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Isolation of *Cryptosporidium parvum* Oocyst From Infected Feces

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Abstract

Objective: 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. Since its diagnosis in the 1970s, *Cryptosporidium* has been attributed an increasingly important role in the neonatal diarrhea syndrome of humans and newborn ruminants. It is very important to diagnose *C. parvum* disease and isolate *C. parvum* oocysts for different scientific targets.

Materials and Methods: In the present study different methods for isolation of *C. parvum* oocysts were examined and a suitable method for preparation and isolation of *C. parvum* oocysts from infected feces was defined. Based on this method *C. parvum* infected watery feces were gathered; same size of potassium dichromate added and stored at 4°C. Fecal specimens were washed and filtrated, respectively with 52, 100, 150 screens. Separated and filtered solution was centrifuged at 2500 rpm for 5 minutes.

Results: In the present study different methods were examined and evaluated. The defined method of Lorenzo et al, was distinguished as the most suitable method for preparation and isolation of *C. parvum* oocysts from infected feces.

Conclusion: This work describes the successful development of method for the recovery and isolation of *Cryptosporidium* oocysts in feces.

Keywords: *Cryptosporidium*, Feces, Oocyst

Introduction

Cryptosporidium is a genus of Apicomplexa phylum parasites that can cause a respiratory and gastrointestinal illness (cryptosporidiosis) that primarily involves watery diarrhea (intestinal cryptosporidiosis) with or without a persistent cough (respiratory cryptosporidiosis) in both immunocompetent and immunodeficient humans (1-3). A number of *Cryptosporidium* species infect mammals (1,4). Recently, this protozoan has caused several waterborne outbreaks of diarrhea (5-7). *C. parvum* has a wide host range and infected animals can be sources of contamination for food and water supplies (7,8). There are many different methods for isolation of *C. parvum* oocyst and it is important to use rapid technique for producing highly purified *C. parvum* oocysts (9,10). In the present study different methods were examined and evaluated and then a suitable method for preparation and isolation of *C. parvum* oocysts from infected feces was defined.

Material and Methods

For preparation and isolation of *C. parvum* oocysts, infected feces were recognized and used to isolate *C. parvum* oocysts. Different proposed methods were used for *C. parvum* oocyst isolation but Lorenzo et al method had the best results (11-13). Based on this method *C. parvum* infected watery feces were gathered. Then same size of potassium dichromate was added and stored at 4°C. Feces specimens were washed and filtrated respectively with 52, 100 and 150 screens. Separated and filtered solution was centrifuged at 2500 rpm for 5 minutes. This work was done twice for good washing and deleting potassium dichromate. To the sediment 20 mL distilled water and 20 mL of diethyl ether was added and then mixed and again centrifuged at 2500 rpm for 5 minutes and this work was done twice. Lastly, prepared sediment was washed with distilled water and saturated water with sugar was added and then was centrifuged at 2500 rpm for 5 minutes. In this method *C. parvum* oocysts were floated and gathered with pipette and stored in distilled water with 0.5% sodium hypochlorite.

Results

In the present study different methods were examined and evaluated. The defined method of Lorenzo et al, was distinguished as the most suitable method for preparation and isolation of *C. parvum* oocysts from infected feces. Modifications to existing oocyst recovery protocols were explored to increase sample size for analysis or to improve overall recoveries.

Discussion

Cryptosporidium parvum, a common opportunistic protozoan, causes severe, protracted and potentially life threatening diarrhea in immunocompromised patients

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(1- 3). Cryptosporidiosis is an important cause for chronic diarrhea and death in HIV/AIDS patients. Among common *Cryptosporidium* species in humans, *C. parvum* is responsible for most zoonotic infections in industrialized nations. Nevertheless, the clinical significance of *C. parvum* and role of zoonotic transmission in cryptosporidiosis epidemiology in developing countries remain unclear (14). In immunocompetent individuals infection by *C. parvum* leads to self limiting diarrhea (1,4,15). Recently, this protozoan has caused several waterborne outbreaks of diarrhea (5,6,7,16). *C. parvum* has a wide host range and infected animals can be sources of contamination for food and water supplies (7,8,17-19). Molecular tools have been developed to detect and differentiate *Cryptosporidium* at the species/genotype and subtype levels. These tools have been increasingly used in characterizing the transmission of *Cryptosporidium* spp. in humans and animals (20,21). One of the important materials in molecular research of cryptosporidium is the suitable isolation of *Cryptosporidium* oocyst for DNA extraction in different studies. The aim of this study was to determine the best route of *Cryptosporidium* oocysts isolation.

In the present study different methods were examined and evaluated and then the most suitable method for preparation and isolation *C. parvum* oocysts from infected feces was defined.

Suresh and Jerold developed and evaluated three methods for isolating *C. parvum* oocysts from the feces of infected rats. In these procedures, oocysts were first isolated from a discontinuous sucrose gradient, and then purified further by being passed through glass beads or Percoll or by dialysis. Their 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 (22).

Based on Suresh and Jerold results, 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. In our research different methods were examined and evaluated and then Lorenzo et al method was defined as the best method for preparation and isolation *C. parvum* oocysts from infected feces. (22)

Conclusion

In conclusion, the method reported in this paper demonstrates that acceptable oocyst recoveries can be achieved.

Ethical Issues

None to be declared.

Conflict of Interests

There is no conflict of interests.

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