نتائج موجبة لأختبار الروزبنكلال في الخيول

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ملاحظات قصيرة

لوحظت حالات مترفقة في مجموعة من الخيول شملت ناسور الحارك، التهاب المفاصل وتورم كيس الصفن. سحب عينات دم من بعض هذه الحالات ومن خيول تبدو سليمة لقياس خضاب الدم وأجراء اختبار الروزبنكلال (Rose Bengal test) . كذلك تم سحب نموذج من السائل في كيس الصفن. أظهرت الفحوصات أن مستوى خضاب الدم كان ضمن الحدود الطبيعية (11.6-16.2) غ/ 100 ملم 3. وان خمسة خيول من عشرة (50%) أظهرت نتيجة موجبة لأختبار الروزبنكلال وكانت من الجنسين وبأعمار مختلفة ، ولم تقتصر على الخيول التي أظهرت ناسور أو تورم . ولم تعزل جراثيم من السائل المصلي الرائع والحادي على كريات دم حمراء وخلايا قيحية والمسحوب من كيس الصفن لأحد الخيول.
EXPERIMENTAL STUDY ON SOME PROTECTIVE METHODS AGAINST HYDATID CYST INFECTION IN MICE
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SUMMARY

Experimental study albion mice as a host was conducted in on attempt to inactivate the larval stage of *Echinococcus granulosus* by formalin treatment. Animals were divided into three groups. The first group was inoculated subcutaneously with alive ovine hydatid protoscolices , whereas the second group was inoculated similarly with 0.75% formalin- treated protoscolices. The rhird group was remained as negative control. a comparison was held between the three groups from haematological , immunological and histopathological points of view. The haematological picture revealed lymphopenia, eosinophilia and neutrophilia in both treated groups of mice. Infected
mice in groups 1 and 2 responded subcutaneous injection of the antigen by production of antibodies detected by indirect haemagglutination test. Similar responses did not occur in noninfected control mice. Pathologically, results showed that the cellular were concentrated at the site of inoculation and at the draining lymph nodes without spreading to other organs. The formalinized protoscolices failed to induce development or formation of any cysts at the site of inoculation, in comparison with live protoscolices. The challenge of both treated groups at 4 weeks post primary infection showed that 80% of the infected and vaccinated animals resisted infection.

**INTRODUCTION**

Hydatid disease in Iraq is one of the most important endemic diseases (1,2). Sheep and cattle are susceptible to infection and the hydatid cysts are noticed primarily on the liver, lungs, disposal and other organs of slaughtered animals. Faulty disposal of infected organs may lead to recycling of *Echinococcus granulosus* in stray dogs (3).

Marshal et. al. (4) Craig (5) studied the serum antibody response in sheep infected with *E. granulosus* hydatid cysts following parental
immunization with hydatid cyst fluid. The authors found that 60% of the infected animals responded to intramuscular injection of the antigen by rapid production of antibodies which were detected by indirect haemagglutination (1H) test whereas the similar responses did not occur in any of the non infected controls. Moreover, the 1H technique was the most suitable one for detection of Hydatid antibodies (4). On the other hand, the studies of Ali-Khan (7) and lightowlers et al. (6) showed that the infection with hydatid cysts is accompanied by substantial lymphopenia and neutrophilia with a reversal in the ratio of lymphocytes to neutrophils. Al-kannany (8) observed lymhopenia, nentrophilia and eosinophilia when the hydatid disease was reproduced experimentally in mice.

Histopathological changes in different infected organs were studied and discussed by many authors (8,12). The refractory or partially susceptible or abort the larval cyst mass by mobilizing a massive inflammatory reaction against the pathogen; in contrast, hypersusceptible hosts show an insignificant tissue reaction to larval cyst mass (10,13).
The objective of the present study was to conduct a preliminary trial for production of formalinized hydatid cyst vaccine by using formalin for the inactivation of ovine hydatid cyst protoscolices.

**MATERIALS AND METHODS**

**Animals:**
Fifty albino mice were divided into there groups. The first group of twenty mice was inoculated with 1250 olive protoscolices subcutaneously, whereas the second group of the same number was inoculated by the same route and dose with formalinized protoscolelices. The third group of 10 mice remained as a negative control.

**Protoscolices:**
Protoscolices were collected from ovine hepatic hydatid cystes according to the procedure of AL-Kannany (8). The viability of the protoscolices was approved by 0.1% aqueous eosin. The protoscolices were counted and a concentration of 1250 cells per 0.1 ml of hydatid fluid was prepared.

**Formalin treatment:**
The protoscolices were treated with 0.75% formalin as a final concentration at 26 °C for 48 hours.
The treated cells were washed five times with phosphate buffer at pH 7.2 to get rid of formalin.

Sampling:
Blood films and sera samples were collected from the heart of 15 mice in groups 1 and 2, and from 5 mice from the negative control at 4 weeks post infection. Thereafter, these mice were sacrificed and examined macroscopically. Pieces of liver, spleen, axillary and brachial lymph nodes, thymus, heart, muscles and skin were collected and fixed in Carnoy's solution for microscopical examination.

Differential leucocytic count (DLC):
The method of Schalm et. al. (14) was carried out for determination of DLC in blood films.

Indirect hemagglutination (1H) test:
1H test was performed according to techniques slightly modified from those described by Marshall et. al. (4), using glutaraldehyde – treated mice red blood cells sensitized by direct contact with sheep hydatid cyst fluid.

Histopathological examination:
The Carnoy's fixed tissue samples were processed by the histokinate paraffin sections were prepared at the
thickness of 4-6. Harris—hematoxylin and eosin stain was used (15).
Challenge test:
At 4 weeks post infection, 5 mice from each group were challenged subcutaneously with 1250 protoscoleices. The animals were necropsied 4 weeks later and examined macroscopically.
Statistical analysis:
Data were statistically analyzed by analysis of variance.
The accepted level of significance was at P < 0.05.

**RESULTS**

Differential leucocytic count:
Table 1 indicate lymphopenia, eosinophilia and neutrophilia in both treated groups of mice. These changes were more significant (P< 0.01) in the first group.
Indirect hemagglutination test:
The results of 1H antibody titer to immunization with
Alive protoscolices (group 1) (titer 1049.1 which is amear of 15 mice) and immunization with formalinized protoscolices (group 2) (titer 559.5 which is amean of 15 mice), High titers of 1H antibodies has been induced by the both treated
groups being highest in the first group which received alive protoscolices. Similar responses did not occur in any of 5 noninfected controls.

Pathological findings:
Mice inoculated with alive protoscolices:
The site of inoculation at 4 weeks post infection appeared as soft palpable subcutaneous lump without any discrete margin. There was enlargement of axillary and bracheal lymph nodes. Cysts proliferation were restricted to small foci in the fibroblastic corona. The cyst foci were vascular and lacked laminated layer. The foci were encircled lightly by mature and immature neutrophils. Several inflammatory foci consisting of lymphocytes and eosinophils were scattered throughout the corona. A number of blood capillaries in the connective tissue around the corona showed lymphocytic cuffs. The muscular layer adjacent to the corona showed myositis and interstitial edema (Fig.1). Histological examination of axillary and bracheal lymph nodes showed depletion of lymphocytes and replacement by plasma cells. The medulla and subcapsular sinuses showed plasmacytosis and histiocytosis (Fig.2).

Mice inoculated with formalinized protoscolices:
There was no cyst formation at the site of inoculation. The subcutaneous response was evaluated macroscopically and by palpation. Histological examination of the subcutaneous tissues revealed the presence of a granulomatous lesions consisted of connective tissue infiltrated by inflammatory cells and showed degenerative changes. Microscopically, axillary and brachial lymph nodes revealed lymphocytic depletion and increasing in capillary sinuses. No histopathologic alteration were observed in other organs examined in all the other groups of animals.

Challenge test:
Results of the challenge for the three groups showed that one mouse from groups 1 and 2 four mice from group 3 exhibited a cysts in the site of inoculation.

DISCUSSION
The results showed that the immunological hematological and pathological reactions were some what the same when albino mice were inoculated either with alive or formalinized protoscolices of E. granulosus, and the animals were stand against the challenge one month post primary infection.
The results of blood investigation indicated that there was lymphopenia in both infected groups of mice except that the lymphopenia was much clearer in the group which infected with alive protoscolices. This may be due to the depletion of T-lymphocytes in the axillary and bracheal lymph nodes. The results indicated too that there were eosinophilia and neutrophilia which are in accordance with the results of AL- kannany (8) but not with those of Ali- khan (7) who did not observe any neutrophilia although he stated that the hydatid disease is always accompanied by substantial lymphopenia and neutrophilia.

In 1986 Marshall et. al. (4) applied the 1H technique for detection the immune response against the hydatid disease. The same technique with slight modification was applied in this study and the results showed a good level of immunity in both treated groups of animals. The titer in case of alive protoscolices was 1049, while in case of formalinized one was 559.5. It has been stated stated that a titer less than 559.5 is quite enough for protection against infection with the hydatid disease (4,16).

The pathological examination showed that the cellular reactions were concentrated at the site of inoculation
and at the draining lymph nodes only without wide spreading into other organs which were examined. In the group which was inoculated with alive protoscolices the formation of the cyst was clear in the second group, the formalinized protoscolices did not succeed in forming any cyst. This observation suggested the presence of an antigens in host responsible for resistance to establishment of protoscolices. The cellular and histological reactions appeared in this study were more or less in agreement with the findings of other workers (8-13). Further study is in progress to manifest the other parameters must be adopted to confirm this. Thereafter this trial should be applied in ovine and bovine species.
Figure (2) . Section of the axillary lymph node . Note the depletion of germinal centers and the infiltration of plasma histiocytes H & E x 250 .
Figure (1). Section of the subcutaneous tissue revealing myositis and interstitial edema. H & E x 250.
Table 1: Differential leucocytic count in mice inoculated by alive and formalinized protoscolices.

<table>
<thead>
<tr>
<th>Cells</th>
<th>No. of cells %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control * Untreated</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>85 ± 0.6</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>2 ± 0.5</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>11 ± 0.7</td>
</tr>
<tr>
<td>Monocytes</td>
<td>3 ± 0.9</td>
</tr>
<tr>
<td>Basophils</td>
<td>1 ± 0.9</td>
</tr>
</tbody>
</table>

*Mean of 5 mice ** Mean of 15 mice ± SD
# p < 0.05          # # P < 0.01
REFERENCES


12- Riley EM, Dixon JB, Kelly DF, C ox DA. Immune response to Echinococcus granulosus, histological
دراسة تجريبية حول بعض طرق التمتعض ضد الإصابة بالأكياس الصدرية في الفئران
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الخلاصة
اعتمدت هذه الدراسة التجريبية محاولة لأبطال فاعلية الدور اليدوي لطفي النسيج والكائنات الحيوانية Echinococcus granulosus بالفولامدين وتم استخدام الفئران البيضاء كمضيف. قسمت الحيوانات التجريبية إلى ثلاثة مجموعات - حققت المجموعة الأولى بالرئوس الأولية أذنًا وتقلصت المجموعة الثانية بدراسة أولية معالمة بمادة الفولامدين بنسبة 0.75% ، بينما استعملت المجموعات الثلاثة من الحيوانات كمجموعة سيطرة سالبة. أجريت المقارنة بين المجموعات الثلاثة من الناحية الدموية والمناعية والنفسية. أظهرت الصورة الدموية للفئران المجموعة الأولى والثانية وجود نقص في الخلايا الليفية زيادة في كل من الخلايا الحمضية والعدلات مقارنة مع المجموعة الثالثة غير المعالمة ، كذلك بينت هذه الدراسة بأن فئران المجموعتين المعاملتين أظهرت استجابة مناعية بعد حقن الروهوسات الأولية تحت الجلد حيث تم قياس تلك الاستجابة باستخدام اختبار التلازم الدموي غير المباشر ، بينما لم تظهر فئران المجموعة الثالثة أي استجابة لهذا الاختبار. أما من الناحية العرضية ، فإن النتائج بينت بأن الاستجابة السلبية تركزت عند موقع الحقن وكذلك في العقد المحيطة بالحقن ولم يكن هناك أي انتشار للخلايا في الأعضاء الأخرى ، لقد فشلت الروهوسات الأولى المعالمة بالفولامدين من إثبات أي كيس عدي في موقع الحقن مقارنة مع الروهوسات الأولية الحية.

إذن اختبار التحدي لفئران المجموعة الأولى والثانية وبعد أربعة أسابيع من الحقن الأولي أظهر 80% من هذه الفئران قد قاموا بحقن.