Molecular Copro-prevalence of *Cryptosporidium* in Egyptian Children and Evaluation of Three Diagnostic Methods

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Objective: To determine molecular prevalence of *Cryptosporidium* in a cohort of Egyptian children and compare three diagnostic tests. **Methods**: Stool samples from children with diarrhea (n=150) and from apparently healthy children (n=100) were examined for *Cryptosporidium* using microscopy, enzyme linked immuosorbant assay (ELISA) and nested polymerase chain reaction (nPCR). **Results**: nPCR detected *Cryptosporidium* in 22.4% of children. Acid–fast stain and ELISA showed false negativity but 100% specificity with nPCR as gold standard. **Conclusion**: *Cryptosporidium* is a common cause of diarrhea in children in Egypt.

Keywords: *Diarrhea, Etiology, ELISA, Nested PCR.*

*C*ryptosporidium is an emerging zoonotic pathogen with potentially life-threatening diarrhea in immuno-compromised patients [1]. Conventional methods for its identification include microscopic examination of its oocyst in fecal smears with acid-fast staining [2], and immunoassays that detect copro-antigen by ELISA. There are conflicting reports about the sensitivity of immuno-detection methods over microscopy [3]. Polymerase chain reaction (PCR) is being increasingly used for diagnosis [4]. The present study was designed to determine the molecular prevalence of *Cryptosporidium* in a cohort of Egyptian children, and to compare the utility of three diagnostic methods.

METHODS

A total of 250 children (150 had diarrhea and 100 were non-diarrheal controls) attending the outpatient clinics of Abou El-Reesh Pediatric Teaching Cairo University Hospital, from February to July 2012, were enrolled in this cross-sectional study. The study was approved by Ethical Committee of Faculty of Medicine, Cairo University.

A single fecal sample from each child was collected and examined microscopically. Acid-fast staining was done from concentrated stool samples and examined for *Cryptosporidium* oocysts. Frozen fecal specimens were processed for fecal sandwich ELISA (RIDASCREEN *Cryptosporidium*-C1201- R-Biopharm AG, Germany) and nested-PCR (nPCR) assay. The nPCR was done by analysis of *ssu rRNA* gene. Copr-DNA was extracted,

using Favor stool DNA spin columns isolation Mini Kit, incubated at 95°C for one hour. Extracted DNA was amplified by nPCR [5] after adjusting annealing at 55°C. Laboratory procedures were performed in Clinical Chemistry Unit, Faculty of Medicine, Cairo University.

Diagnostics were evaluated and ranked by Multi-attribute utility theory [6] and Analytical hierarchy process [7] to help in decision-making, based on laboratory utility instead of being limited to the diagnostic yields. The six attributes were given a rank order from 1 to 3 and every attribute was prioritized by its importance over the other as per the laboratory's infrastructure. The results were analyzed using the SPSS software, Version 15.0 (Chicago, IL, USA).

RESULTS

Cryptosporidium copro-DNA and copro-antigen were detected in both diarrheal and non-diarrheal fecal samples, while *Cryptosporidium* oocysts were detected in diarrheal fecal samples only (**Table I**). Considering nPCR as the reference method, both acid-fast staining and ELISA had no false positivity (100% sensitivity and positive predictive value); however, they gave false negative results (32% and 55%, respectively). Using Multi-attribute utility, ELISA had the highest rank (2.39), followed by nPCR (1.96) and acid-Fast staining (1.73) (**Table II**).

DISCUSSION

Our results demonstrated a high prevalence of Cryptosporidium in diarrheal and non-diarrheal fecal

TABLE I DIAGNOSTIC YIELD OF FECAL ACID-FAST STAIN, ELISA AND nPCR IN DETECTION OF *CRYPTOSPORIDIUM*.

	ELISA		Microscopy	
	+ve	−ve	+ve	−ve
PCR Positive				
Diarrheal group (*n*=38)	22	16	18	20
Non-Diarrheal group (*n*=18)	9	9	0	18
PCR Negative				
Diarrheal group (*n*=112)	0	112	0	112
Non-Diarrheal group (*n*=82)	0	82	0	82

samples. *Cryptosporidium* were more frequent in children with diarrhea than in those with no diarrhea.

Our results were consistent with findings of other studies from developing world [8-11]. Although children without diarrhea are seldom tested for the presence of enteric pathogens, asymptomatic cryptosporidiosis may lead to growth retardation through mucosal damage causing nutrient malabsorption [12].

Many PCR primers using various target genes have been constructed to detect *Cryptosporidium* but 18 S rRNA (ssu-rRNA) gene loci are widely used [13]. PCR-inhibitors in feces and oocysts resistant to lysis lead to false-negative PCR results [14]. Despite these caveats, we obtained higher rates and this may be attributed to thermal treatment of samples during DNA extraction with elution of DNA in 2 steps using less amount of elution buffer. The wide use of PCR is hindered by its relative high cost and time taken to extract DNA from stool. However, PCR still holds importance for the identification of *Cryptosporidium* DNA in samples with low number of oocysts, and for detection of species.

We conclude that *Cryptosporidium* is an important enteric pathogen in Egyptian children. Immunodiagnosis of cryptosporidiosis may be of benefit, and molecular detection should also be an option. Cryptosporidiosis is underdiagnosed because clinicians fail to consider this diagnosis in immunocompetent patients with diarrheal illnesses (particularly children) and do not request stool analysis for *Cryptosporidium*.

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TABLE II MULTI-ATTRIBUTE EVALUATION OF DIAGNOSTIC TECHNIQUES FOR CRYPTOSPORIDIUM

Evaluation Items	Priority value	Acid-fast stain	ELISA	nPCR
Cost-effectiveness	0.95	3	2	1
Sensitivity	0.35	1	2	3
Specificity	0.35	3	3	3
Ease of use	0.9	1	2	3
Ease of interpretation	0.15	1	3	2
Ability to perform batch testing	0.5	1	3	2
Ability for species specification	0.07	0	0	3
Total*		1.73	2.39	1.96

Priority values were multiplied by the ranks given for each attribute for every diagnostic technique.

WHAT THIS STUDY ADDS?

• *Cryptosporidium* is commonly isolated from fecal samples of Egyption children.

• Molecular methods and ELISA results in higher yield as compared to acid-fast staining.

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