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

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Abstract:
We evaluated the diagnostic performance of microscopical 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.

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 >10%) than in developed countries (1 to 3%).[2]

It is well known that detection of *Cryptosporidium* oocysts in faecal samples is made by modified acid fast staining technique, which requires the presence of large number of oocyts, 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 parts 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 the cryptosporidiosis among patients in most of clinical laboratories.[5-7] However, the assay has not been used for the screening of cryptosporidiosis in large-scale epidemiological surveys.

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.

Materials 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 centres (Mansoura, Talkha, Belgas 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 form concentrated pellet from each sample were prepared on two slides, air-dried and stained separately by modified Ziehl-Neelson technique[9] and Auramine-Rhodamine stain.[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 eluate: Faecal supernatant for each sample was prepared in a proportion of 1:1 (1gm of stool thoroughly mixed with same volume of distilled water). The mixture was

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centrifuged at 1500 rpm for 5min. The supernatant was recovered and transferred to fresh tube with the addition of 0.2% mentholated that act as preservative for coproantigen, and stored at - 50ºc until used for detection of Cryptosporidium-specific coproantigen.

Enzyme immunoassay: All faecal supernatants were processed according to the instructions guide by stool antigen enzyme linked immunosorbent assay (cryptosporidium – CELISA callable, Australia) to detect *Cryptosporidium* specific coproantigens.

Statistical analysis: chi-square was used to compare detection efficiency of stool microscopy and immunoassay for cryptosporidiosis diagnosis and p valve of less than 0.05 was considered significant.

Results:

The comparative sensitivity of stool microscopy using modified Ziehl-Neelsen and Auramine-Rhodamine techniques) 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 result was found in the sensitivity of copro-ELISA verses modified Ziehl – Neelsen (p=0.006) and Auramine-Rhodamine (p=0.01) techniques. 20.18, 10.09 and 9.17% stools showed co-exisiting of *Cryptosporidium* with one, two or more than two intestinal parasities.

Discussion:

In the present study, 1768 random stool specimens were screened for cryptosporidiosis diagnosis, using two staining techniques (modified Ziehl-Neelsen and Auramine-Rhodamine) and Copro-ELISA. The overall prevalence of *Cryptosporidium* was 10.07% by copro-ELISA, 5.49 and 6.17% by modified Ziehl – Nelsen and Auramine-Rhodamine staining methods respectively. Similar results have been reported by other.[6,7] The present findings suggest significant prevalence of cryptosporidiosis among random population of Dakahlia province, Egypt. Similar results have been observed in other developing African countries.[12]

In the present study, both Auramine-Rhodamine and Copro-ELISA appeared to be suitable for the screening of cryptosporidiosis in large number of stool samples in a short time period. The assay could be useful for rapid diagnosis of cryptosporidiosis in busy clinical laboratories. To avoid false negative results in commonly used modified Ziehl-Neelsen technique, both Auramine-Rhodamine and copro-ELISA can be used when a patient is complaining clinical symptoms for the diagnosis of *Cryptosporidium* infection.

Table 1: Comparison of sensitivity of staining techniques and enzyme immunoassay for diagnosis cryptosporidiosis.

Method	No. 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-Neelsan 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).

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