IJBPAS, February, 2015, 4(2): 708-712

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ISOLATION OF *CRYPTOSPORIDIUM PARVUM* OOCYST FROM INFECTED CALVES FECES

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ABSTRACT

Cryptosporidiosis is a parasitic disease caused by *Cryptosporidium parvum*, a widely distributed protozoan parasite, which infects both wild and domesticated. Animals, as well as humans, chiefly immunocompromised individuals (O'Donoghue, 1995; Griffiths, 1998). Since its diagnosis in the 1970s, Cryptosporidium has been attributed an increasingly important role in the neonatal diarrhoea syndrome of newborn ruminants. It is very important to diagnose *Cryptosporidium parvum* disease in animals and isolation of *Cryptosporidium parvum* oocysts to different scientific targets.In present study different methods for isolation *Cryptosporidium parvum* oocysts were examined and defined a suitable method for preparation and isolation *Cryptosporidium parvum* oocysts from infected feces. Based of this method *Cryptosporidium parvum* infected watery feces were gathered were added with same size of Potassium Dichromate and stored in 4̊ C. Feces specimens are washed and filtrated respectively with 52, 100, 150 screen. Separated and filtered solution was centrifuged with 2500 rpm for 5 minute. This work was done two time for good washing and deleting Potassium Dichromate. Sediment of centrifugation was added with 20 ml distilled water and 20 ml of Diethyl Ether and then they were mixed and again were centrifuged with 2500 rpm for 5 minute and this work was done twice. Last prepared sediment was washed with distilled water and then was added with saturated water with sugar and then was centrifuged with 2500 rpm for 5 minute. Based of this

rout *Cryptosporidium parvum* oocysts were floated and were gathered with pipet and stored in distilled water with o.5% Sodium Hypochlorite.

Keywords: *Cryptosporidium parvum,* Oocyst, Infected Calves Feces

INTRODUCTION

Cryptosporidium parvum, is a protozoa and obligate intracellular parasite. It has been given additional species names when isolated from different hosts **[1, 3, 4, 6]**. It is currently thought that the form infecting humans is the same species that causes disease in young calves. *Cryptosporidium parvum*, a common opportunistic protozoan, causes severe, protracted, and potentially life threatening diarrhea in immunocompromised patients **[6, 9]**. In immunocompetent individuals infection by *C. parvum* leads to selflimiting diarrhea **[9]**. Recently, this protozoan has caused several waterborne outbreaks of diarrhea **[9]**. *Cryptosporidium parvum* has a wide host range, and infected animals can be sources of contamination for food and water supplies **[9]**. There in many different methods to isolation of *Cryptosporidium parvum* oocyst and it is important to use rapid technique for producing highly purified Cryptosporidium *parvum oocysts* **[2, 5]**. In present study different methods were examined and evaluated and then defined a suitable method for preparation and isolation *Cryptosporidium parvum* oocysts from infected feces.

MATERIAL AND METHODS

For preparation and isolation of *Cryptosporidium parvum* oocysts, infected calves were recognized and there feces were used to *Cryptosporidium parvum* oocysts isolation. Different proposed methods were used to Cryptosporidium parvum oocysts isolation but Lorenzo et.al method had the best results **[8]**.

Based of this method *Cryptosporidium parvum* infected watery feces were gathered were added with same size of Potassium Dichromate and stored in 4ºC. Feces specimens are washed and filtrated respectively with 52, 100, 150 screen.Separated and filtered soloution was centrifugated with 2500 rpm for 5 minute. This work was done two time for good washing and deleting Potassium Dichromate.Sediment of centrifugation was added with 20 ml distilled water and 20 ml of Diethyl Ether and then they were mixed and again were centrifugated with 2500 rpm for 5 minute and this work was done twice. Last prepared sediment was washed with distilled water and then was added with saturated water with sugar and then was centrifuged with 2500 rpm for 5 minute. Based of this

rout *Cryptosporidium parvum* oocysts were floated and were gathered with pipet and stored in distilled water with o.5% Sodium Hypochlorite.

RESULT AND DISCUSSION

Cryptosporidium parvum, a common opportunistic protozoan, causes severe, protracted, and potentially life threatening diarrhea in immunocompromised patients **[1, 3, 4, 6]**. In immune-competent individuals infection by *Cryptosporidium parvum*leads to selflimiting diarrhea [**9]**. Recently, this protozoan has caused several waterborne outbreaks of diarrhea **[9]**. *Cryptosporidium parvum*has a wide host range, and infected animals can be sources of contamination for food and water supplies **[4, 6, 9**]. There in many different methods to isolation of *Cryptosporidium parvum* oocyst and it is important to use rapid technique for producing highly purified *Cryptosporidium parvum oocysts* **[2, 5, 7, 10, 12]**. In present study different methods were examined and evaluated and then defined a suitable method for preparation and isolation *Cryptosporidium parvum* oocysts from infected feces.

Suresh and Jerold developed and evaluated three methods for isolating *Cryptosporidium parvum*oocysts from the feces of infected rats **[11]**. In these procedures, oocysts are first isolated from a discontinuous sucrose gradient, then purified further by being passed through glass beads or Percoll or by dialysis.

Suresh and Jerold results defined that Percoll gradient purification yields a pure fraction of oocysts and relatively fewer oocysts are collected by this method and large volumes cannot be efficiently processed by this method **[11]**.

Based of Suresh and Jerold results the ability to effectively recover oocysts from rat feces suggests that the laboratory rat may be a convenient substitute for ruminants in the propagation and maintenance of *C. parvum* oocysts for in vitro and in vivo use **[11]**.

In present study different methods were examined and evaluated and then defined that Lorenzo et.al method is a suitable method for preparation and isolation *Cryptosporidium parvum* oocysts from infected feces. Based of this method *Cryptosporidium parvum* infected watery feces were gathered were added with same size of Potassium Dichromate and stored in 4ºC. Feces specimens are washed and filtrated respectively with 52, 100, 150 screen. Separated and filtered solution was centrifuged with 2500 rpm for 5 minute. This work was done two times for good washing and deleting Potassium Dichromate. Sediment of centrifugation was added with 20 ml distilled water and 20 ml of Diethyl Ether and then they were mixed and again were

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ACKNOWLEDGMENTS

This study was supported financially by Tabriz Branch, Islamic Azad University, Tabriz, Iran

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