

Determination of the effective component on hatching media of *Echinococcus granulosus* eggs

Ismail A.W.Al-Hadithi , Muntaha M.H.Al-Alousi
and Hameed M.Al-Falahi

College of Medicine , Al-Anbar University – Iraq

تحديد العامل المؤثر على فقس بيوض طفيلي الأكياس العذرية في الوسط الزراعي

اسماعيل الحديثي ، منتهى الألوسي ، حميد الفلاحي
كلية الطب - جامعة الانبار

الخلاصة :

اظهرت دراسة العامل المؤثر في الفقس أن هناك فروقات كبيرة في نسبة الفقس عند استخدام محاليل مختلفة ، حيث كانت نسبة الفقس (% 42.83 , 14.00 % , 10.2 %)
53.3% باستخدام (سائل الصفراء، بيكرينات الصوديوم ، ألترسين ومادة البيرياكتين) على التوالي بينما لا يوجد أي تأثير على نسبة الفقس عند استخدام مادتي حامض الهيدروكلوريك والبيسين.
أن استخدام المحلول المعوي الصناعي ضروري جدا لأكمال عملية الفقس وأن غياب أحد مكوناته له تأثير سلبي على الفقس. أشارت الدراسة لوجود فرق معنوي في نسبة الفقس تبعاً لكل عامل .

Summary

The present study deals with the determination of effective component in the hatching media . it revealed that there is a large variability in the effects of different solutions on the percentage of hatching .

The hatching index of the different solutions showed that bile was most effective component and respond for 55.3% of hatching . The second factor was sodium bicarbonate (NaHCO_3) solution 42.83%, trypsin gave 14.0% of hatching and then the pancreatin which gave 10.2% . Each of hydrochloric acid and pepsin solution doesn't affect the percentage of hatching.

The component of Artificial Intestinal Solution (AIS) is very important for the completion of hatching process , where the absence of each component resulted in a change of the hatching rate . There were significant differences between the percentage of hatching.

Introduction :-

Wantland (1953) was subjected ova of *Taenia* at body temperature to an artificial gastric juice , but no visible effect of the embryophore was demonstrated after four hours of the treatment,when the eggs were placed in digestion medium consisting of ox-bile and Pancreatin adjusted to PH from 7.2 – 7.6 . embryophores disintegrated in 5 – 20 min . releasing active oncospheres .

Silverman (1954) described an in vitro technique capable of demonstrating the hatching of teaniid eggs and activation of the hexacanth embryo.It was found that intestinal juices of sheeps and cattles, artificial pancreatic juice and bile caused rapid disruption of the embryophorse and activation of the oncospheres (3).

Meymerian (1961) found that both NaHCO₃ and NH₄OH removed onco-spheres of eggs with out activation of oncospheres and both Pancreatin and Trypsin caused somes activation of the free oncospheres. Heath and Smith (1970) described a technique for hatching and subsequent in vitro activation of onospheres of *E.granulosus* and other teaniid to cystic larva , the hatching media consisting of acid pepsin following by alkaline Pancreatin with bile .The aim of this study is to determine the effective component of hatching medium of *E.granulosus* eggs .

Materials and Methods :-

Fertile human hydatid cysts were removed surgically from infected patients in Ramadi general Hospital in Al anbar province . The viability of protoscolices was measured using eosin as a vital stain and flam cell activity .

The experimental infection was done by feeding a human hydatid cysts to two dogs which were kept under controlled condition for 60 days.

The dogs were killed after using oral dose of strychnine sulphate and they were brought to the laboratory and dissected within 30 min . The intestine was removed and cut into pieces ranged between 10 – 15 cm , incised longitudinally by cisor and blaced in worn (40 c) normal saline (7) . The Adult worms dislodged carefully from mucosal lining by using spatula . They are usually harbored the upper and the middle thirds of the difinitive (Saeed , 1983) .

Gravid segment was removed by cutting with sterile needle and trans-ferred to a petridish containing normal saline , eggs also teased from gravid segment and stored at 4 ± 1 C in saline solution (5).Containing :

Penicillin G	1000 U / ml
Streptomycin sulphate	1000 mg / ml
Nystatine	1000 U / ml

The hatching media for *E.granulosus* eggs representing artificial gastric and intestinal solution , which was described by Heath and Smith (1970) as a modified technique of Silverman (1954) , which consist of :

1- Artificial gastric solution (AGS) which contain :

HCl	1 ml	1 %	} Dissolved in Pepsin 1 gm 1 % of distilled water
100ml			
NaCl	0.85 gm	0.85%	

2- Artificial intestinal solution (AIS) which contains :

Trypsin	0.2 gm	0.2%	} Dissolved in 100ml of distilled water
Pancreatin	0.5 gm	0.5%	
NaHCo3	0.5gm	0.5%	
NaCl	0.85gm	0.85%	
Bile	5.0ml	5.0%	

Determination of the effective component in the hatching media :-

- 1- Each component of hatching media prepared as single solution , then the eggs were treated with this solution for one hour .
- 2- Six sets of hatching media were prepared each one lose one component of the media to determine the role of each component .
- 3- The control was contain all components of the hatching media .

The work was designed as a multiple experiments each was designed to test certain factors in three replicat with one for control . The same procedure was used in all experiments with some differences according to testing factors . All experiments were done at a constant temperature of (38 ± 1 C) . The eggs and the hatching solution all brought to this temperature before the hatching solution were added to the eggs . About 3000 of eggs were used in each replicat and Incubated for 1 hour in each solution .

Results and Discussion

Both tables 1 & 2 indicate that there was a large variability in the effects of different solutions on the percentage of hatching .

The hatching index of the different solutions showed that bile was the most effective component and respond for 55.3% of hatching . The second factor was sodium bicarbonate (NaHCO_3) solution 42.83% Trypsin gave 14.0% of hatching and then the pancreatin which gave 10.2% . Each of hydrochloric acid and pepsin solution doesn't affect the percentage of hatching . fig .1 shows the differences in the percentage of hatching according to different solution .

The component of AIS is very important for the completion of hatching process, where the absence of each component resulted in change of the hatching rate .

The results of this study revealed that bile is the most important factor within AIS in hatching process, this agreed with Silverman(2), Meymerian (4) and Huffman & Jones (9) . The absence of bile from hatching media resulted in reduction of hatching rate to (25.7%) The role of bile could be in one of two ways: first it could cause denaturation of protein that render them more accessible for proteolytic enzyme . (10). Secondly ; bile aids through its ability to catalyse pancreatic enzymes (2).

We believe that bile may act through all mechanisms because the treatment of eggs with bile alone, or within AIS caused the occurrence of hatching but the addition of other components of media (AGS) increased the percentage of hatching from 56.11% - 93.55%.

Sodium bicarbonate (NaHCO_3) is one of the important components of pancreatic juice , it provides a large quantity of alkaline ion in the pancreatic juice , the hatching index of it equal to 46.3% , its absence from hatching media decreases the percentage of hatching to 36.7% , this is due to reduction of the pH of AIS toward the acidity which leads to inactivation of all enzymes activated in alkaline medium in range of 7.5 – 8 .

Pancreatin is an extraction of mammalian pancreas containing enzymes protease , lipase and amylase activity (11) .

The absence of Pancreatin from hatching media decreased the hatching rate to 10.34% pancreatic enzymes are very important in digestion of embryo, but it couldn't act alone without other contents of AIS which represent the medium of small intestine , bile salt and acids catalyse the reaction of pancreatic enzymes , so the hatching index of Pancreatine was 10.34% .

Trypsin is a proteolytic enzyme secreted by pancreas within pancreatic juice, the results showed that the absence of Trypsin decreased the percentage of hatching from 84.8 % - 81.4 %. The treatment of eggs with Trypsin alone caused disruption of chorionic membrane and caused hatching 14.0 % of eggs.

The present study showed that all components of hatching media have a role in the hatching process but in varying degree and bile is a major effective factor in this process.

Table 1 : The relationship between each component of hatching medium and the percentage of hatching & hatching index :

Substance	Replicate	No. of eggs	No. of hatched eggs	% of hatched eggs	Mean X	Hatching index
HCl	1	3000	Nil	0	0	0
	2	3000	Nil	0		
	3	3000	Nil	0		
Pepsin	1	3000	Nil	0	0	0
	2	3000	Nil	0		
	3	3000	Nil	0		
Trypsin	1	3000	330	11.0	14.0	14.05%
	2	3000	420	14.0		
	3	3000	510	17.0		
Pancreatin	1	3000	320	10.6	10.2	10.34%
	2	3000	360	12.0		
	3	3000	420	8.0		
Sodium bicarbonate	1	3000	1300	43.3	42.83	46.5%
	2	3000	1460	48.6		
	3	3000	1400	36.6		
Bile	1	3000	1600	53.3	55.3	55.5%
	2	3000	1760	58.6		
	3	3000	1620	54.0		
Control	1	3000	2970	99.0	95.1	100%
	2	3000	3000	100.0		
	3	3000	2690	86.3		

Table 2 : Omitting of some component hatching media and percentage of hatching .

Hatching media without	Replicate	No. of eggs	No .of hatched eggs	%of hatched eggs	Mean X	Hatching index
HCl	1	3000	1400	46.6	52.1%	52.2%
	2	3000	1700	56.6		
	3	3000	1600	53.3		
Pepsin	1	3000	2700	90.0	91.3%	91.4%
	2	3000	2720	90.6		
	3	3000	2800	93.3		
Trypsin	1	3000	3500	83.3	81.4%	84.8%
	2	3000	2430	81.0		
	3	3000	2400	80.0		
Pancreatin	1	3000	1620	54.0	52.4%	52.4%
	2	3000	1600	53.3		
	3	3000	1500	50.0		
Sodium becarbonate	1	3000	830	27.6	33.2%	36.7%
	2	3000	920	30.6		
	3	3000	1250	41.6		
Bile	1	3000	770	25.6	25.6%	25.7%
	2	3000	780	26.0		
	3	3000	760	25.3		
Control	1	3000	2990	99.6	99.8%	100%
	2	3000	3000	100		
	3	3000	3000	100		

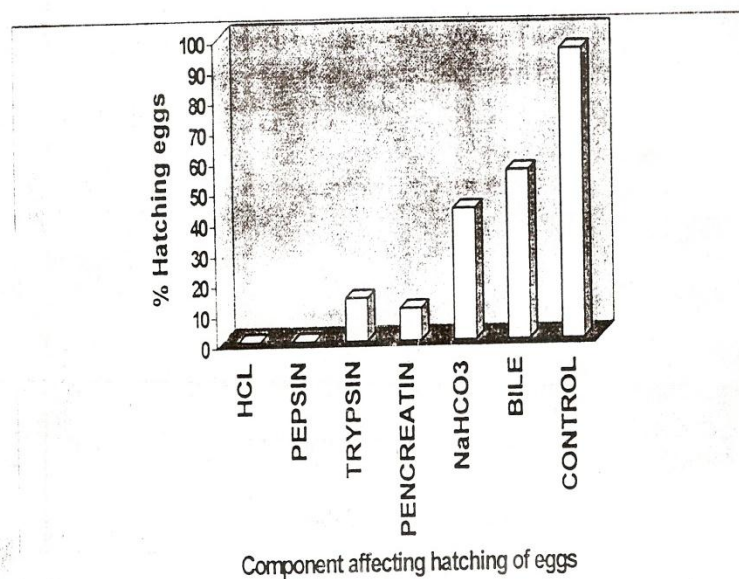


FIG (1) . The difference in the percentages of hatching according to the different solutions of hatching media .

References

- 1- Wantland,W.W .(1953) . Cysticercus fasciolaris In the Syrian hamster. J . parait.39 : 667 – 668 .
- 2- Silverman , P.H.(1954a) . Studies on the boilogy of some tapeworm of the genus Taenia . I . Factors affecting hatching and activation of teaniid ova ,and some criteria of their viability. Ann . Trop . Med . parasit . 48:207 – 215.
- 3- Berberian , (D.A.(1957) . Host specificity and the effects of digestive juices on ova of E.granulosus in "Tenth Annual Report " Orient hospital , Beirut . p . 33 – 43.
- 4- Meymerian , E.(1961) . Host-parasite relationship on Echinococcosis . VI.Hatching and activation of E . granulosus ova in vitro . Amer. Trop.Med . Hygi . 10:719-726 .
- 5- Heath , D.D and Smyth J . D .(1970) . in vitro cultivation of Echinococcosis granulous , Taenia hydatigena , T.ovis,T.pisitormis and T.serialis from oncosphre to cyctic larva . parasitology . 16 :329 – 343 .
- 6- Smyth , J.D and Bareet , N.J.(1980) . Procedures for testing the viability of human hydatid cysts following surgical removal , especially after chemotherapy . Tran .Roy.soci.Trop.Med.Hygi.74(5) : 649 – 652 .
- 7- McManus,D.P. (1981).A biochemical study of adult and cystic stagesof E.granulosus of human and animals origin from Kenya. J.Helminth .55:21 – 27.
- 8- Saeed.I.S(1988) Immuization of mice against Echinococcus granulosue by using protoscoleces exposed to Ultraviolet irradiation M.SC.Thesis.Coll.Unvi.Salahaddin .
- 10- Glassman ,H.N.(1984).Surface active agents and their application in bacteriology .Bact . 12 : 105 – 148 .
- 11- Martindal , W . (1989) . The extra pharmacopoeia . By: Reynolds , J . E . The pharmaceutical press . London . 24th end P : 32.

Recived (28/9/2009)
Accepted (16/2/2010)