Evaluation of immunomagnetic separation and the sucrose flotation methods coupled with immunofluorescence or PCR for detection of Cryptosporidium and Giardia (oo)cysts in water samples

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Abstract

Introduction: Detection of Cryptosporidium and Giardia parasites in water samples is usually performed by US Environmental Protection Agency 1623 method. Nevertheless, the USEPA1623 method still need improvement, to prevent and control the water borne parasitic disease. Therefore, we undertook the present study.
Materials and methods: Totally 48 surface water samples were collected. Four samples from 12 sites and samples of each site were evaluated by IMS-IFA, SF-IFA, IMS-PCR and SF-PCR. These typically involve sample filtration by membrane filter, separation by Sucrose flotation or immunomagnetic separation (IMS) methods and detection of (oo)cysts by PCR or immunofluorescent staining.
Results: Same samples were evaluated by the different techniques at the same time showing a rate of Cryptosporidium oocysts detection of 8 (66.6%) by IMS-IFA, 7 (58.3%) by SF-IFA, 10 (83%) by IMS-PCR and 0% by SF-PCR.Giardia cysts detected in, 5 (41.7%) by IMS-IFA, 3(25%) by SF-IFA, 7 (58.4%) by IMS-PCR and 2 (17%) by SF-PCR.
Conclusion: Data analysis showed a higher sensitivity of IMS-PCR for the detection of Giardia and Cryptosporidium (oo)cysts respectively in comparison with others techniques used in this study. IMS prior to DNA extraction showed a higher sensitivity to eliminate or reduce PCR inhibitors that presence in water samples.

Keywords: Giardia, Cryptosporidium, IMS, Flotation methods, PCR, IFA, Water

Introduction

Immunological and molecular methods used to assess the prevalence and sources of waterborne protozoa. The recovery yield of Cryptosporidium and Giardia (oo) cysts from water depends on the purification and identification methods used. Recently, immunomagnetic separation (IMS) purification method recommended by US Environmental Protection Agency (US-EPA) is widely used, but this method still has some limitations and need improvement (1-4). Although IMS are standard procedures for purification of Cryptosporidium oocysts and Giardia cysts in water samples but it has some limits. Apart from IMS for purifying oocysts from water samples, some other methods, such as sucrose floatation and Percoll-sucrose centrifugation were also applied (5-7).
Plus purification methods, the recovery yield of Cryptosporidium and Giardia (oo) cysts from water depends on the detection methods too.

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Nested PCR appears to be more sensitive than IFA for detecting of oocysts in water concentrates (4, 8, 9). Compared to method 1622/1623, the PCR methods have the ability to differentiate Cryptosporidium species that are infective to humans from those that are not infective to humans (10). But, PCR inhibitors present in water samples are major problem in the molecular detection of microorganisms in environmental samples (11-15).

The presence of Giardia and Cryptosporidium in different water sources in Iran makes it imperative to develop Standard methods to maximise public health surveillance of waterborne protozoa like Giardia and Cryptosporidium .

To authors Knowledge, no comparison of IMS or SF coupled with IFA or PCR for recovering the Cryptosporidium and Giardia (oo)cysts in same surface water samples was reported .

Therefore, in this paper, we compared the efficiencies of different methods for purification and detection of Cryptosporidium and Giardia (oo)cysts, in field river water samples.

Materials and methods

Totally 48 surface water samples were collected from 12 sites. Four samples from each site that were evaluated by IMS-IFA, SF-IFA, IMS-PCR and SF-PCR. For each sample, five liters of environmental water samples from river water were filtered through a 142 mm diameter membrane filter with a pore size of 1.2 µm. The filter was rinsed two times by 50 ml of 0.1% PBS-Tween 80. Then, the entire sample was transferred into a 50-ml Falcon tube and concentrated by centrifugation in at 3000 g for 10 min. The supernatant was discarded, and the pellet with the (oo)cysts was subjected to different purification and detection methods. These typically involve separation by Sucrose flotation or immunomagnetic separation (IMS) methods and PCR or immunofluorescent staining, for detection of oocysts .

Sucrose floatation & Immunomagnetic separation (IMS): All samples were treated with sucrose flotation (SF) method according previous study (16) and IMS methods. All IMS kits do not perform equally well , since, great recoveries have been obtained previously with the Dynal IMS procedure (17) so, in present study this kit use for imounomagnetic procedure. IMS procedure was performed according to the manufacturer's instructions (Dynabeads G/C combo IMS kit; Dynal A.S., Oslo, Norway), as performed in our previous study (16).

DNA extraction and PCR methods: The DNA was extracted with the QIAamp DNA minikit as recommended Jiang et al. (2005) (15).

A nested–PCR was used to amplify a 825-bp fragment of Cryptosporidium oocyst 18s RNA (18). PCRs reaction were performed as described in our previously published paper (19). A Semi-nested PCR assay, using the primers to amplify a 432-bp fragment of the Giardia glutamate dehydrogenase gene (GDH) (20). The PCR reactions performed as described in our previously paper (16).

IFA methods: A previously published IFA protocol was performed to detect Cryptosporidium and Giardia (oo)cysts (21, 22). Cryptosporidium and Giardia (oo)cysts were identified on the basis of their size, shape, and structure, according to guideline described in method 1623.

Results

Totally 25/42 samples were positive for Cryptosporidium oocysts and 17/25 samples for Giardia cysts. Same samples were evaluated by the different techniques at the same time showing a rate of Cryptosporidium oocysts detection of 8 (66.6%) by IMS-IFA, 7 (58.3%) by SF-IFA, 10 (83%) by IMS-PCR and 0% by SF-PCR.

Giardia cysts detected in, 5 (41.7%) by IMS-IFA, 3(25%) by SF-IFA, 7 (58.4%) by IMS-PCR and 2 (17%) by SF-PCR.

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Table 1. Results of different purification and detection of (oo)cyst in water samples.

	Giardia		Cryptosporidium	
Methods	Negative	Positive	Negative	Positive
IMS-PCR	5/12 (41.6%)	7/12 (58.4%)	2/12 (17%)	10/12 (83%)
SF-PCR	10/12 (83%)	2/12 (17%)	12/12 (100%)	0/12
IMS-IFA	7/12 (58.3%)	5/12 (41.7%)	4/12 (33.4%)	8/12 (66.6%)
SF-IFA	9/12 (75%)	3/12 (25%)	5/12 (41.7%)	7/12 (58.3%)
Total	31/48	17/48	23/48	25/48

Data are shown as ratio or percent.

Discussion

The recovery yield of Cryptosporidium and Giardia (oo)cysts from water depends on the purification and identification methods used. Purification by IMS, and detection by IFAUSEPA method 1623 has been widely used with recovery rates varying from 40.0 to 100% (23-26).

However for some organisms there are no IMS procedures, and IFA detection method is unable in identification of Cryptosporidium or Giardia species.

Therefore some others purification methods, such as sucrose floatation and Percoll-sucrose centrifugation (5-7) and detection methods like PCR (4, 8, 9) were also applied.

In the present study, we found that IMS method enhanced with PCR assay (IMS-PCR) showed slightly higher positive results than IMS-FA, SF-IFA and SF-PCR. Also others studies have shown, that nested PCR is more sensitive than microscopy (4, 8, 9).

As shown in this study, mainly DNA extracted from (oo)cysts purified by IMS from water samples produced the most PCR amplification and SF-PCR gives less positive results than IMS-PCR so PCR inhibitors were more present in oocyst purified by SF method.

Although, our investigations showed that IMS appears to be more sensitive than flotation procedures but that not all IMS procedures yield the same results (27).

However, IMS had some advantages, such as rapidity in processing and less personnel skill required than sucrose floatation technique.

Although our data show, sucrose floatation technique gives less positive results than IMS, but SF was cost-effective and easier to perform as the IMS technique so sucrose floatation is an alternative way when IMS method is not suitable. As in a study, Koompapong et al 2009 suggest using SF-IFA technique for detecting oocysts in water samples especially in water with high turbidity, low or high pH, and high iron particle in water samples. (28). In present study, SF technique enhanced with FA give more positive results than IMS-IFA and SF-PCR methods that may due PCR inhibitor because SF method couldn't eliminate or considerably reduce substances that might be inhibitory to DNA amplification by PCR.

As mention above, Plus purification methods, the recovery yield of Cryptosporidium and Giardia (oo)cysts from water depends on the identification methods too.

In IFA detection method, some object cross react with commercial antibodies resulting false positives (29). IFA cannot differentiate Cryptosporidium species or strains from humans and animals (10). Although PCR has some advantages over IFA but PCR is susceptible to many inhibitors present in samples (11-15). Also empty oocysts cannot be detected by PCR methods, So IFA adds significant value to PCR-negative results (30, 31).

As mention above, must keep in mind, each method has each own advantages and disadvantages, so dependent to aim and the design of the study, a combination of techniques should be used to make sure that water samples is or is not contaminated and infectious. High

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efficiency, reasonable cost and Aim of study are important items in the selection of the method. However, each of these methods has some limits thus, the development of other purification and detection technique is necessary for the assessments of these waterborne pathogens in environmental samples.

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