

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

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### Abstrak

Tujuan Untuk mengetahui frekuensi infeksi *Cryptosporidium* sp pada anak bawah tiga tahun (batita) dengan deteksi

gen 18S rRNA dari tinja yang sudah dipreservasi lama dan membandingkannya dengan modifikasi metode tahan

asam (MTA) dari tinja hasil konsentrasi.

Metode Sejumlah 188 feses anak batita yang telah tersimpan selama 13 bulan di 40C, dikonsentrasikan dengan teknik air eter,

selanjutnya dibuat sediaan, dipulas dengan pewarnaan MTA; sisa konsentrat diekstraksi DNA dengan teknik kejut panas dingin

dan penambahan proteinase K, lalu dilakukan PCR langsung terhadap gen 18S rRNA.

Hasil Proporsi sampel positif *Cryptosporidium* adalah 34.6% dengan PCR gen 18s rRNA dan 4.8% dengan pulasan

MTA dari tinja konsentrasi. Secara statistik perbedaan kedua hasil tersebut bermakna.

Kesimpulan Frekuensi infeksi *Cryptosporidium* sp di batita tinggi sekali dan penyimpanan tinja dalam larutan kalium

dikromat selama 13 bulan, tampaknya tidak mempengaruhi hasil PCR. Tingginya frekuensi infeksi *Cryptosporidium* di

populasi itu menunjukkan tingginya transmisi di daerah tersebut sehingga berpotensi menular ke kelompok yang rentan

misalnya imunokompromais.

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<b>Abstract</b><br>

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 &#8804; 3 years old, were stored for 13 months in 2.5% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution at 40C. *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 &#8804; 3 years old was very high and stool storage in K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> 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.