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Immuno-monitoring of Echinococcosis Cysts development:

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Abstrat Early diagnosis of Echinoccosis disease can aid in significant improvements and in the quality of both of the management and treatment of the disease.The infected mice did not show any sign of infection until the end of the fifteenth week. However, the antibody (against the echinoccosis cysts) titre showed a different pattern where, the titre continued to increase from the end of the first week up to the fourth week (1:64, 1:256, 1:512, 1:1024 respectively) followed by a decline until the eighth week and stabilized (1:256, 1:128,1 : 32, 1:16, respectively) then after until the appearance of the cyst at the sixteenth week of infection.The cysts were removed from infected mice starting from week 16th until week 20th and their diameters were measured to be ranging from 0.5 to 1ml at week 16th and reached 1.5 to 2.5 at the 20th week, respectively .In conclusion, the use of immuno techniques used enabled us to determine the time of growth and development of the cyst as well as follow-through Histologically show that the target member and the most affected is the liver

Introduction

Echinococcusis cysts are caused by infestation with the parasite *Echinococcus* . This parasite is found worldwide .The adult tapeworm lives in the digestive tract of carnivores , such as dogs and wolves .Eggs are released into the stool and inadvertently ingested by the intermediate hosts ,such as sheep cattle and humans etc . The egg larvae invade the bowel wall and mesenteric vessels of the intermediate host , allowing circulation to the liver or lungs . In the liver or lungs or both , the larvae grow and become encysted . The hydatid cyst develops an outer layer of inflammatory tissues and inner germinal membrane that produces daughter cysts . When carnivores ingest the liver of the intermediate host , the scolices of the daughter cysts are released in the small intestines and grow into adult worms ,thus completing the life cycle of worm (Hegglind et al ; 2007) .

Early diagnosis of echinococcusis disease can aid in significant improvements and in the

quality of both of the management and treatment of the disease. The definitive diagnosis for most human cases of hydatid disease can be achieved by some physical imaging methods, such as radiology, ultrasonography, computed tomography (CT scanning) and magnetic resonance imaging (Wenbao et al., 2003).

There are more spaefic methods for diagnosis of hydatid disease in the definitive host namely, DNA recognition methods. Copro-DNA has proven to be of value for the diagnosis of Echinococcosis in animal definitive hosts. DNA isolation from the faeces, however, is laborious. PCR is a technically demanding and expensive technique. It is currently used mainly for confirmatory testing of coproantigen-positive samples or for identification of taenid eggs recovered from faeces.And also can not locate the way for the emergence of the first Echinococcosisand a member (target).(Bretagne et al., 1993.,Lymbery , 1995.,Mcmanus , 2006 and Varcasia et al., 2007).

Several techniques for the immunological diagnosis have been evaluated during the last few decades (Pauluzzi 1970 ; Giunchi et al., 1972 ; Farag et al., 1975 ; Pinon, 1976 ; Matossian, 1977 ; Matossian et al., 1979 ; Kagan and Norman , 1979 ; Chemtai et al., 1981 and Dottorini et al., 1985). In addition to the intradermal casoni test and complement fixation test a number of other tests were used for the serodiagnosis in recent years , which includes indirect haemagglutination test (IHA), the bantonite flocculation test (BFC), latex agglutination test (LA) ,indirect fluorescent antibody test (IFA) , countercurrent immunoelectrophoresis (CIE) and enzyme-linked immunosorbent assay test (ELISA) (Kagan , 1968., OIE , 2008). ELISA test has been adopted widely and was found superior to most of the other serological tests (Speiser and Weiss , 1979 ; Speiser , 1980 ; Gebreel et al., 1983; and Dottorini et al., 1985).

The aim of our study is to try to follow up patients using the an immune test, to see the member's homepage, and the period ofearly growth and the development of the bag, which may facilitate the treatment of the disease.

Materials and Methods

The EchinococcosisFumouze IHA (LaboratoiresFumouze, Levallois-Perret, France), was used for the monitoring the *Echinoccus*cyst development in mice. In this experimental model 220 (40 days old) Albino Swiss Mice divided into two groups: 1- the laboratory infected group consisted of 200 mice (100 females, 100 males) and 2- the a control group consisted of 20 (10 males, 10 females) according to the manufacturer instruction as recommended by Zhang et al . (2001). The mice were infected with 2,500 onchosphereOrally. Blood samples were collected starting one week after infection until week 20, by heart puncture of 5 females and 5 males of infected mice and left at 4°C for overnight for clotting. Sera were separated from

clots by centrifugation at 1500 × g and stored at -20°C until use. In the same time, the appearance, location and size of the hydatid cysts were examined in the sacrificed mice.

The EchinococcosisFumouze IHA kit (LaboratoiresFumouze) was used according to the instructions of the manufacturer. Briefly, the test procedure was as follows: 64uL of an initial 1:64 dilution of each serum

was subjected to further 2-fold serial dilutions to a maximum of 1:1024, and 1 drop (25 uL) of sheep red blood cells sensitized with an *E. granulosus* antigen, provided with the kit, was added to each diluted sample. After incubation for 2 h at room temperature, the total antibody titer in the test serum was recorded as 1 dilution before that which yielded a clear, sharp, dark spot similar to those in the negative control wells. Titers were expressed as reciprocal values. The results were evaluated with a cutoff titer of 1:1024, as recommended by the manufacturer, and also with a cutoff titer of 1: 64.

Results:-

The infected mice did not show any sign of infection until the end of the fifteenth week. However, the antibody (against the hydiated cysts) titre showed a different pattern where, the titre continued to increase from the end of the first week up to the fourth week (1:64, 1:256, 1:512, 1:1024 respectively) followed by a decline until the eighth week and stabilized (1:256, 1:128,1 : 32, 1:16, respectively) then after until the appearance of the cyst at the sixteenth week of infection (Table,18 Figur,50). The cysts were removed from infected mice starting from week 16th until week 20th and their diameters were measured to be ranging from 0.5 to 1ml at week 16th and reached 1.5 to 2.5 at the 20th week, respectively (Figures 47,48).

.Table (19): Titers antibody in sera of mice infection by (IHA) test

Weeks	Male$^{(x)}$	Female$^{(x)}$
First week $^{(*)}$	1:64	1:64
Second week$^{(*)}$	1:256	1:256
Third week$^{(*)}$	1:512	1:512
Forth week$^{(*)}$	1:1024	1:1024
Fifth week	1:256	1:256

Sixth week	1:128	1:128
Seventh week	1:32	1:32
Eighth week (*)	1:16	1:16
Ninth	1:16	1:16

There were significant differences P<0.005=*

X= There were no significant differences P=0.915.

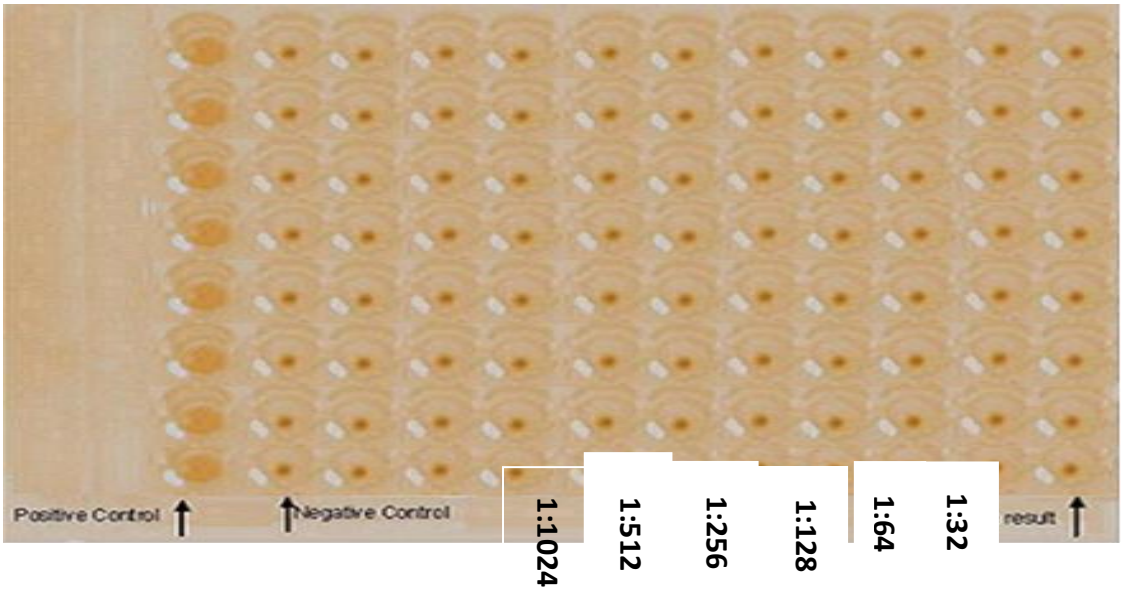

Fig. (46): A photograph showing some negative results for sample from sera of mice before infection Laboratory by IHA test

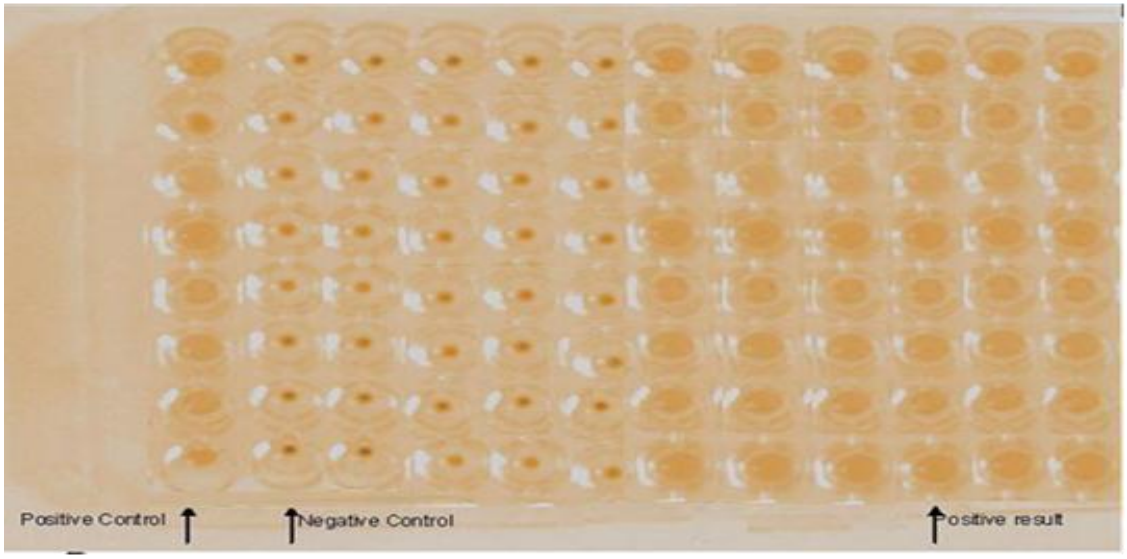

Figure (46). The IHA results from week 1-4positive titres (appearing at as indicated above the columns as compared to the control and negative samples.

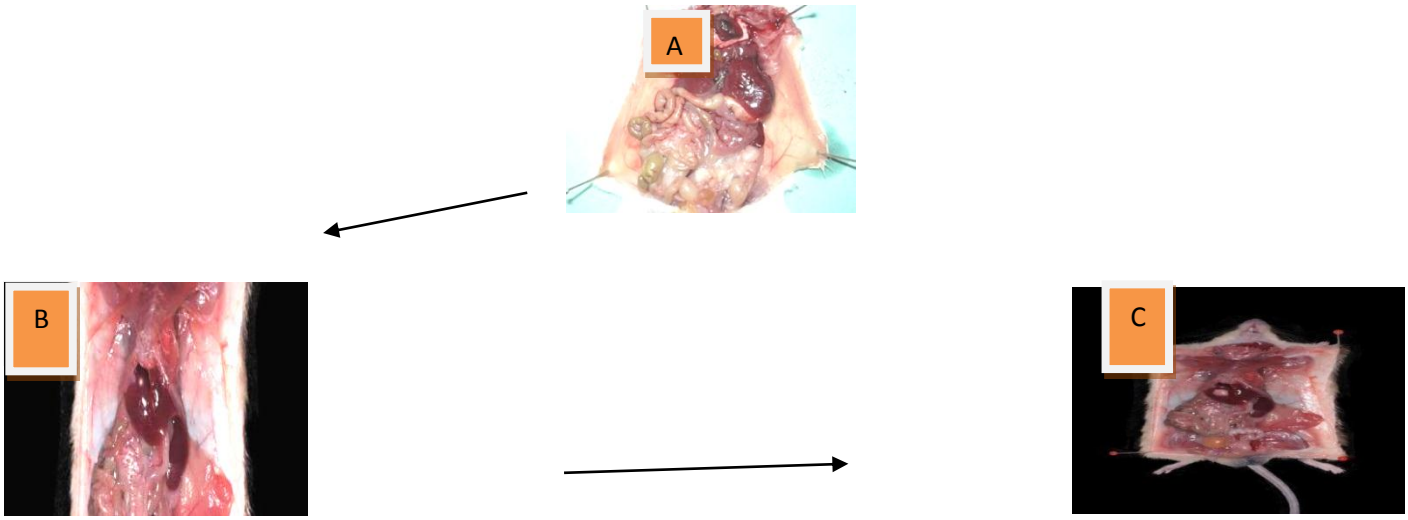

Figure (47) A dissected mice showing the cysts. A:the control mice, B: infected mice at week 16 and C: infected mice at week 20. Arrows pointed to the formed cysts.

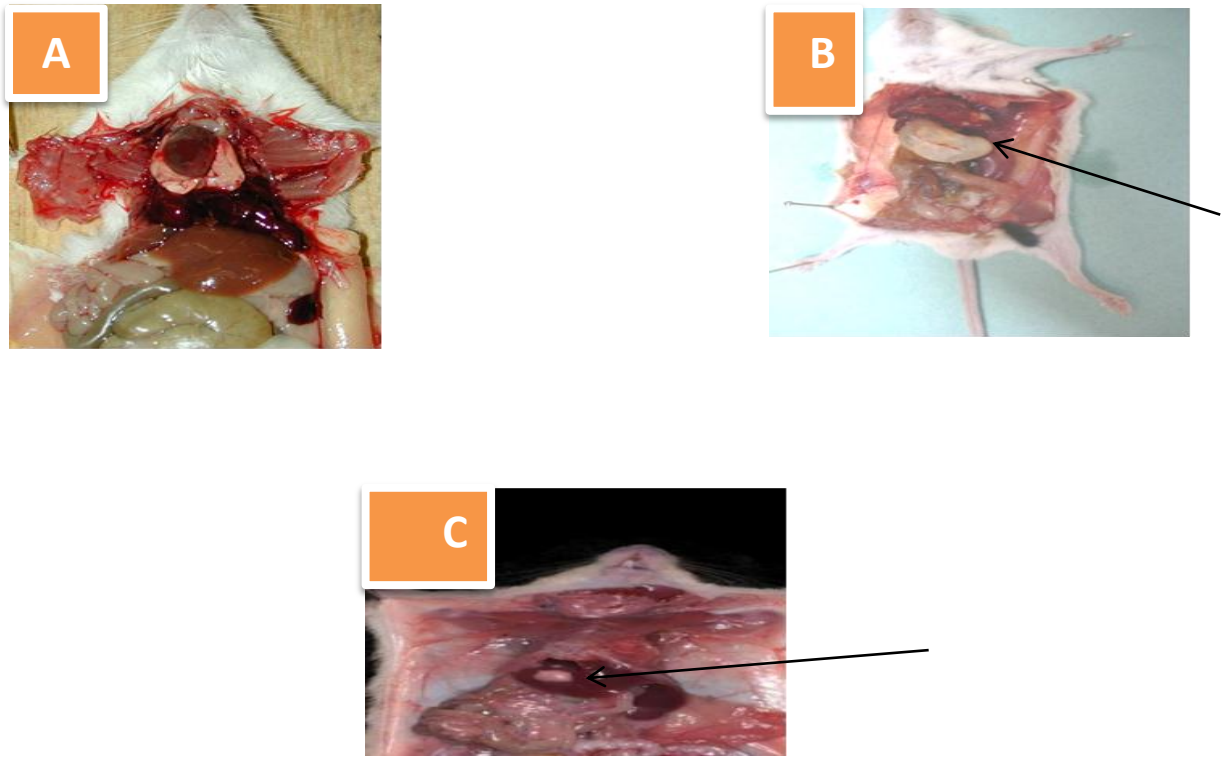

Fig. (50): Photographs Anatomy mice to continue with the first emergence of the cysts, A: A photograph of mouse male non-infected (CONTROL), at week 20, B: A mouse male laboratory infected in week 20 after

infection, C: A mouse female laboratory infected in week 20 after infection ,where the size of incoming infections ranging from 1.5 to 2.5 mm.

Dissection

Use of higher titers (1 : 400 or more) , as . suggested by Kagan,(1968) and used by various workers, which have minimized the non specific reaction, may increase the number of false negative cases. We have observed that by considering 1:64 or more titre the IHA test (with GFACL"'-SRBCs reagent) is found to be suitable and reliable for serodiagnosis based on normal healthy control (92 per cent positivity). If we consider 1:32 dilution as cut off titre the result is 100 percent positive while 88 and 60 per cent positivity are observed by using 1:128 and 1:256 cut off titrerespectivly. Thus by considering higher dilution as cut off titre, the number of the false negative cases increases. The dilution 1:64 is found to be best cut off point with our reagent as it is clear from our findings, with of negative sera and positive. IHA is ideal test for routine diagnosis of hydatid disease. It is sensitive and technically mucomplicated. They further mentioned that ELISA would probably be of particular value for large scale screening , sines sera can be tested at one dilution , while complement .fixation (CF) reaction remains the most reliable and helpful method for indicating reinfection or continuation of infection (Pauluzzi, 1965).

The lyophilized reagent can be prepared . and supplied on large scale for sero-epidemological studies and for routine diagnosis in the laboratories and hospitals of the contry . The metacestode develops from the oncosphere and is a cystic structure typically filled with a clear fluid (hydatid fluid) . The postoncospheral development takes 10-14 days. By this time, the bladder (measuring 60 µm-70 µm in diameter) consists of a nucleated germinal layer and a thin laminated layer which lacks nuclei. Most of the cysts grow slowly in size and become surrounded by host tissue (pericyst) encompassing the endocyst of metacestode origin. The endocyst consists of the outer laminated layer and the inner cellular germinal layer, which may form brood capsules and protoscoleces. The minimum time required for the development of protoscoleces in cysts in humans is not exactly known, but based on data from animals, it is expected to be 10 months or longer after infection (Ammann& Eckert,1995 andPawłowski ,1997). Protoscoleces can be already formed in small cysts of 0.5 cm-2.0 cm diameter. In the same patient, fertile (with protoscoleces) and sterile (without protoscoleces) cysts may coexist. Quite frequently, smaller daughter cysts are formed within a larger mother cyst . Several small single cysts growing in close proximity to each other may form clusters, thus presenting a ‘polycystic’ or ‘multivesicular’ appearance which has to be distinguished from AE and PE. Echinococcusgranulosus cysts have a variable natural course of development. According to an ultrasound study in 66 human patients in Turkana area of Kenya, about 30% of cysts grew slowly (1 mm to 5 mm per year), 43% showed a moderate growth (6 mm to 15 mm per year), 11% exhibited a more rapid increase (average: 31 mm, maximum: 160 mm per year), and 16% of cysts did not expand or had collapsed (Romig,1990 , Romig et al;1986). Partially or totally calcified cysts are not uncommon. The size of cysts is variable and ranges usually between 1 cm and 15 cm, but much larger cysts containing 48 l of cyst fluid have been noted (Ammann& Eckert,1995). Spillage of viable protoscoleces or small daughter cysts after cyst rupture may result in secondary echinococcosis. In our study, after seven days of the titers antibodies in the serum of animals infected experimentally 1:64, which indicates the arrival of active onchosphere to the

blood and then become the standard continues to increase today, 14,21,28 In the 1:256 ,1:512 ,1:1024 respectively and then started to decrease from 35 to 56 days even settled on a titers 1:16, This indicates that the active onchosphere begun to reach the organ target , and practiced camouflage properties that work on the inhibition of host immunity and so membrane that surrounds himself with an external component of the protection of the host tissue, namely (particulate materials) (Morseth , 1967)

The follow-up to be the cyst and its development in the internal organs by anatomy of the seventh day as the first week to fifteenth week, is not note any be of the bags in spite of making sure the incidence of test serum in the week XVI was observed first catapulted Casey diameter ranging from 0.5 to 1 mm, and the nested on what size it until the twentieth week, the measurements ranged between 1.5 to 2.5 mm as described (Marquardt et al., 2000 and Gottestein, 1992, and Manson & Bell, 1987). When the larva became stable in the host(in bladder, for example) it grew slowly up to about 10 mm in five months and once the cyst is formed it produces a reaction by the host against the cyst's wall for life long. Moreover, the size and shape of the cyst is determined by the site and the host in which it grows (Morseth, 1967).

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