

Studies on Using Cattle and Sheep Hydatid Cyst Fluid Instead of the Fetal Calf Serum in Leishmania Culture

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Article information	Abstract
<p>Article history: Received: 23 July 2012 Accepted: 14 Sep 2012 Available online: 12 Mar 2013 ZJRMS 2013; 15 (12): 9-12</p> <p>Keywords: Hydatid cyst fluid Fetal calf serum Leishmania culture</p> <p>*Corresponding author at: Dept. of Laboratory Science, Faculty of Paraveterinary Sciences, Bu-Ali Sina University, Hamadan, Iran E-mail: hrezvan@gmail.com</p>	<p>Background: Leishmania is a single cell parasite causing leishmaniasis, which is a common disease between humans and animals. Due to the importance of in-vitro culture of the parasite in leishmania research, developing new methods for in-vitro cultivation of the parasite has always been a goal for leishmania researchers. The main objective of this study was to use sheep and bovine hydatid cyst fluids as alternatives for fetal calf serum (FCS) in leishmania in-vitro culture.</p> <p>Materials and Methods: A total of 1 million leishmania promastigotes were added to 4 flasks as follow. A flask containing DMEM medium with 10% fetal bovine serum, a flask containing DMEM and 10% sheep hydatid cyst fluid, a flask containing DMEM medium with 10% bovine hydatid cyst fluid and a flask containing DMEM medium alone. After 2, 4, 7, 9, 11, 21 and 24 days, the number of parasites were counted and compared.</p> <p>Results: The result of this study showed that, DMEM medium enriched with 10% sheep hydatid cyst fluid in 168 hours and medium enriched with 10% bovine hydatid cyst fluid in 96 hours can act as a good alternative for fetal bovine serum in the culture <i>Leishmania major</i>.</p> <p>Conclusion: The results showed that sheep and bovine hydatid cyst fluid can be used as alternatives to FCS for dense cultivation of leishmania. The results also showed that, the growth of promastigotes in medium enriched with bovine cyst fluid is more rapid than the medium enriched with sheep cyst fluid in the beginning of cultivation.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Leishmaniasis is a parasitic disease common between humans and animals and caused by genus *Leishmania* [1-3]. The disease has been yet reported from 88 countries including 16 developed ones. Now, twelve million people are infected to leishmaniasis worldwide and a further population of three hundred million is at risk of acquiring the disease [1-5]. The importance of leishmaniasis and its complications such as: long lasting wound, facial ulcer, the risk of secondary infection, heavy expenses of medications for the society, long term treatment and chemotherapy side effects are so high and that the world health organization financially supports the research on various aspects of this disease [2-6].

The basic step in studying any pathogenic agent is its in-vitro culture in the laboratory that facilitate subsequent works on any aspect of the pathogen such as detection and vaccines development [7].

Now days, leishmania is in-vitro cultivated in basic or specific media enriched with fetal calf serum (FCS). FCS is the remaining part of the plasma proteins after coagulation and conversion process of fibrinogen to fibrin and it is obtained from the fetal bovine blood in the slaughterhouse [8,9]. The most important feature of FCS is, having low levels of antibody and more growth factors.

The importance of adding the FCS into the leishmania medium culture is as high as its absence in the media results in the parasite not to grow and finally die [7, 10, 11]. Nowadays this material is commercially being produced and sold widely by biological companies and its sale rate in 2008 is estimated to be around 7×10^5 liters throughout the world [8, 9]. Using FCS in leishmania culture has been always accompanied with problems such as the need for extensive facilities for the preparation of FCS, contamination of FCS with viruses, bacteria and prions, variation of FCS due to the genetic variation of calves being used and its high price [12-14]. Thus, in recent years many studies have been conducted to replace other materials with FCS in leishmania culture.

Hydatid cyst fluid ingredients and microelements are very similar to those of serum [15]. So that, and due to the easy preparation of hydatid cyst fluid on butcheries or slaughterhouses, it is proposed to be a good candidate to be used instead of FCS in leishmania culture.

Materials and Methods

Leishmania parasite: *Leishmania major* parasites were kindly gifted by Dr. Habibi in Razi Vaccine and Serum Research Institute Karaj/Iran.

Hydatid fluid: Cattle and sheep hydatid infected livers were obtained from Hamadan's slaughterhouse. To remove the hydatid fluid, the outer surface of the liver was first disinfected with ethanol and the hydatid cyst fluid was taken by a 5cc syringe. The hydatid cyst fluid was then put on ice, transferred to the parasitology lab at the school of paraveterinary sciences in Bu-Ali Sina University and centrifuged at 3000 rpm for 15 min. The supernatant was removed and stored at -20°C until required. In the experiments, the hydatid cyst fluid was sterilized by passing through $0.22\ \mu\text{m}$ filters and used.

Leishmania culture: Leishmania promastigotes were first cultured in DMEM medium supplemented with 10% complement disabled FCS (Bio Idea Group), 100 IU/ml and Streptomycin 100Mg (Bio Idea Group) at 25°C to stationary phase. The parasites were then counted using neobar slide and put in four separated T75 flasks containing medium culture at 10^6 cell/flask as follow:

The first flask was containing DMEM medium, 100 IU/ml penicillin, 100 μg streptomycin and 10% FCS. The second flask was containing DMEM medium, 100 IU/ml penicillin, 100 μg streptomycin and 10% bovine hydatid cyst fluid. The third flask was containing DMEM medium, 100 IU/ml penicillin, 100 μg streptomycin and 10% sheep hydatid cyst fluid. The last one was used as control containing DMEM medium and 100 IU/ml penicillin and 100 μg streptomycin.

The number of parasites in each flask was counted on day 2, 4, 7, 9, 11, 21, 24 and compared to each other.

Results

One million promastigotes of *L. major* in the logarithmic phase were cultured in an order of different flasks using the culture medium supplemented with FCS, bovine hydatid cyst fluid and sheep hydatid cyst fluid (see materials and methods). The cells were counted on day 2, 4, 7, 9, 11, 21 and 24. On day 2, the number of parasites increased in the order of 5.4×10^6 , 8.4×10^6 , 6.5×10^6 and 4.4×10^6 (Table 1). After 4 days of culture, the number of parasites in FCS supplemented medium was 6.8×10^6 , in medium containing bovine hydatid cyst fluid was 8.7×10^6 , in medium containing sheep hydatid cyst fluid was 6.9×10^6 , and in DMEM alone was 3.6×10^6 (Fig. 1). Similarly, on days 7, 9, 11, 21 and 24 the parasites were counted (Table 1).

Discussion

Leishmaniasis is an important tropical disease with a wide spread in the world and no effective vaccine to control it [3]. Mass cultivation of leishmania parasites is essential for biochemical, physiological or immunological studies for which different media supplemented with FCS are used [12]. However, high density of FCS in the culture media is toxic for the parasite and its preparations is associated with difficulties such as extensive facility requirement, the elimination of animals to obtain the serum and the cost [7, 10, 11]. Hydatid cyst fluid is a clear liquid that contains proteins, glucose, triglycerides and various salts such as sodium, chlorine, phosphorus, calcium and magnesium [10].

Table 1. Comparison of leishmania major promastigote counting in DMEM medium supplemented with FCS, bovine hydatid cyst fluid, sheep hydatid cyst fluid and DMEM alone on different days of culture

Supplements	Days after culture							
	0	2	4	7	9	11	21	24
FCS*	1×10^6	5.4×10^6	6.8×10^6	13.3×10^6	10.8×10^6	3.4×10^6	2×10^6	2.0×10^6
Bovine HCF**	1×10^6	8.4×10^6	8.7×10^6	8.1×10^6	2.8×10^6	2.5×10^6	1.0×10^6	4.0×10^6
sheep HCF**	1×10^6	6.5×10^6	6.9×10^6	9.3×10^6	3×10^6	2.7×10^6	4.0×10^6	1.0×10^6
Control	1×10^6	4.4×10^6	3.6×10^6	2.2×10^6	3.9×10^6	4.2×10^6	1.1×10^6	2.0×10^6

* FCS = fetal calf serum

** HCF = hydatid cyst fluid

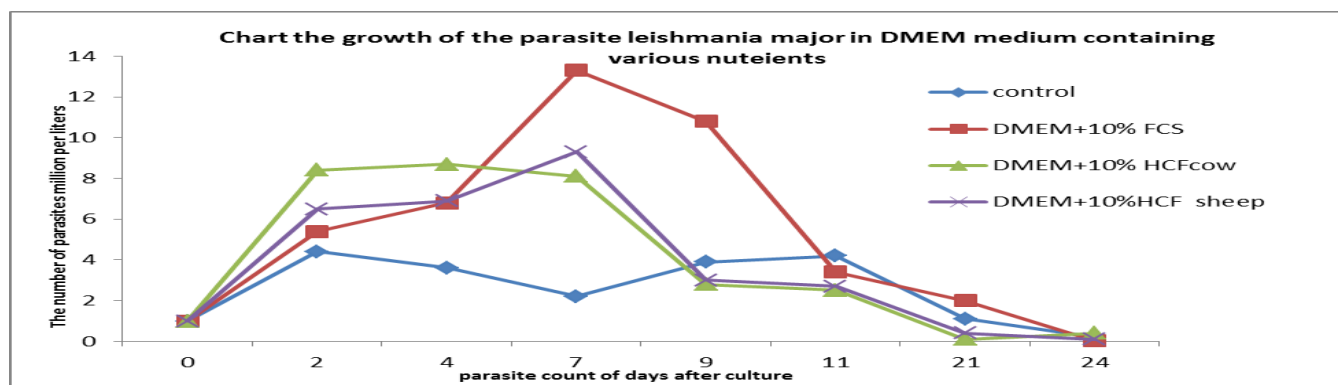


Figure 1. One million logarithmic phase promastigotes of *Leishmania major* were cultured in four T75 cell culture flasks containing DMEM medium supplemented with 10% of FCS, bovine hydatid cyst fluid, sheep hydatid cyst fluid and DMEM medium alone. The parasites were then counted using a neobar slide on days 2, 4, 7, 9, 11, 21 and 24 after the culture and compared to each other

Studies on chemical contents of different animal's hydatid cyst fluid showed that their ingredients are remarkably different [12, 16, 17]. Other studies have shown that the level of glucose, protein and triglycerides in sheep liver and lung's hydatid cyst fluid is higher than those of the cattle [18]. Also, the level of sodium and chlorine ions in bovine hydatid cyst fluid was higher than that of sheep, while the level of calcium ions and trace elements such as iron, copper and phosphorus in sheep hydatid fluid is higher than that of bovine's [18]. Furthermore, the chemical compounds of the fertile and infertile hydatid cyst fluid are different [16, 17].

In the present study, for the first time the bovine hydatid cyst fluid was used as an alternative to FCS and compared with that sheep in leishmania major culture. According to the results obtained, in the first 48 hours, the number of cells counted in DMEM medium enriched with FCS, bovine hydatid cyst fluid, sheep hydatid cyst fluid and DMEM alone was in the order of 5.4×10^6 , 8.4×10^6 , 6.5×10^6 and 4.4×10^6 showing a significant increase in the number of parasites counted in medium containing cattle or sheep hydatid cyst fluid compared to that of the parasites in DMEM alone. These results were confirmed by the number of the parasites counted after 96 hours. Therefore, it can be concluded that cattle and sheep hydatid cyst fluids contain appropriate growth factors that efficiently stimulate proliferation mechanisms. After 96 hours of the culture, optimum growth of leishmania major promastigotes in bovine hydatid cyst fluid enriched medium occurred and that shows sufficient levels of glucose, proteins, triglycerides and salts such as sodium and chlorine in bovine hydatid cyst fluid [12, 16-18]. After 7 days the maximum growth of leishmania major in all media supplemented with FCS, sheep hydatid cyst fluid was obtained, however, the number of leishmania major promastigotes in media containing bovine hydatid cyst fluid was similar to that of day 5.

The results were similar to those obtained in previous studies. In a study by Fakhar, the growth of leishmania major promastigotes in medium of brain and heart extract containing %10 sheep hydatid cyst fluid until 72 hours was very excellent and approximately was equal to that of FCS [10]. Some other studies have also been conducted on leishmania growth stimulants and minerals necessary for the culture of leishmania parasites as well as finding an appropriate alternative to the FCS [19- 25]. Nassiri showed that the growth of *L. major* promastigotes in RPMI medium supplemented with 10% chicken serum is approximately similar to that of FCS supplemented media [17]. Muniaraj successfully used the cow, goat and buffalo milk to stimulate the cultivation of *Leishmania donovani* [19].

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In other studies, rat serum, horse serum, egg yolk, beef intraocular fluid, human serum and human urine were used to enrich the media in leishmania culture with and without FCS [20-25].

The results of this study were in line with those of others and showed that sheep and cattle hydatid cyst fluid can act as good alternatives for FCS in *L. major* medium culture. Some advantages of using hydatid cyst fluid in leishmania culture is its availability, low cost, nontoxicity, high efficiency, easy preparation, high level of nutrients and no need for ethical permission. Moreover, in Iran, hydatid cyst is hyper endemic and the prevalence of the disease is very high so that, the hydatid cyst fluid is freely available in all slaughterhouses [10, 13, 27].

The results clearly showed that the parasite growth in medium enriched with 10% bovine cyst fluid is faster than that supplemented with sheep cyst fluid in the first few days. As the bovine hydatid cyst fluid is sterile and infertile compared to that of sheep. This makes it easier to collect and reduces the risk of infection for the researchers.

So, it can be concluded that the growth of leishmania major in DMEM medium supplemented with sheep or cattle hydatid cyst fluid is feasible. As appropriate alternatives for FCS in DMEM medium, the cattle hydatid cyst fluid can be used for the first 96 hours while the sheep hydatid cyst fluid can grow and keep the parasite for 168 hours. Moreover, the bovine cyst fluid supplemented medium induces the parasite to grow faster than the medium containing the sheep cyst fluid in the first few days after the culture.

Further studies needs to determine the qualitative and quantitative analysis of hydatid cyst fluids from different animals and various organs for replacing the FCS in leishmania culture.

Acknowledgements

We would like to thank BU-Ali Sina University for their financial support. The school of Paraveterinary Sciences, Bu-Ali Sina University, Prof. Zolfigol, Dr. Fayzi for all their help.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Bu-Ali Sina University, Hamadan.

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Please cite this article as: Afsahi A, Aeini Z, Rezvan H, Aazami S. Studies on using cattle and sheep hydatid cyst fluid instead of the fetal calf serum in leishmania culture. *Zahedan J Res Med Sci (ZJRMS)* 2013; 15(12): 9-12.