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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 modifi kasi metode tahan
asam (MTA) dari tinja hasil konsentrasi.
Metode Sejumlah 188 feses anak batita yang telah tersimpan selama 13 bulan di 4oC, 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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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 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 ≤ 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.