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SOME BIOLOGICAL AND SEROLOGICAL STUDIES ON HYDATIDOSIS IN EXPERIMENTALLY INFECTED RABBITS

(With 1 Plate and 1 Figure)

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بعض الدراسات الحيوية والسيرولوجية لأرانب مصابة معمليا بالحويصلات المائية

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تم عمل عدوى معملية لأرانب باعطائها بويضات الدودة الشريطية القزمية (اكينوكوكس جرانيولوزس) مع تجميع أمصال من هذه الأرانب أسبوعيا وذلك لدراسة بداية ظهور الأجسام المناعية المضادة للحويصلات المائية. وقد تم ذبح الأرانب بعد ١٣ أسبوعا وذلك لدراسة مكان ونوع الحويصلات المائية المتكونة. بينت الدراسة وجود حويصلات مائية صغيرة في أكباد ورئات الأرانب المصابة فقط مع خلو باقي الأعضاء الأخرى والعضلات المختلفة من أى حويصلات مائية. كما أوضحت الدراسة أن الأجسام المناعية المضادة المتكونة في أمصال الأرانب المصابة بالحويصلات المائية يمكن الكشف عنها بعد ٣ أو ٥ أسابيع من بدء العدوى طبقا لنوع المولد المضاد المستخدم وذلك باستخدام الاختبار المناعي الانزيمي المترابط (الاليزا). وقد أوضحت الدراسة أن المولد المضاد النقي المحضر من السائل الموجود داخل الحويصلات المائية أفضل من المولد المضاد المحضر من بويضات الدودة الشريطية المذكورة في الكشف على الأجسام المناعية المضادة في أمصال الأرانب المصابة حيث أمكن الكشف عنها بعد مرور ثلاثة أسابيع من بدء العدوى مع استخدام المولد المضاد النقي وبعد خمسة أسابيع عند استخدام المولد المضاد المحضر من البويضات.

SUMMARY

The present work was designed to infect rabbits experimentally with *E. granulosus* eggs and to study the location of hydatid cysts in these rabbits as well as the appearance of specific antibodies in their sera. Macroscopical examination of all internal organs and different muscles of the experimentally infected rabbits 13 weeks post-infection revealed the presence of small hydatid cysts in livers and lungs only.

Microscopically, these cysts contained protoscolices. Sera from the infected rabbits were used to evaluate the diagnostic sensitivity of *E. granulosus* eggs and purified hydatid fluid (arc 5) antigens by ELISA. The antibody levels appeared at 5 and 3 weeks p.i. with eggs and arc 5 antigens, respectively. The antibody levels increased gradually till 8 weeks p.i. and nearly remained in constant levels till 13 weeks p.i.(end of experiment). The arc 5 antigen gave optical density (O.D.) higher than that of the eggs indicating high levels of sensitivity.

Key words: *Biological, Serological, Hydatidosis, Experimentally, Rabbit*

INTRODUCTION

Hydatidosis is a cyclozoonotic infection having a world- wide distribution and variable geographic incidence. It is considered as a public health problem and meat economic losses of the Middle East and North Africa (Schmidt and Roberts, 1977 and Gallardo, 1995). The biological mechanism in hydatidosis is still quite unknown to explain host-specificity or host-resistance (Yamashita, 1969).

Diagnosis of hydatidosis is still difficult, which is based mainly on serodiagnostic techniques. So, in recent years, the validity of serological techniques for diagnosis of hydatidosis have been improved. A variety of techniques are applied for such diagnosis in different animals and human as Indirect haemagglutination (IHA), Counter immuno-electrophoresis (CIE), Immuno- Fluorescence (IF), ELISA and Immuno-Blot (EITB) (Iacona et al., 1980; Salim, 1982; French and Ingera, 1984 and Mousa, 1992). These serological techniques are still lacking the diagnostic specificity, especially in endemic areas with fascioliasis and schistosomiasis. Therefore, evaluation and purification of antigens are needed to increase the sensitivity of these serological techniques for the detection and confirmation of the disease in its early stages.

The rabbit can act as a suitable intermediate host for *E. granulosus* (Thompson, 1986 and Jenkins and Thomson, 1995). The description of development of hydatid cysts in rabbits would be possible to define an experimental model suitable for further study of biological and serological hydatidosis.

So, the aim of the present study was to determine the distribution and the type of hydatid cyst in experimentally infected rabbits with *E.*

granulosus eggs as well as to evaluate the *E. granulosus* egg and purified hydatid fluid (arc 5) antigens for sero-diagnosis of experimental rabbit hydatidosis by ELISA.

MATERIALS and METHODS

Collection of E. granulosus eggs:

The *E. granulosus* eggs were collected from experimentally infected dogs with fertile hydatid cysts (camel origin) by concentration floatation method (Mathis et al., 1996). The eggs were washed several times in sterile saline, divided into two parts, the first part was used freshly to infect rabbits and the second part was used for preparation of egg antigen.

Experimental infection:

Nine young adult female New Zealand rabbits, each of about 1.5 k gm body weight, were kept in separate clean and dry cages and fed on concentrates. Fecal samples were examined daily for 15 days before infection to detect any parasitic infection. The rabbits were divided into two groups, the first group included 6 rabbits which were infected with 5000 *E. granulosus* eggs orally. The second group included 3 rabbits as non infected control. The serum samples from both infected and control rabbits were collected weekly from zero day till 13 weeks p.i. for serological study. Meanwhile, the fecal samples were collected from infected and control rabbits every 3 days to ensure that the rabbits were free from intestinal parasites. The rabbits were slaughtered after 13 weeks p.i., the different muscles and all internal organs were examined macroscopically to determine the number and location of the hydatid cysts.

Serological study:

Serum samples:

Serum samples from both infected and control rabbits were collected weekly during a period of 13 weeks.

Preparation of antigens:

***E. granulosus* egg antigen:**

Egg antigen was prepared according to Mousa et al. (1996). The eggs were homogenized for 15 minutes on ice using a tefflon glass homogenizer followed by sonication for 5 minutes to disrupt the remaining intact eggs. The homogenates were centrifuged at 20,000 rpm for 45 minutes at 4°C. The protein content of the supernatant was

determined using Lowry`s method (Lowry et al., 1951). The preparation was aliquoted and saved at – 70°C until used.

Purified hydatid fluid (arc 5) antigen:

The purified hydatid fluid antigen was prepared using the method described by Oriol et al. (1971) and modified by Mousa (1992). Fresh hydatid cysts from camels were collected and the hydatid fluid was aspirated using a sterile syringe. The collected fluids were centrifuged at 1500 rpm for 30 minutes, The supernatant was concentrated using UM 0.5 membrane filter. The concentrated fluid was dialyzed against 0.005 M acetate buffer, pH 5.0, then centrifuged at 10,000 rpm for 60 minutes. The precipitate was dissolved in 0.2M phosphate buffer, pH 8.0, then centrifuged at 10,000 rpm for 60 minutes. The supernatant was saved and salted out with saturated ammonium sulphate, then centrifuged at 10.000 rpm for 60 minutes. The supernatant was dialyzed against 0.2 M phosphate buffer, pH 8.0, for 48 hours, then against 0.005 M acetate buffer, pH 5.0 and then centrifuged at 10.000 rpm for 60 minutes. The precipitate was dissolved again in 0.2 M phosphate buffer, pH 8.0 and the protein content was determined using Lowry`s method.

Enzyme linked immunosorbent assay (ELISA):

ELISA was performed according to Iacona et al., (1980). ELISA plates, 96 flat bottom wells (Linbro, ICN Inc, USA) were coated with 50 µl of 5 µg soluble egg and arc 5 antigens in carbonate buffer, pH 9.6, separately. The plates were incubated overnight at room temperature. Following blocking with 0.1 % bovine serum albumin (BSA) in coating plates, 50 µl of rabbit sera diluted at 1:100 in PBS was added for 2 hours at 37°C in a shaking water bath. After washing the plates 5 times with PBS containing 0.05% Tween-20, 50 µl of alkaline phosphatase labeled anti-rabbit IgG antibodies diluted at 1000 in PBS were added for 1 hour at 37°C in a shaking water bath. The chromogen paranitrophenyl phosphate, at 1 mg per ml substrate buffer was added and the absorbance of the colored reaction was read within 30 minutes at 405 nm using a titertek multiskan ELISA reader.

RESULTS

Macroscopical examination of all internal organs and different muscles from 6 rabbits experimentally infected with 5000 *E. granulosus* eggs, 13 weeks post-infection (p.i.) revealed the presence of 24 and 18 small hydatid cysts in livers and lungs, respectively (Table1). These

cysts were 2-5 mm. in diameter being partially embedded in the liver and lung tissues, but clearly visible on the liver and lung surfaces. Microscopically, these cysts were fertile and contained protoscolices (Plate 1B,C,D). Other internal organs and the different muscles were free from hydatid cysts

Table 1: The location of hydatid cysts from experimentally infected rabbits with *E. granulosus* eggs (13 weeks post-infection).

	Liver	Lungs	Other internal organs	Different muscles
1	4	7	-	-
2	4	4	-	-
3	5	-	-	-
4	5	-	-	-
5	3	4	-	-
6	4	3	-	-
Total	25	18	-	-
Mean	4	3	-	-

The sensitivity of *E. granulosus* eggs and arc 5 antigens using sera from experimentally infected and control rabbits was studied by ELISA. The analysis of the obtained ELISA data illustrated in Fig. (1) showed significant antibody levels at 5 and 3 weeks p.i. with eggs and arc 5 antigens, respectively compared with negative control rabbit sera. The antibody levels increased gradually till 8 weeks p.i. and nearly remained in constant levels till 13 weeks p.i. (the end of experiment) with both antigens. The Arc 5 antigen gave optical density (O.D.) higher than the eggs one in all tested positive sera indicating high levels of sensitivity.

DISCUSSION

In the present investigation, biological studies were conducted through experimental infection of rabbits with *E. granulosus* eggs to determine the distribution and the type of hydatid cyst in rabbits. The present study revealed that the hydatid cysts appeared in the livers and lungs only. These results agreed with Jenkins and Thomson (1995) who reported that the hydatid cysts appeared in lungs of wild rabbits experimentally infected with *E. granulosus* eggs. While, these results

were contrary to Higashi and Derhalli (1986) who failed to infect rabbits experimentally with secondary hydatidosis from camel origin. Such variations might be related to the developmental stage used for infection, they inoculated protoscolices intra-peritoneally, while in the present study *E. granulosus* eggs were used orally (the natural rout).

Also, the present study revealed that the hydatid cysts were fertile, small size and embedded partially in the liver and lung tissues. These data agreed with those observed by Thompson (1986) and Jenkins and Thomson (1995) who reported nearly the same results.

In Egypt, the present study proved that the rabbits is a suitable intermediate host for *E. granulosus*. Hence it could be added among the list of intermediate hosts for *E. granulosus*.

The evaluation of *E. granulosus* eggs and purified hydatid (arc 5) antigens for diagnosis of experimental rabbits hydatidosis by ELISA was studied. The present study revealed that the purified antigen was more sensitive than egg antigen for the diagnosis of rabbit hydatidosis. These data agreed with those reported by Iacona et al. (1980); Gottstein et al. (1987); Njeruh et al. (1989) and Mousa (1992) who observed that the antigen prepared from hydatid cysts is more suitable for diagnosis of human hydatidosis by ELISA. Also, the present study revealed that the antibody level of experimentally infected rabbits appeared at 5 and 3 weeks p.i. using both eggs and arc 5 antigens, respectively. However, Derhalli et al. (1981) reported that the antibody level appeared at 6 weeks p.i. from rabbits sera experimentally infected with protoscolices by the intra-peritoneal rout. Such variations might be related to the material of infection as well as to the sero-diagnostic techniques used, where they used counter electrophoresis, while in the present study ELISA was used. So, we can say that the arc 5 antigen is more suitable for early diagnosis of rabbit hydatidosis.

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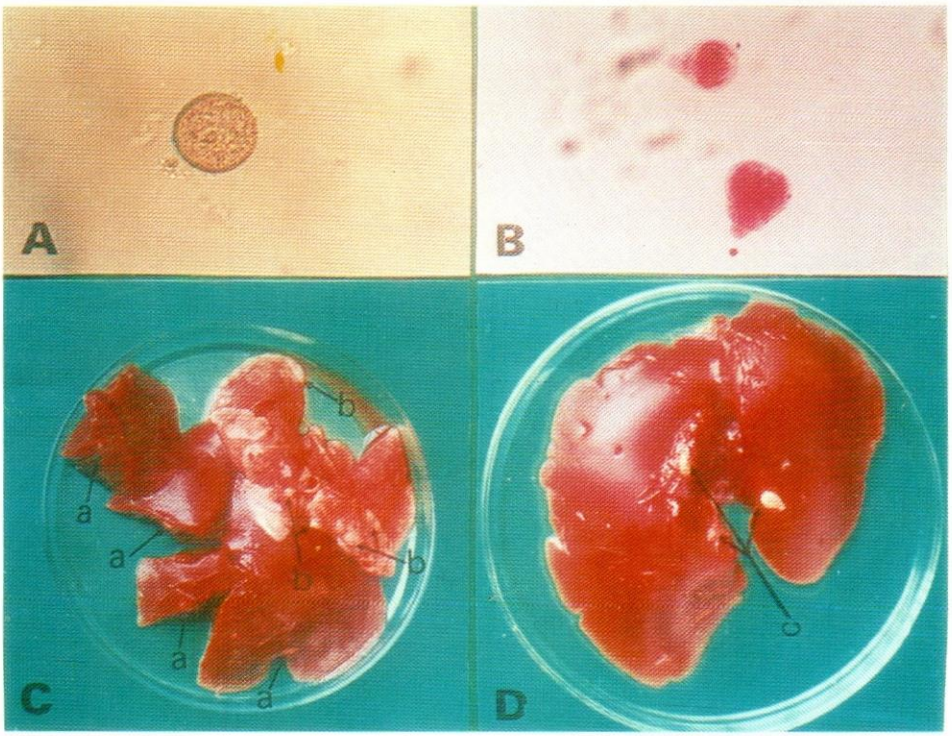
Plate (1) : Shows the following .

(A): *E. granulosus* egg (X400)

(B): Hydatid scolices formed in experimentally infected rabbit (X100).

(C & D): Small size hydatid cysts appeared in rabbit livers and lungs.



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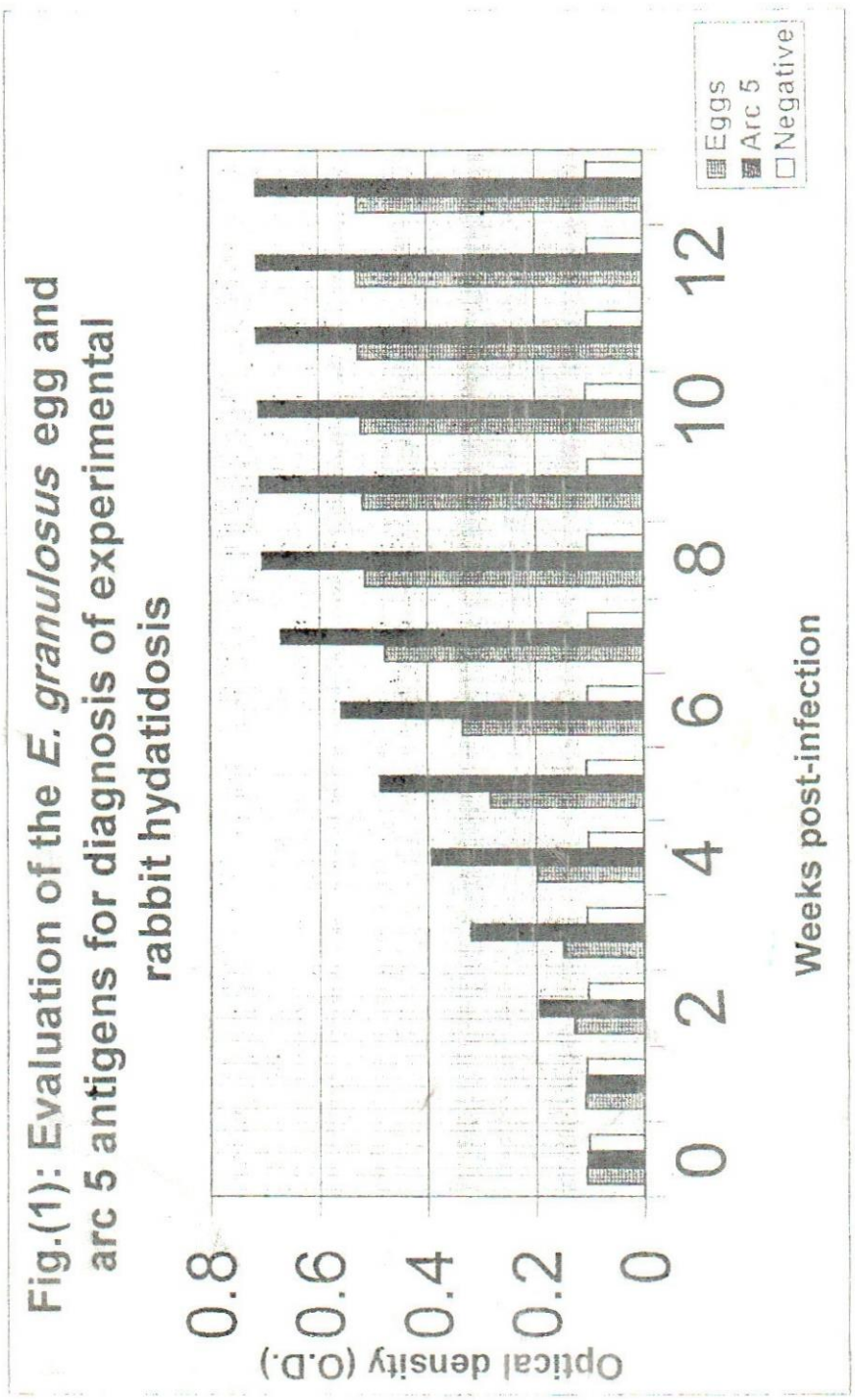

Fig.(1): Evaluation of the *E. granulosus* egg and arc 5 antigens for diagnosis of experimental rabbit hydatidosis