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

administered as Lack-MBP and A2-HIS recombinant proteins associated or not to adjuvants (rIL-12 and/or allumen) or as DNA plasmids (pCDNA3-A2 and pCI Neo-Lack) associated or not to pCI-IL-12 DNA, as adjuvant. BALB/c mice were immunized in their left hind footpads with 2 doses of proteins in 15 days interval. Mice immunized with plasmid DNA, received *intramuscularly* 2 doses in 21 days interval. One month after, mice were infected in their right hind footpads with $1x10^5$ or $1x10^6$ stationary phase promastigotes of *L. amazonensis* or *L. major*. Immunization with recombinant proteins, associated to adjuvants or with DNA plasmids, was able to induce a robust Th1 immune response prior to challenge infection. However, only mice immunized with A2 antigen were protected against *L. amazonensis* challenge. A high and sustained IFN-γ production, increased levels of A2-specific IgG2a antibodies and low levels of parasite-specific IgG antibodies were detected. A2 protein administered alone did not induce protection against infection; however, A2 DNA associated or not by IL-12 DNA induced protection against *L. amazonensis* challenge. In contrast, mice immunized with Lack protein or Lack DNA, associated or not by adjuvants, had low levels of IFN-γ and high levels of both Lack-specific and parasite-specific IgG1 antibodies and were not protected against *L. amazonensis* infection. However, mice immunized with Lack antigen were protected against *L. major* infection. Curiously, the association between A2 and Lack antigens in the same vaccine completely abrogated the A2 specific immune responses and, consequently, the protective effect of this antigen against *L. amazonensis* challenge. We concluded that A2, but not Lack, fits the requirements to compose a safe vaccine against tegumentary leishmaniasis.

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Study of quantification and validation methodologies of soy proteins and collagen and technological uses in meat products

Della Torre, J.C.M. **Proteínas de soja e colágeno: validação das metodologias de quantificação e avaliação tecnológica do uso em produtos cárneos.** Campinas – SP. 2004. [Dissertação de Doutorado – Área: Tecnologia de Alimentos – Faculdade de Engenharia de Alimentos – UNICAMP Campinas] Orientador: Dr. Nelson José Beraquet.

Comminuted meat products, such as frankfurters, fresh ground sausages and hamburgers are exposed to fraud by the abusive use of protein extensors of the types collagen and soy proteins, used in meat products because of their technological benefits, with reduced processing costs, although they are considered limiting, with regard to essential amino-acids, and producing adverse effects in sensory characteristics. The effective control of addition levels is not properly controlled by the Sanitary Surveillance bodies in Brazil, due to the lack of a validated quantitative analytical methodology for soy proteins and because there are no legal limits to collagen inclusion in meat products. As a contribution to the establishment of maximum contents and effective control of soy proteins and collagen, this project sought: a) to validate intra-laboratorially the official AOAC methodologies in the quantification of hydroxyproline (collagen) and soy proteins in meat products; b) to evaluate proximate composition and technological influence of extensors addiction to Lyoner sausage, and c) to present both sensorial and physicochemical Adolfo Lutz Institute analytical routine results of commercial products like frankfurters, fresh ground sausages and minced beef meat. The formulation and processes were the commercially used for meat emulsion batter, Lyoner sausage and canned emulsion. Beef meat, pork backfat and ice quantities were balanced in order to keep constant the 4.7 humidity : protein ratio as well as the 20% lipid level, replaced with either texturized soy proteins (TSP, Maxten E-100), texturized concentrated (CSP, Proteimax TR-120) and isolated (ISP, Supro 500E) in concentration of 0 to 6% or connective tissue from cooked pork rind or fresh beef connective tissue recovered from cuts obtained by using the Skyner machine, in the range 0 to 15%. The color parameters L* (luminosity), a* (red) and b* (yellow) were measured using a spectrophotometer (Minolta) and the compression hardness was measured using a Texture Analyser (TAXT2i/25). The proximate composition and pH were carried out according to Adolfo Lutz Institute's Analytical Norms. Emulsion stability was reported as percent fat and gelatin released from sterilized canned emulsion. A trained 9-member panel evaluated the sensory attributes using an unstructured 10 cm line scale, following the randomized complete block design, with replicate judgments. Laboratorial