

## ORIGINAL ARTICLE

## MODIFIED ACID FAST STAINING: A BETTER DIAGNOSTIC TOOL IN CHRONIC DIARRHOEA DUE TO CRYPTOSPORIDIOSIS

Shahina Mumtaz, Jawad Ahmed\*, Liaqat Ali, Hamid Hussain\*\*

Department of Pathology, Khyber Medical College, \*Institute of Basic Medical Sciences, Khyber Medical University,

\*\*Department of Community Medicine, Khyber Medical College, Peshawar

**Background:** *Cryptosporidium parvum* is an emerging pathogen responsible for chronic diarrhoea in children and immuno-compromised individuals, especially AIDS patients. Currently, there is no effective therapeutic strategy for treating cryptosporidiosis, therefore control and supportive treatment of cryptosporidiosis depends upon rapid and accurate diagnosis of this infection.

**Methodology:** A descriptive study was conducted in the Pathology Department of Khyber Medical College and Pathology Laboratory of Khyber Teaching Hospital over a period of one year March 2007–April 2008. A total of 200 stool samples were tested for the presence of *C. parvum* oocysts from children <5 years age suffering from diarrhoea for >5 days. Total and differential leukocyte count was determined to assess immune status of the patients. Modified Ziehl-Neelsen (Z-N) staining, a rapid, sensitive and easy test, was used successfully for the detection of *C. parvum* oocysts in stool specimen.

**Results:** *Cryptosporidium* oocysts were found in 18 (9.0%) samples. Out of 18 positive cases, 13 (72.2%) children had lymphopenia hence their immune status was impaired. Infection was common in children between 1–24 months of age. Mean duration of diarrhoea was 11 months. Most of *C. parvum* infected children were consumers of well water (77.8%).

**Conclusion:** Cryptosporidiosis, although a self-limiting disease, rarely investigated routinely, can become chronic and life threatening in immuno-compromised individuals. Majority of affected patients are immuno-compromised. Modified Z-N is a sensitive and rapid method which can explore the gravity of this infection even further if used routinely and may control morbidity and mortality associated with this infection.

**Keywords:** Chronic diarrhoea, modified Ziehl-Neelsen staining, immuno-compromised

## INTRODUCTION

*C. parvum* is one of the most common waterborne intracellular human parasitic protozoa that has emerged as an important cause of diarrhoea in human and animals.<sup>1,2</sup> Acute and persistent diarrhoea are not two separate diseases but form a continuum. Acute diarrhoea is defined as increased frequency of defecation ( $\geq 3$  times/day or at least 200 gms of stool/day) lasting less than 14 days, may be accompanied by abdominal cramps, nausea and vomiting.<sup>3</sup> Persistent diarrhoea is defined by WHO as an episode which starts acutely and continues at least for 14 days. Persistent diarrhoea most often occurs in children <2 years of age. Death from persistent diarrhoea is frequently associated with several host factors such as pre-existing under nutrition, micronutrient deficiency, inappropriate therapy of the most recent acute diarrhoeal episode and immunodeficiency. The common outcome of diarrhoea appears to be mucosal injury and ineffective repair.<sup>4</sup>

In the United States, Giardiasis and Cryptosporidiosis are frequently associated with persistent diarrhoea. Giardiasis responds well to metronidazole but *Cryptosporidium* infection is notoriously difficult to manage, particularly in patient with low CD4 cell count. In such patients the most effective strategy is focused on to improve immune response.<sup>5</sup> HIV infected patients with CD4 count

<50/mm<sup>3</sup>, when develop Cryptosporidiosis are at high risk for biliary symptoms and death usually ensues within a year of infection.<sup>6</sup>

*C. parvum* exist as small oocysts in the environment and is responsible for spread of infection to new host(s). Oocysts are highly resistant to disinfectants such as chlorine and other commonly used domestic disinfectants. Oocysts are of tiny size (4–5  $\mu$ m) and may breach filters. However, filtration system, especially one that is not well maintained and well operated may not provide absolute protection. Eleven outbreaks of Cryptosporidiosis have been reported in the United States during 1991–2000 as a result of water contamination.<sup>7</sup> Oocysts in stool specimen (fresh or in stored media) remain infective for long period of time, thus stool samples should be preserved in 10% buffered formalin or sodium acetate acetic acid formalin (SAF) to render oocysts non-viable. According to Gaash<sup>8</sup> the contact time with formalin necessary to kill oocysts is approximately 18 to 24 hrs.

Pakistan is a developing country. Diarrhoea and protein energy malnutrition (PEM) are commonly encountered problems in this country. AIDS and PEM severely impair immune system.<sup>9</sup> According to a study conducted by Banwat *et al*<sup>10</sup> there is a high prevalence rate of cryptosporidium in young children suffering from PEM.

The aim of this study was to investigate immune status of patients suffering from diarrhoea and highlight the importance of quick and easy method of detection of this parasite in routine stool examination in patients suffering from chronic diarrhoea. Such patients are not routinely investigated for oocysts of *C. parvum* as specific diagnostic methods are not available for stool examination. This study will help in the health education and awareness of clinicians about importance of cryptosporidiosis testing. It will not only help to explore the real situation of this infection but also help in the control of spread of this disease. This can reduce the length of illness, risk of dehydration and can also reduce mortality.

## MATERIAL AND METHODS

A prospective, descriptive study conducted from March 2007 to April, 2008 in the Pathology Department, Khyber Medical College (KMC) and Khyber Teaching Hospital (KTH) Peshawar. Faecal carriage of *C. parvum* oocysts was investigated in 200 stool specimens collected from children (<5 year of age) who were suffering from diarrhoea >5 days. All the relevant nurses and technical staff were trained for collection and processing of (stool) specimen.

There are many techniques used for detection of *C. parvum* oocysts in the stool. However, modified Z-N staining is effective, cheap, non-invasive and easy to perform lab test. This modified Z-N staining has proved useful in laboratory diagnosis of cryptosporidiosis.<sup>1,8</sup>

Screening of the stool specimen was done by wet mount preparation along with 1% Lugol's Iodine. By using this method, *Cryptosporidium* oocysts can be easily differentiated from yeast cells. *C. parvum* oocysts do not accept iodine stain and appear as transparent discs while yeast cells accept iodine stain and appear deep yellow.<sup>11</sup> Presumptive oocysts were confirmed by using modified Z-N technique. Prior to Z-N staining, stool specimens were concentrated by formalin ethyl acetate method.<sup>12</sup>

Faecal smears were made from concentrated specimens along with positive control slides. Slides were air dried, fixed in methanol for 3 minutes. Slides were stained with strong carbol fuchsin for 15–20 minutes, thoroughly rinsed in tap water and then decolorised in acid alcohol for 15–20 seconds. Finally these slides were rinsed in tap water and then counter stained with 0.4% methylene blue (or Malachite green) for 30–60 seconds. Slides were rinsed thoroughly and air dried. The oocysts appeared pink against blue background of debris.<sup>11</sup> According to Morgan *et al*<sup>13</sup> microscopy is a reliable diagnostic method than immunologically based methods for detection of oocysts.

## RESULTS

Out of 200 faecal specimens examined, 18 samples (9.0%) were positive for oocysts of *C. parvum*. Majority of children with *C. parvum* infection were less than 24 months of age. Mean age of the patients with *C. parvum* infection was 20 months with a SD=15.29 months (minimum 1 month and maximum 60 months). Mean duration of diarrhoea was 11 days in the positive cases (Table-1). According to the onset of illness, 16 children (88.9%) presented with persistent diarrhoea and the remaining 2 children (11.1%) had acute diarrhoea. The source of drinking water in majority of cases (14, 77.8%) was well water while only 4 (22.2%) children were using municipal water supply for drinking. Out of 18 positive cases of cryptosporidiosis, 13 (72.2%) cases had lymphopenia, hence were immuno-compromised (Table-2).

**Table-1: Carriage of *C. parvum* in children of different age groups**

Age	Cases (%)	Controls (%)	p
1–12 Months	5 (27.78)	20 (10.9)	0.004
13–24 Months	11 (61.1)	72 (39.7)	0.012
25–60 Months	2 (11.1)	90 (49.4)	-

**Table-2: Immune status of patients investigated for cryptosporidiosis (n=200)**

	Oocyst positive	Oocyst negative	Total
Immuno-compromised	13 (72.22%)	-	
Immuno-competent	5 (27.78%)	-	
Total	18	182	200

## DISCUSSION

Diarrhoeal diseases affecting children between 1–5 years of age have long been considered as major causes of morbidity and mortality in developing countries associated with poverty and malnutrition.<sup>1,14,15</sup> A study conducted in Brazil has identified that *C. parvum* is highly endemic with 95% of children and adults were sero-positive for cryptosporidium antigen. Immune system is considered as a major factor in determining the duration and severity of infection.

The incidence of cryptosporidiosis in various countries of the world has been reported as under:

- 8.9% in Bangladesh<sup>16</sup>
- 8.8% in Iraq<sup>17,18</sup>
- 7.3% in India and 13.5% in Bethlehem, Palestine<sup>18</sup>
- 5.3% in Nigeria<sup>19</sup>

*C. parvum* is an important cause of chronic diarrhoeal disease in patients with AIDS.<sup>20</sup> It can cause severe symptoms in person who are immuno-compromised.<sup>21</sup> Despite its wide distribution in the environment and obvious relevance to public health, its detection in the stool is not routinely done in cases of chronic diarrhoea.

In this study, we found that 18 (9%) children of <5 years of age had *C. parvum* oocysts in their

faeces. The incidence of *C. parvum* infection in our study is consistent with study from Rawalpindi, Pakistan by Iqbal *et al*<sup>12</sup> who has reported higher rate (10.3%) in children with diarrhoea than in children without diarrhoea (3.3%).

In our study, *C. parvum* oocysts were frequently detected in stool samples collected from patients between 13–24 months of age (SD=15.29). This increased infection rate may be due to immunologic immaturity compounded by lymphopenia recorded in the majority of patients who were positive for cryptosporidiosis (72.2%). Similar observation was made by Nagamani *et al*<sup>22</sup> from Indonesia and Ghana.

## CONCLUSION

Majority of patients suffering from cryptosporidiosis were immuno-compromised. Modified acid fast staining is an economical and easily applicable method for the detection of *C. parvum* oocysts in faecal samples. This test should be made available routinely as a screening tool of chronic diarrhoeal stool specimens that come to the laboratory.

Reasons for not requesting a *Cryptosporidium* test, include the perception that cryptosporidiosis is rare and self limiting disease. It is not considered as risk to others and that no specific treatment is available for it. It may be rare, because it is rarely investigated. It is usually self-limiting but can be chronic and life threatening in immuno-compromised patients. The diagnosis of *C. parvum* is not difficult and can be achieved by simple conventional staining methods like modified Z-N staining. However, depending upon the facilities available, Z-N staining is more sensitive and easy to use for the detection of *C. parvum* oocysts. Routine testing of stool will help to explore the gravity of the situation even further and help to reduce morbidity and mortality associated with *C. parvum* infection.

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## Address for Correspondence:

**Dr. Jawad Ahmed**, Professor of Microbiology & Director, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar. **Phone/Fax:** +92-91-5862519  
**Email:** j62ahmed@yahoo.com