Detection of *Cryptosporidium* sp infection by PCR and modified acid fast staining from potassium dichromate preserved stool

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**Abstract**

**Aim** To identify the frequency of *Cryptosporidium* infection in children below 3 years old by examining concentrated long term preserved stool using PCR detection of 18S rRNA gene and compared with modified acid fast staining technique.

**Methods** Hundred eighty eight stools from children ≤3 years old were stored for 13 months in 2.5% K2Cr2O7 solution at 4°C. *Cryptosporidium* oocysts were isolated by water-ether concentration technique. The concentrates were smeared onto object glass and stained with modified acid fast staining, and the rest of the concentrates were DNA extracted by freezing and thawing cycles and proteinase K digestion, then direct PCR was done to detect 18S rRNA gene.

**Result** The proportion of positive stools for *Cryptosporidium* sp by acid fast staining from concentrated stools and 18S rRNA PCR were 4.8% and 34.6% respectively, which showed statistically significant difference.

**Conclusion** The frequency of *Cryptosporidium* infection among children ≤3 years old was very high and stool storage in K2Cr2O7 for 13 months did not affect the PCR result. High prevalence of *Cryptosporidium* infection indicated high transmission in that area and the potential to be transmitted to other individuals such as the immunocompromised. (Med J Indones 2009; 18: 149-54)

**Key words:** 18S rRNA, cryptosporidiosis

DNA can be isolated from any biological specimens; the most often widely used specimen is blood and hair because they are easily available. The DNA will be used to identify an organism by PCR, which is a method firstly introduced by Mullis in 1985. This method was developed further by the Department of Human Genetics, Cetus Corporation, California for the amplification of β-globin human gene to diagnose prenatal genetic disorder such as sickle cell anemia.¹

The rRNA is present in ribosomes of all organisms, the pro and eucaryote, which consist of small and large subunits. The 18S rRNA is present in the small ribosome subunit in the cytosol of eucaryotes.² In medicine, rRNA is the target of antibiotics, while in evolution, rRNA can be used in the taxonomy of an organism, to calculate the distance of relationship between one organism to another, and to calculate species divergence.³ Identification of 18S rRNA gene was used to study a number of eukaryotes such as plants, animals and protozoa as well as *Cryptosporidium*.⁴

*Cryptosporidium* sp. is an intestinal coccidian protozoa, which infects animal as well as human and causes