AJPS, 2009, Vol. 6, No.1

A brief study about the preservation of *Echinococcus granulosus* protoscolices in vitro for a long periods.

Ahmed A. M. Al-Azzauy
College of Pharmacy, Dept. of Medical Microbiology & Biotechnology

---

الخلاصة

أجريت محاولة بسيطة للإحتفاظ برؤيسات دودة المشوكات الحبيبية *Echinococcus granulosus* المزالة من اكياس البشر المائية بكامل حيويتها في الزجاج in vitro وذلك باستخدام ثلاثة اوساط زرعية مختلفة هي (المادة الزرعية RPMI –1640 ومحلول رنكر الفسلجي Ringer's Solution ومحلول الملح الفسلجي الاعتيادي المعقم Normal Saline Solution) كما درست درجة الحرارة المثلى لحفظها من بين ثلاث درجات حرارة تم اختيارها وهي (4, 25, 37) ْْْم.

اظهرت النتائج ان أفضل مادة لتنمية الرؤيسات الاولية مع احتفاظها بحيوية عالية هي محلول الملح الفسلجي الاعتيادي، حيث امكن المحافظة على الرؤيسات الاولية المستزرعة فيه لفترة وصلت الى (75) يوماً وذلك عند استعمال درجة حرارة (25) ْم. اما درجة الحرارة المثلى للحفظ فتباينت تبعاً للمادة الزرعية المستعملة.

Abstract

Simple attempt were applied to keep the protoscolices of *Echinococcus granulosus* removed from human hydatid cysts alive with full activity in vitro using different culture media [RPMI-1640, Physiological Ringer's solution(RS), Physiological normal saline solution(NS)], the best temperature for cultivation in this media were also investigated between three degrees (4, 25, 37)°C .

The best medium for cultivation with a high activity was (NS) solution where the protoscolices continue for (75) days in (25)°C. Whereas the best temperature for cultivation was variable according to the cultivation medium.

Introduction

Unilocular echinococcosis is a cyclozoonotic infection produced by the metacestode stage of *Echinococcus granulosus*; it has a world-wide distribution and causes large economic and public health problems [1, 2, 3, 4, 5]. Iraq is one of the areas where the disease spreading, moreover, the disease considers hyperendemic in Iraq [6]. It has public health importance not only in areas of endemicity but also in countries or regions without endemicity due to the migration of infected people [7].

The human infects with hydatid cysts throughout the accidental ingestion of the worm eggs evacuated in the faeces of the dogs infected with this worm [5], throughout the contaminated food, drink and articles; so that the human considers accidental intermediate host. The high epidemiology of the disease related to the high resistance of the two infected stages for the intermediate and final hosts (the ova and hydatid cysts); the cyst producing very large numbers (reach to millions) of the protoscolices which have an importance not less than the eggs because they have the ability to infect the final host and develop into an adult worm (after being ingested by a suitable definitive host) and continue the life cycle [4,8,9].

The development of the protoscolex is charming biologically because of its ability of the exceptional differentiation into two lines depending on the host; if it is ingested by a suitable final host it will produce adult worm, but if the primary cyst ruptured inside the intermediate host, it will causes like a dissemination of the protoscolices in the same host. Moreover, each protoscolex has the ability to remove the differentiation and transform toward the production of secondary cysts [4, 8].

To facilitate the different research studies on the protoscolices, many researchers tried to isolate this protoscolices from their hydatid cysts, and they try many times to cultivate it in their laboratories in vitro using different culture media; they get benefits in many fields, for example they studied on the viability of the protoscolices and the influencing of the drugs on the respiration of their in vitro [10]. Others studied about the ability to preserve the protoscolices viable (or alive) in vitro and inside the hydatid cyst using different temperatures [11]. Others studied the isolation of different antigens from the protoscolices [12, 13, 14]; and also biochemical studies were applied [2], they were study about the amino acids transportation system from and to the in vitro cultivated protoscolices [15] and others. From this point we could recognize the importance to find a suitable method to preserve the protoscolices alive and active in vitro as they are in their habitat after the removing and isolation from their natural microenvironment inside the hydatid cyst.

Materials and method

1-The isolation and cultivation of the protoscolices in vitro:

The most possible quantity of the hydatid fluid free of protoscolices were sucked from the hydatid cyst sample to leave the protoscolices in the bottom, then the cyst opened to suck the requested fluid which contain the protoscolices; it is putted in a flask for precipitation. After (10)min., the infiltrated sucked to leave the protoscolices, which they suspended then with a known volume of normal saline solution; the viability and the concentration of the protoscolices were investigated using the microscope and eosin stain. The viable protoscoices were distributed upon the cultivation flasks in an equal numbers (≈500

protoscolex in each flask) and divided into three main groups (A, B, C) subdivided to subgroups as the following:

Group A:

Include (9) flasks, divided into (3) subgroups each one include (3) flasks; the flasks of each subgroup contain one culture medium (RPMI-1640, Ringer's solution and normal saline solution) consecutively. The flasks of the main group (A) were putted in (4)˚C.

Group B:

Also include (9) flasks, divided as in the above, but the protoscolices incubated in (25)˚C.

Group C:

Also include (9) flasks divided as in the above, but the incubation done in (37)˚C.

Results

The results showed that the normal saline solution (NS) were the best culture medium for preservation with full activity, the average number of the preservation days were (75) days in (25)˚C, whereas the protoscolices were still viable (67) days in (37)˚C and (57) days in (4)˚C.

The most suitable temperature for cultivation were varied according to the used medium, however, it was (25)˚C with (NS), (4)˚C with (RPMI-1640) and (37)˚C with (RS), the results clarified in table (1) and figure (1).

Culture medium	Temperature	Average no. of viability days
RPMI-1640	4˚C 25˚C 37˚C	31 28 22
Ringer's solution	4˚C 25˚C 37˚C	41 35 57
Normal saline solution	4˚C 25˚C 37˚C	57 75 67

Table 1: clarify the relationship between the average no. of viability days and the culture media in (4, 25, 37)˚C.

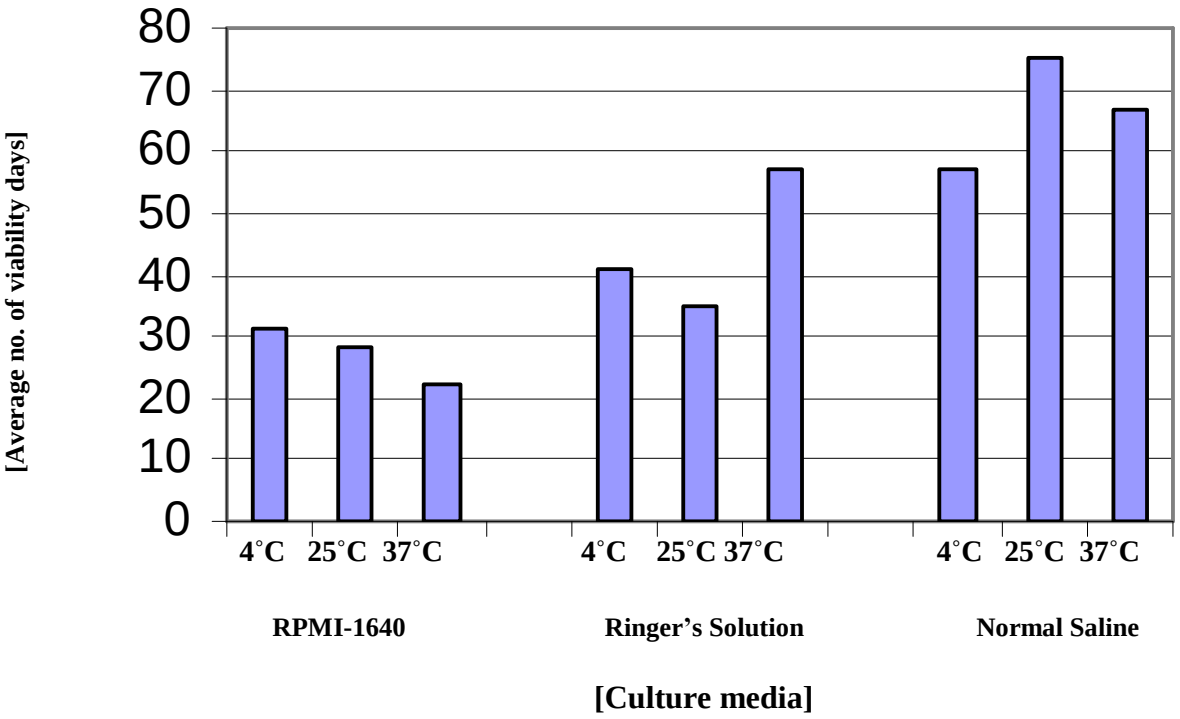

Fig (1): clarify the relationship between the average no. of viability days and the culture media in (4, 25, 37)˚C.

Discussion

In this experiment, the influencing of three culture media and three temperatures on the viability period of *Echinococcus granulosus* protoscolices in vitro was observed. The results showed that each of the culture medium and the temperature of cultivation influencing in the viability lasting of the cultivated protoscolices.

This cultivation method providing adequate time to the searcher to study on their. The importance of this method could be summarized in the following:

1 - The preservation of the protoscolices with full activity for along time providing adequate time to the searcher to isolate adequate quantity of the somatic and metabolic antigens in vitro, and help in the study of the effects of different factors and substances on their viability as the effects of the drugs and chemicals in a time which may be difficult to get a sample for study. Also the normal saline providing a suitable medium for cultivation because it isn't contain amounts of nutrient substances which may contradict with the used research materials of the experiments.

2 - If this preservation method carried out successfully it will help the searcher to use their to induce the experimental infection with secondary hydatid cyst in a time which may be difficult to get fresh sample of hydatid cysts.

The samples that are mentioned above are the human hydatid cysts, because the surgical removal doesn't carry out in any time because the surgery depends on the public health status of the patient, which make the collection of the samples difficult and not easy in any time, as well as the hydatid cysts from animal sources doesn't provide a real substitution for the human hydatid cysts because of the probability of the difference in the strain of the worm that caused the infection is possible (conceivable) as it is mentioned in many references, which make the study on the causative agent in human not exact (strict).

References

1 - Conder, G.A.A .; Andersen, F.L. and Schantz, P.M. (1980). Immunodiagnostic test for Hydatidosis in sheep: An evaluation of double diffusion, immunoelectrophoresis, indirect haemagglutination, and intradermal test, Am. J. parasitol. 66(4): 577-584.

2 - McManus, D.A. (1980). Biochemical study of adult and cystic stage of *Echinococcus granulosus* of human and animal origin from Kenya, J. Helminthol. 55: 21-27.

3 - Fauser, S. and Kern, P. (1997). T-lymphocyte cytokine RNA expression in cystic echinococcosis, Acta Trop. 64 (1, 2): 35-51.

4 - Holcman, M. and Heath, D.D. (1997). The early stages of *Echinococcus granulosus* development, Acta Trop. 64 (1, 2): 5-17.

5 - Shambesh, M.K. ; Craig, P.S. ; Wen, H. ; Rogan, M.T. and Paolillo, E. (1997). IgG1 and IgG4 serum antibody responses in asymptomatic and clinical expressed cystic echinococcosis patients, Acta Trop. 64 (1,2): 53-63.

6 - Al-Dabagh, M.A. and Al-Janabi, T.A. (1990). The science of hydatid cysts, 1st ed., Al-Etedal Company limited, Baghdad.

7 - Mamuti, W. ; Yamasaki, H. ; Sako, Y. ; Nakaya, K. ; Nakao, M. ; Lightowlers, M.W. and Ito, A. (2002). Usefulness of hydatid cyst fluid of *Echinococcus granulosus* developed in mice with secondary infection for serodiagnosis of cystic echinococcosis in humans, Clin. Diagn. Lab. Immunol. 9(3): 573–576.

8 - 8-Gottstein, B.Rev. (1992). Molecular and immunological diagnosis of echinococcosis, Clin. Microbiol. 5(3): 248-261.

9 - Eckert. J. and Deplazes, P. Rev (2004). Biological, Epidemiological, and Clinical aspects of echinococcosis, a zoonosis of increasing concern, Clin. Microbiol. 17(1): 107–135.

10 - Schwabe, C.W.N. ; Hadidian, L. and Roussa, M. (1963). Host-parasite relationships in echinococcosis IX, in vitro survival of hydatid scolices and the effects of drugs upon scolex respiration, Am. J. Trop. Med. Hyg. 12: 338-345.

11 - Andersen, F.L. ; Loveless, R.M. (1978). Survival of protoscolices of *Echinococcus granulosus* at constant temperatures, J.Parasitol. 64 (1): 78-82.
12 - Kagan, I.G. and Agosin, M. (1968). Echinococcus antigens, Bull. Wld. Hlth. Org. 39: 13-24.

13 - Williams, J. F. (1979). Recent advances in the immunology of cestode infections, J. Parasitol. 65: 337-349.
14 - Auer, H. and Aspock, H. (1986). Studies on antigens from in vitro cultivated protoscolices of Echinococcus multilocularis and their possible use in the serodiagnosis of human Echinococcosis, Taeniasis (cysticercosis) and Hydatidosis (Echinococcosis), Second Int. Symp. (2-7) Dec.: 7-15.
15 - Jeffs, S.A. and Arme, C. (1986). *Echinococcus granulosus* specificity of amino acid transport system in protoscolices, J. Parasitol. 95: 71-78.