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A Comparative Evaluation of Stool Microscopy and Coproantigen-ELISA in the Diagnosis of Cryptospridiosis

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Abstract

We evaluated the diagnostic performance of microscopically examination of staining techniques (Modified Ziehl-Neelsen and Auramine-Rhodamine) and Coproantigen Enzyme Linked Immunosorbent Assay (Copro-ELISA) for cryptosporidiosis diagnosis. Copro-ELISA appeared to be most sensitive than staining techniques. The commercial Copro-ELISA and Auramine-Rhodamine proved to be valuable diagnostic tools for *Cryptosporidium* infection.

Key Words: Cryptosporidiosis, Auramine-Rhodamine stain, Copro-ELISA

Introduction

Cryptosporidium parvum, are important agents of parasite-induced diarrheal disease, which is a serious health problem in tropical regions [1], Cryptosporidiosis is an important worldly distributed infection of livestock and humans. Epidemiological studies have demonstrated that cryptosporidiosis is more prevalent in developing countries (5 to 7%) than in developed countries (1 to 3%) [2].

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It is well known that detection of *Cryptosporidium* Oocysts in fecal samples is made by Modified acid fast staining technique, which requires the presence of large number of Oocyst, costly, time consuming, often difficult being depend upon trained and expert knowledge of morphologic differentiation of *Cryptosporidium spp* [3, 4].
In view of increasing number of malignancies and AIDS in humans in different part of the world, studies on cryptosporidiosis diagnosis might assume further significance, especially among random subjects, who are apparently asymptomatic carriers of this disease, and are important reservoir for spread of infection in

the region. This evidence strongly supports the needs to detect *Cryptosporidium* infection and treat the asymptomatic infections.

Stool antigen immunoassay has been successfully applied for the diagnosis of cryptosporidiosis among patients in most clinical laboratories [5, 7]. However, the assay has not been used for the screening of cryptosporidiosis in large scale epidemiological diagnosis.

The present study was undertaken to investigate the diagnostic sensitivity of stool microscopy using Modified Ziehl-Neelsen and Auramine-Rhodamine stains and a commercially available immunoenzymatic assay for cryptosporidiosis diagnosis.

Material and Methods

Stool samples:

The study was carried out from September 2009 to march 2010. A single stool specimen was collected from 1768 random subjects from four centers (Mansoura, Talkha, Belqas and Aga) of Dakahlia province, Egypt.

Stool Microscopy:

To demonstrate *Cryptosporidium* Oocysts, all stool specimens were processed for formalin-ether concentration method [8]. Two thin smears from concentrated pellet from each sample were prepared on two slides, air-dried and stained separately by Modified Ziehl-Neelsen Technique [9] and Auramine-Rhodamine stains [10, 11]. The whole smears were examined under oil emersion for detection *Cryptosporidium* Oocysts. The later stained smears were examined in fluorescence microscope for the presence of this organism.

Presence of other intestinal parasites was established by direct smear microscopy after formalin-ether concentration method of stool in normal saline and iodine preparations.

Faecal elute:

Faecal supernatant for each sample was prepared in a preparation of 1:1 (1 gm of stool thoroughly mixed with same volume of distilled water). The mixture was centrifuged at 1500 rpm for 5 min. The supernatant was recovered, transferred to fresh tube with the addition of 0.2% mertholate that act as preservative for coproantigens, and stored at -50 C° until used for detection of *Cryptosporidium* specific coproantigens.

Enzyme immunoassay:

All faecal supernatant were processed according to the instructions guide by stool antigen Enzyme Linked Immunosorbent Assay (*Cryptosporidium*- C ELISA Cellabs, Australia) to detect *Cryptosporidium* specific coproantigens.

Statistical analysis:

Chi-square that was used to compare detection efficiency of stool microscopy and immunoassay for cryptosporidiosis diagnosis and p-value of less than 0.05 was considered significant.

Results

The comparative sensitivity of stool microscopy using Modified Ziehl-Neelsen and Auramine-Rhodamine Technique and Coproantigen Enzyme Linked Immunosorbent Assay (Copro-ELISA) is shown in Table 1. Copro-ELISA appeared to be more sensitive than staining techniques. A significant different was found in the sensitivity of Copro-ELIZA verses Modified Ziehl-Neelsen (p=0.006) and Auramine-Rhodamine (p=0.001) Technique. 20.18, 10.09 and 9.17% stool samples showed co-existing of *Cryptosporidium* with one, two and more than two intestinal parasites.

Table1: Comparison of sensitivity of staining techniques and enzyme immunoassay for diagnosis of cryptosporidiosis.

Method	Number of cases positive (%)
Ziehl-Neelsen	97 (5.49%)
Auramine-Rhodamine	109 (6.17%)
Copro-ELISA	178 (10.07%)
P=0.389, Chi square=0.742 (Ziehl-Neelsen verses Auramine-Rhodamine)	
P=0.006, Chi square=9.99 (Ziehl-Neelsen verses Copro-ELISA)	
P=0.001, Chi square=18.05 (Auramine-Rhodamine verses Copro-ELISA)	

Discussion

In the present study, 1768 random stool samples were screened for cryptosporidiosis diagnosis, using two staining techniques (Modified Ziehl-Neelsen and Auramine-Rhodamine) and Copro-ELISA. The overall prevalence of cryptosporidiosis was 10.07% by Copro-ELISA. Modified Ziehl-Neelsen and Auramine-Rhodamine staining methods detected 5.49 and 6.17% Cryptosporidium Spp. Oocysts respectively. The results of this study suggest significant prevalence of cryptosporidiosis among random immmunocompetent population of Dakahlia province, Egypt. Similar results were reported by others in developing African countries [7, 12].

Arrowood and Sterling [11] and Tortora *et al* [13] reported that Auramine-Rhodamine staining technique is a dependable and efficient of examining faecal smears for the presence of *Cryptosporidium* Oocysts in a high risk population. Moreover, recently Khurana *et a*l [14] and Cetinkaya *et al* [15] found that both Copro-ELISA and PCR methods were found to be equally preferable for the detection of *Cryptosporidium* oocysts in stool samples.

The results of present study showed that Auramine-Rhodamine and Copro-ELISA appeared to be suitable for the screening of cryptosporidiosis in a large number of stool samples in a short time. Copro-ELISA could be useful for rapid diagnosis of cryptosporidiosis in busy clinical laboratories. To avoid false negative results both Modified Ziehl-Neelsen and Auramine-Rhodamine Techniques can be used when a patient is complaining gastrointestinal symptoms for the diagnosis of cryptosporidiosis.

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