for the rabbit serum that immunization with the second antigen(Protoscoleces) has reached (1:256) by using IHAT and (1:512)when use ELISA, The highest titter of third antigen (Germinal and Laminated layer)has reached (1:128) by using IHAT and (1:256) when we used ELISA method.

Conclusion: The highest antibodies titer could be found when use the Protoscoleces antigen more than Cyst fluid and Germinal and Laminated layer antigen.

Key words: Hydatid cyst, *E.granulosus*

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Introduction:-

Hydatid cyst is one of the most dangerous healthful and epidemic diseases in the most of the world (1).this disease considers Hyper-endemic in many countries such as Iraqi , Syria, Palestine , labanon , north Africa , Arabian island , Sudan and in some south America countries-(2) .Inspite of the proceeding that followed by these countries to stop spread this disease but they can not control on it therefore these countries are suffering from losses on both sides humanity and economy , therefore many researcher have turn to study Hydatid cyst disease from different sides (epidemic, chemistry , physiological structure for Hydatid cyst constriction and study the steps of genic development.(3; 4;5). Also there are many attempts to find out the treatment to stop the parasite without need to surgery by using (Al-bendazol, Mebendazole) beside to abstraction some antigens from parasite and using them to immunize the animals in attempt to raise the immune response that stop the disease and remove the parasite. The researcher use Hydatid cyst fluid antigen, secretion and excretion antigen and Protoscoleces antigen (6). Heath *et al*., 1998 (7) com to have gained the lambs a high immunity against infection with *E.granulosus* by inject onchospheres under skin Therefore the goal of the research is to know which type of the following antigens (protoscoles, Hydatid cyst fluid and germinal and laminated layer antigen) have a higher ability to induce the immune system and then to be able in future use it in immunizing study with some biological adjuvant.

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Material and methods:

A- Farm animals:We use sheep RBC red blood corpuscles to treat and sensitize them with these three antigens which were used it in indirect hemagglutination test (IHAT).

B-Laboratory animals: - (12) new Zealand rabbit were used from Al-razy centre for research and diagnostic tools

C- Source of Hydatid cyst : The samples of Hydatid cyst gain from livers and lungs of sheep's that naturally infected with *E.granulosus* parasite after slaughter these sheep's in massacre these samples are moved in freezing container to medical research centre laboratory in medicine collage .

D-The Preparation of antigens

All hydatid cyst were gathered in a big sterile dishes then sterilization the surface of hydatid cyst by Alcoholic Ethel 75% and by using (10ml) medical syringe the hydatid cyst fluid was aspirated in inactive way to get Protoscoleces which were gathered in sterile flask that washed by physiological normal slain (sodium chloride 85%) and added the antibiotics (Penicillin and streptomycin) the rest of the hydatid cyst that representation by cyst layers the outer fiber layer (origin host)were removed and only the laminated and germinal layers were put in sterile flask and added to it the antibiotics and stored it up in (-20c)

1-The first antigen: - This antigen is represented by hydatid cyst fluid which empty from protoscleces which obtain it after centrifugation (300 C/M) for 10 minute then concentrate the protein in it by using dialysis tube against the polyethylene glycol 6000 with (4c) temperature.

2-The second antigen: - Represent the protoscleces antigen Wen and Crag ,1994(8). method was followed to prepare the antigen the protoscleces suspending with phosphate buffer saline (0.15m) with PH(7.2) and exposure it to freezing and thawing three time and to expose the suspensions to crack by sonication for (10 second) and repeat practicability for ten time by stopping alteration for one minute then filter the solution by centrifuge with high speed (15000 minute/ cycle) with (4c) temp. for half hour the distribute in sterile tubes and store it at (-20c) after add antibiotics.

3-The third antigen: This antigen represented by two layers of hydatid cyst wall (germinal and laminar layer) is done first by crushing the tissue with fictile modar . Then suspension it with phosphate buffer saline (PH:7.2) then expose the mixture to cracking by sonication like in second antigen preparation .

D-Protein concentration measurement:- We measured the protein concentration according to (9) method which depend on attachment with Coomassie Brilliant Blue G-250 stain with protein that help to appear the colour and hyper absorption.

E-The Preparation of antibodies against the three antigens:- 12 New Zealand rabbits have been used (two and half month age). Nine of them are used in immunization against the three antigens (three for each antigen) while the rest rabbit (three) are considered as control group. The rabbit were immunized by antigen with concentration 1 mg/ml on three main dose as the following.

1-The first week:-Dose scope is 1ml for each antigen is given to animals in so much as two doses. The first 0.5ml sub-Cutaneously after three days do the second dose 0.5ml in intravenously.

2-The second week: - Dose scope is 2ml for each antigen. First dose antigen (1ml) that inject in intravenously, after three days inject with second dose in sub-Cutaneously**.**

3-The third week:- Dose scope (3ml) for each antigen. First dose antigen (1.5ml) that inject in intravenously, after three days inject with second dose in sub-Cutaneously**.** After seven days from the last inject dose .The blood collected from the rabbits by heart puncture then put it in sterile tube and left the tube for one hour and then separation the serum by centrifuge at 3000 cycle /minute for 10 minute then keep the serum in (-20c)temp. after add Merthiolate to it.

-TestsF

Indirect hemagglutination test for antibodies titter measurement:-

Farshy and Kagan ,1970(10) were used the fallow up method for evaluate the serum titter for immune rabbit by three antigens. First we pulled blood from cervical vein of sheep and in sterile circumstances in glass beaker contain glass ball for getting out the connective fibers for corpuscles then filter blood by layers of gauze for getting away from clotted blood then washed them by phosphate buffer saline and then the blood corpuscles were fixed by using formalin (40%) with rate represent debility of arranged of packed corpuscles volume by using dialysis tube , then washed it again with phosphate buffer saline and divided the quantity in two parts. The first one undergo to sense operation with three antigens every one in privately while the last part is left without any thing to use it as control cells. Sense operation to bloody cells start first by tannage with tannic acid with the same volume and concentration (1: 20000) then put it in water bath for 20 minute and stir it from time to time. Then washed the blood corpuscles twice with phosphate buffer saline (7.2) then attached the corpuscles with antigen with measure and concentration (1:20,1:40,1:80) and with primary concentration (2mg/ml) then put it in water bath for (30) minute with stirring after that washed it by centrifuge 1% of normal rabbit serum solution and is kept on 4C Tem. After add Merthiolate with 1:10000 concentration .

Enzyme linked Immunosorbent assay :-

This assay was done according to Voller *et a*l., 1976(11) method whereas the serums that contain the antibodies are put between the attachment antigen on the solid surface and ant immunoglobulin that label with enzyme so that we evaluate the interaction program when incubate the enzyme with substrate this test is done on isolated antigens in the same time by using Nunc plate.

The procedure:-

1. We added 100 µ L from isolated antigens that melted in coating buffer (pH=9.6) (0.25 µ g/wall) then coverd the plate and put it in (37) tem. For 24 hour.
2. Washing the contents with washing buffer by washing system.
3. We added 100 µ L from diluted serum sample (1:2, 1:4, 1:8…….) and incubate with 37 tem. For one hour.
4. Washing it again like article 2 .
5. We added 100 µ L from anti Rabbit immunoglobulin that conjugate with peroxides enzyme which preparing in pig in each weal and then covered the plate and put it in 37 tem. For one hour.
6. The plate was washed like article 2 .
7. We added 100 µ L from Orthophenyle Diamine 2HCL (OPD) to each weal and left it for 20 minute in dark place.
8. We stoped the interaction after 20 minute by adding 50 µ L from H2SO4 acid . Then The absorption degree with wave length 492nn.

Results:-

Estimation the titter of indirect hemagglutination.

The measurement depends on the attachment between the antibodies that presence in immunize rabbit serum and antigen that coated on the red blood corpuscles surface then measure the qualitative agglutination which explain the presence or not the antibodies that belonging against Hydatid cysts antigens, moreover, it measure the quantitative agglutination which explain to us the antibodies titter that produce in immunize Rabbit serum. We observe during the immunizing program period the agglutination titter is raising (look at table 1)

Table (1):- The result of indirect hemagglutination test from immunized rabbit serum by hydatid cyst antigens

Sample	The immunized serum with hydatid cyst fluid antigen	The immunized serum with protoscleces antigen	The immunized serum with hydatid cyst wall antigen	Serum control
Serum before immunization	-	-	-	-
Serum after 2nd week from dose	1:16	1:64	1:32	-
Serum after the end of immunizing program	1:64	1:256	1:128	-
Serum after 3 weeks from the end of immunizing program	1:32	1:128	1:64	-
Serum after 5 weeks from the end of immunizing program	1:8	1:64	1:32	-

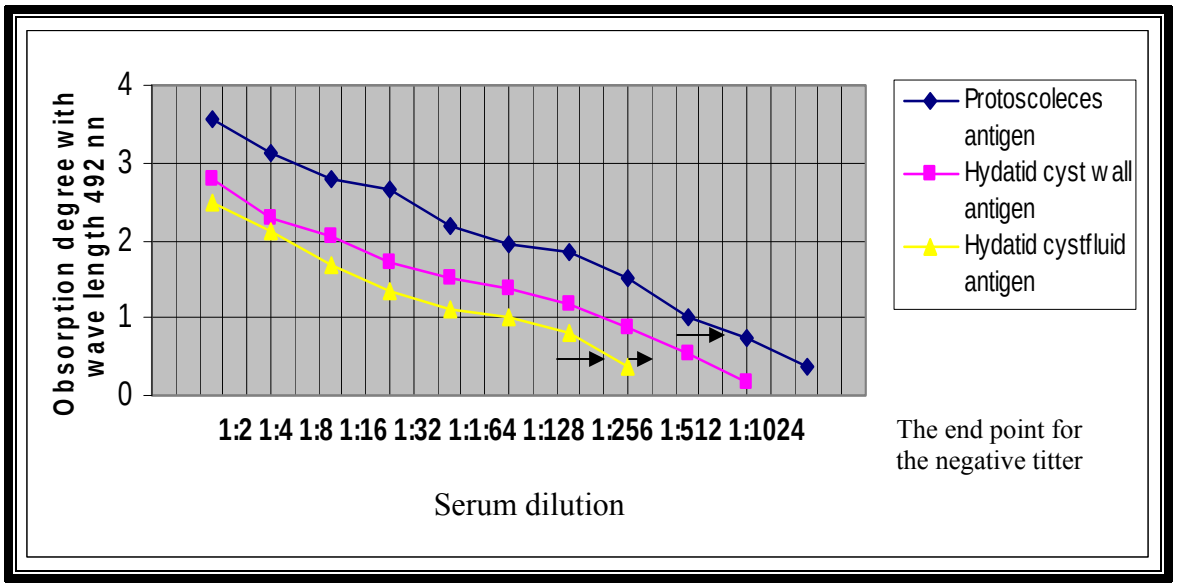
The activity of the immunized rabbit serum with Hydatid cysts antigen result by ELISA.

The test results were appeared that there is differences in antibodies titter between the animals that immunized with Hydatid cysts antigens and these differences depend on the type of antigen quality that use in injection. The antibodies titter in immunized serum by Hydatid cyst fluid antigen reach at (1:128) according to shape 1 . While the antibodies titter in immunized rabbit serum by Protoscoleces reach at (1:512) and the antibodies titter of immunized rabbit serum by Hydatid cyst wall antigen reach at (1:256).

The positive results for ELISA test appeared with orange colour on the bottom of the plastic plate and the colour indicate that there were an interaction between peroxides and OPD substrate while the negative results appeared with limpid colour.

Discussion:-

These result showed that two test (Indirect hemagglutination test and Enzyme linked Immunosorbent assay) indicate that the higher titter of antibodies scored in immunized rabbit serum when injected by Protoscoleces the antibodies titter reached at (1:216 and 1:512) respectively. and followed this antigen that have ability to induced the immnuo response system to produce antibodies is the Hydatid cyst wall (germinal and laminal layer) which score higher titter of antibodies when used it (1:128 and 1:216) respectively. While the Hydatid cyst fluid antigen score was look lower titter of antibodies when used it (1:64 and 1:128). The results of this research lead to the differences in antigen ability to attach with the antibody that depend on the antigenisity and the last depends on the molecular size and the number of epitopes in it that have the ability to attach with antibodies and form antigen- antibodies complex. Therefore the ability of Protoscoleces antigen was higher than Hydatid cyst wall (germinal and laminal layer) antigen to induce the immune system to produce antibodies and the last antigen was higher than Hydatid cyst fluid antigen. These results were agree with Wattal et al., 1986(12) who used two type of antigens the first one was Hydatid cyst fluid antigen and the second was Protoscoleces antigen which the last were scored the higher antibodies titter by using indirect hemagglutination test.



Shape (1):-The result of ElISA tests for measurement the activity of the immunized rabbit serum with Hydatid cysts antigen.

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