Standardization and evaluation of molecular methods to detect oocysts of *Cryptosporidium* spp. (Apicomplexa: Cryptosporiidae) in faecal samples: extraction of genomic DNA and PCR (polymerase chain reaction)

Carvalho-Almeida, T. T. **Padronização e avaliação de métodos moleculares para detecção de oocistos de *Cryptosporidium* sp. (Apicomplexa: Cryptosporiidae) em amostras fecais: extração de DNA genômico e PCR (reação em cadeia pela polimerase)**. São Paulo - SP. 2004. [Tese de Doutorado – Área: Práticas em Saúde Pública – Faculdade de Saúde Pública – USP]. Orientadora: Profa. Dra. Maria Helena Matté.

The protozoan parasite *Cryptosporidium parvum* has become recognised as important emerging human pathogens. For molecular studies, most of the techniques to extract genomic DNA require the use of imported kits to concentrate, rupture the very resistant oocyst wall, and purify the DNA from the samples matrix. The aim of this study was to develop a simple and rapid method based on polymerase chain reaction (PCR) to detect *Cryptosporidium* in preserved faeces. Oocysts were concentrated from faecal specimens by flotation on sucrose gradient. Genomic DNA was prepared from purified oocysts by adding a lysis buffer containing 70 mM β-mercaptoethanol, digested with proteinase K and extracted with phenol-chlorophorm-isoamyl. The standardization was started by performing a one step PCR to detect *Cryptosporidium* spp. using a generic set of primer (AWA). To detect *C. parvum* a one step PCR was assayed using the specific primer (LAX). To increase the sensitivity of the method, were tested nested-PCR assays, using an outer primer (XIA). Thirty nine DNA samples were analysed from the standard calf, 52 samples from 17 patients and 45 samples from 14 animals. The results were: 54.28% positive samples by single PCR AWA, 71.42% by nested-PCR, 67.74% by single PCR LAX and 44.44% by nested-PCR for the standard calf. The overall positivity for human and animal samples were: 34.48% by single PCR and 54.83% by nested-PCR for *Cryptosporidium* spp. and 16.00% by single PCR and 50.00% by nested-PCR for *C. parvum.* Using Vistra Green for staining agarose gel, yielded the visualisation of the amplicons. These results show that this simple and cheap method could be improved to be used on the routine laboratory work.

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Evaluation of immune response and protection induced by immunization with A2 and Lack antigens against experimental *Leishmania major* and *Leishmania amazonensis* infection

Coelho, E. A. F. **Avaliação dos níveis de proteção e da resposta imune induzida pela imunização com os antígenos A2 e Lack na infecção experimental com *Leishmania (Leishmania) major* e *Leishmania (Leishmania) amazonensis***. Belo Horizonte, MG, 2004 [Tese de doutorado Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais] Orientador: Carlos Alberto Pereira Tavares

In an attempt to select candidate antigens for a leishmaniasis vaccine, we investigated the protective effect of A2 and Lack antigens against *L. amazonensis* and/or *L. major* infections in BALB/c mice. The Lack and A2 antigens were