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Original Article

Microbiology Section

Comparison of ELISA and Microscopy for detection of *Cryptosporidium* in stool

SEEMA MITTAL[1], MADHU SHARMA[2], UMA CHAUDHARY[3], APARNA YADAV[4]

ABSTRACT

Background: Cryptosporidiosis, a diarrheal disease caused by the protozoan parasite *Cryptosporidium spp.* has become recognized as one of the most common causes of water borne diseases in humans.

Aims and Objectives: To compare the sensitivity of ELISA and Microscopy for detection of *Cryptosporidium* in stool samples.

Materials and Methods: The study was conducted in the Department of Microbiology of PT. B.D. Sharma PGIMS Rohtak, between January 2011 to june 2011 on 50 stool samples, which were processed for detection of cryptosporidial antigen by ELISA and detection of cysts by microscopy (Modified Ziehl and Nelsen staining).

Study and Design: This was a prospective study conducted in the Department of Microbiology in PT. BD Sharma, PGIMS, Rohtak, India.

Result: Out of total, 50 stool samples eighteen (36%) samples were found positive for *Cryptosporidium* cysts by microscopy in comparison to 3(6%) stool samples which were found positive for cryptosporidial antigen by ELISA. Samples found positive with ELISA were also positive with microscopy. Sensitivity, specificity, positive predictive value and negative predictive value for ELISA was 16.7%, 100%, 100% and 68% respectively.

Conclusion: The study concludes that stool microscopic Modified acid fast staining is more sensitive method than ELISA for detection of *Cryptosporidium* in stool samples but the specificity of ELISA was more than microscopy.

Keywords: *Cryptosporidium*, ELISA, Microscopy

INTRODUCTION

With the increase in interest in gastrointestinal diseases potentially caused by waterborne outbreak, *Cryptosporidium (coccidian protozoan)* parasite has gained attention as a emerging pathogen in the last few decades.

Till now, more than 26 species of *Cryptosporidium*, are recognized based on host specificity, morphology and molecular biology studies [1].

It causes self limiting diarrheal disease in immunocompetent and severe diarrhea in immunocompromised persons. Presently, the increasing population of immunocompromised persons and the various outbreaks of cryptosporidiosis have placed an even greater emphasis on this pathogen.

In diarrhea, it is important to evaluate the stool samples for *Cryptosporidium* cysts for the presence of cryptosporidiosis (especially in case of immunocompromised person).

Therefore this study was conducted to compare the efficacy of microscopy and ELISA for the detection of *Cryptosporidium* cysts in stool in developing nations.

MATERIALS AND METHODS

The present study was conducted on 50 stool samples from the patients with acute diarrhea of both sexes including all age groups over a period of six months from January to July 2011.

These stool samples were sent in 10% formalin solution for the ova cyst examination, which were concentrated in Sheather's sugar solution fixed on glass slide with polyvinyl alcohol-mercuric chloride and modified Ziehl Nelsen staining was done [2]. Same samples were processed further by ELISA as per manufacturer's guidelines. (*Cryptosporidium* DFO- DiBect florescent LNo. 250050AL150)

RESULTS

Cryptosporidium species (spp.) were detected in 18 out of 50 stool samples by microscopy, while three samples were found positive by ELISA. Those samples which were positive by ELISA were also found positive with microscopy. The other parasitological profile of stool samples showed the presence of *Giardia* spp., *Entamoeba histolytica*, etc [Table/Fig-1]. The sensitivity and specificity of ELISA in detecting Cryptosopridial Ag was found 16.7% and 100% respectively in comparison of modified staining, presuming microscopy as gold standard for detecting the presence of *Cryptosporidium* cysts [Table/Fig-2].

DISCUSSION

In recent period, *Cryptosporidium* spp has been identified as a major cause of self limiting acute enteritis having symptoms of abdominal pain, mild fever and diarrhea of variable severity for about 2-26 d [3]. It has low infecting dose of ≤10-100 oocysts which are ubiquitous in nature [4].

It has emerged as the known cause of water–associated outbreaks of gastroenteritis, even in disinfected water resources. This is because the oocyst of, *Cryptosporidium* can resist chlorination, and can survive for a prolonged period in the environment.

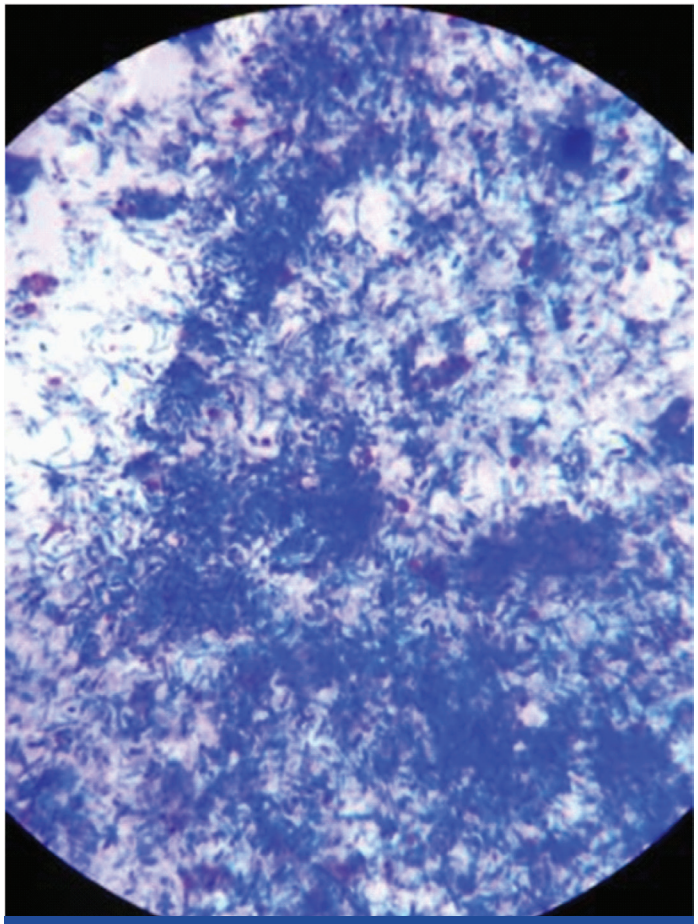
It is a zoonosis and is transmitted via the fecal oral route, this has been implicated as one of the more important opportunistic infections in patients with AIDS (2-5%). Symptomatic intestinal and respiratory cryptosporidiosis has been seen in both immunocompetent and immunocompromised patients of all ages [5]. After the establishment of primary infection, the immune status of host play a important role

Parasite	Total no. of cases	Percentage(%)
E.histolytica	30	60
Giardia spp.	17	34
Hemnolepsis nana (H.nana)	1	2
Ascaris lumbricoides	1	2
H.nana+ E.histolytica	1	2

[Table/Fig-1]: Prevalence of parasite in stool samples by microscopy (n=50).

	Sensitivity	Specificity	NPV	PPV
ELISA	16.7%	100%	68%	100%
Microscopy	100%	100%	100%	100%

[Table/Fig-2]: Sensitivity and specificity of ELISA and Microscopy.



[Table/Fig-2]: Modified acid-fast stain of stool shows red oocysts of *Cryptosporidium* against the blue background of fecal debris

in determining the severity of infection. In healthy individual it causes a self limiting diarrhea, in contrast in immunocompromised it can lead to very severe life threatening cholera like illness.

Now, various methods are available for the detection of Cryptosporidiosis in different clinical specimen, but the method which can be used for routine screening purpose in stool samples from the cases of gastroenteritis should be acceptable in terms of sensitivity and specificity and provide clinically relevant, cost-effective, rapid results, particularly in a potential waterborne diseases prone regions.

In the present study, in modified acid fast staining oocyst appear as bright red in colour [Table/Fig-3] and it was found to be more sensitive than ELISA (16.7%) in detection of *Cryptosporidial* oocyst. However the speciation could not be done by both microscopy as well as ELISA. Both the methods had same specificity and positive predictive value (100%). Similar results has shown by other studies as well [6,7]. But the identification of *Cryptosporidium* species should be done with Polymerase chain reaction, which is a gold standard for the diagnosis.

CONCLUSION

For diagnosing, merely the presence of oocyst in stool samples microcopy was preferable than ELISA in terms of sensitivity, negative predictive value, cost effectiveness and facilities availability.

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PARTICULARS OF CONTRIBUTORS:
1. Senior Resident, Department of Microbiology, Pt. B.D. Sharma PGIMS, Rohtak, Haryana, India.
2. Professor, Department of Microbiology, Pt. B.D. Sharma PGIMS, Rohtak, Haryana, India.
3. Senior Professor and Head, Department of Microbiology, Pt. B.D. Sharma PGIMS, Rohtak, Haryana, India.
4. Professor, Department of Microbiology, Pt. B.D. Sharma PGIMS, Rohtak, Haryana, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Seema Mittal,
718-1/35, Janta Colony, Near Devi Vihar, Rohtak-124001, India.
Phone : 8901386672, E-mail : dr.sima2010@rediffmail.com

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