ELECTROPHORETIC PROTEIN PATTERN OF *Cystoisospora felis* (WENYON, 1923) FRENKEL, 1977 (APICOMPLEXA: CYSTOISOSPORINAE) SPORULATED OOCYSTS*

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ABSTRACT:- CARVALHO FILHO, P.R. de; FLAUSINO, W.; LOPES, C.W.G. **Electrophoretic protein pattern of *Cystoisopora felis* (Wenyon, 1923) Frenkel, 1977 (Apicomplexa: Cystoisosporinae) sporulated oocysts.** [Padrão eletroforético de proteínas de oocistos esporulados de *Cystoisospora felis* (Wenyon, 1923) Frenkel, 1977 (Apicomplexa: Cystoisosporinae)]. *Revista Brasileira de Parasitologia Veterinária*, v. 13, n. 4, p. 173-175, 2004. Universidade Federal Rural do Rio de Janeiro, Instituto de Veterinária, Departamento de Parasitologia Animal. BR 465 Km 07 Seropédica, RJ 23890-000, Brazil. E-mail: lopescwg@ufrrj.br

The technique of SDS-polyacrilamide gel electrophoresis (SDS-PAGE) was used to study the protein pattern of sporulated oocysts of *Cystoisospora felis*. Pure strain of *C. felis* sporulated oocysts was purified, suspended in solubilizing buffer, sonicated and submitted to SDS-PAGE. Zymograms show ten well-identified bands with approximated molecular weights of 20, 25, 33, 39, 42, 55, 64, 73, 81 and 85 KDa. It was revealed bands that can be antigenic markers to *C. felis* infection.

KEYWORDS: *Cystoisospora felis*, protein, oocysts, SDS-PAGE.

RESUMO

A técnica de eletroforese em gel de poliacrilamida-SDS (SDS-PAGE) foi usada para estudar o padrão protéico de oocistos esporulados de *Cystoisospora felis*. Um isolado puro de oocistos esporulados de *C. felis* foi purificado, suspendido em tampão de solubilização, sonicado e submetido ao SDS-PAGE. Através dos zimogramas, foram identificadas dez bandas com pesos moleculares aproximados de 20, 25, 33, 39, 42, 55, 64, 73, 81 e 85 KDa. Foram observadas bandas que podem ser marcadores antigênicos para infecção por *C. felis*.

PALAVRAS-CHAVE: *Cystoisospora felis*, proteína, oocistos, SDS-PAGE.

Cystoisospora felis is a coccidian parasite highly prevalent in cats and wild felids all over the world (DUBEY, 1993; DUSZYNSKI et al., 2000). Its life cycle is classified as facultatively heteroxenous, where cats can be infected with either sporulated oocysts or tissue monozoic cysts containing only one life stage, the hypnozoites (DUBEY; FRENKEL, 1972; FRENKEL; DUBEY, 1972; MARKUS, 1976; MEHLHORN; MARKUS, 1976). Immunological inter-relationship of *C. felis* with other cyst-forming Coccidia have already been identified, especially between *T. gondii* infection in cats (CHESSUM, 1972; DUBEY, 1976, OMATA et al., 1991a, b). The objective of this study was to identify *C. felis* protein groups in extract of sporulated oocysts analysed with SDS-polyacrilamine gel electrophoresis (SDS-PAGE) that can be important antigenic markers for *C. felis* infection in latter studies. A pure isolate of *C. felis* sporulated oocysts have been maintained since May 2003 under laboratorial conditions and was passed twice in Coccidia-free kittens without observation of any other Coccidia species, even *C. rivolta*. Oocysts were sporulated in 2.5% (w/v) potassium dicromate solution and purified using the flotation method on sucrose solution with Petri dishes and followed by discontinuous sucrose gradient

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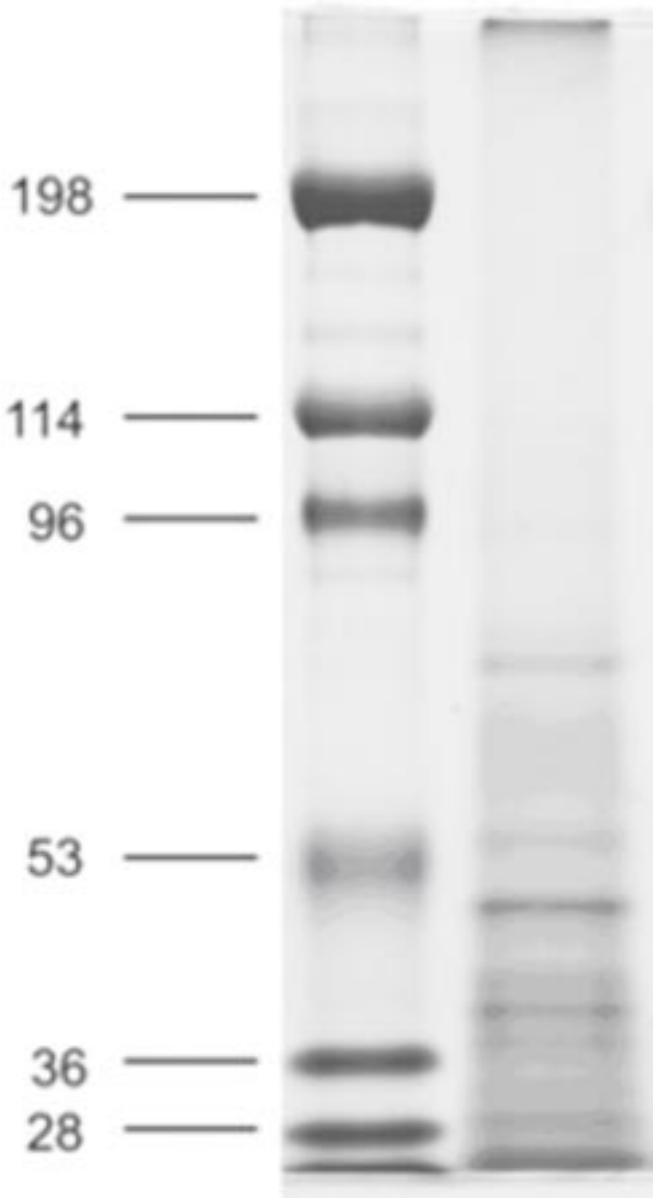

technique (ARROWOOD; STERLING, 1987). Five million (5 x 10^6) purified oocysts were than washed with a 3% (v/v) sodium hypochloride for 15 minutes for removing bacterial or fungal contaminates. Sporulated oocysts were suspended in 500µl of solubilizing buffer [2% 2-mercaptoethanol, 12.5 mM Tris-HCl buffer, 4.6% sodium dodecil sulfate (SDS), 2mM phenyl methyl sulfonyl fluoride (PMSF)] for SDS-PAGE. Suspension were boiled for 10 minutes, sonicated for 1 minute at 60 Hz and filtered throughout a 0.45µ-membrane (Millex™ – Millipore, Bedford, USA). Filtered solution was boiled for more 3 minutes to thermic denaturation of oocyst proteins. Oocyst extract was applied on a 10% polyacrilamine (acrylamide / bis-acrylamide 30:1) gel, electrophoresed according to Laemmli (1970). Molecular weight standards were myosin (198 KDa), â-galactosidase (114 KDa), bovine serum albumin (96 KDa), ovalbumin (53 KDa), carbonic anidrase (36 KDa) and soybean trypsin inhibitor (28 KDa) (Prestained SDS-PAGE Standards, Broad Range, Bio-Rad Laboratories). Following SDS-PAGE, the gel was stained with Coomassie blue 250R, destained, packed within cellophane sheet (Biomed, Pharmacia, Swenden) and dried. Zymograms were integrated by using a 2202 Ultrosean, Laser densitometer, LKB Bromma conected to a Hawlett Packard 3396, serie II Integrator. Ten labeled protein bands were found in zymograms of sporulated oocyst extracts. Bands at 20, 25, 33, 39, 42, 55, 64, 73, 81 and 85 KDa (Figure 1) were identified but many of them can be restrict only to oocyst and sporocyst walls, possibly without any importance in *C. felis* sporozoite infection and host immunological processes. The 85-KDa protein band was highly identified in zymogram, representing 34.95% of extract proteins by densitometric analysis (Figure 2). It is possible that this protein group

Figure 1. SDS-PAGE of *Cystoisospora felis* sporulated oocysts. Left lane with molecular weight standards (KDa) and right lane with *C. felis* sporulated oocyst extract stained by Coomassie blue 250R.

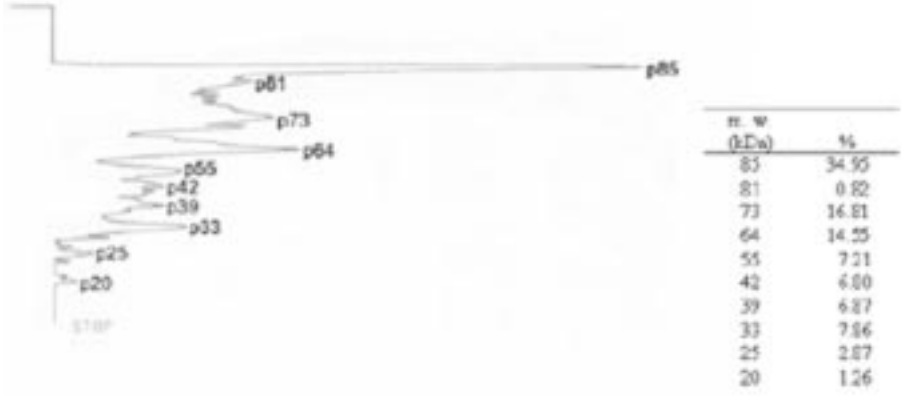

Figure 2. Densitometric analysis of *Cystoisospora felis* sporulated oocyst extract electrophoresed on 10% SDS-PAGE. Peaks with molecular weights (m. w.) and table with percentages of proteins in *C. felis* oocyst extracts.

play a role in the structure of oocyst and sporocyst walls of *C. felis*. Common proteins of *Cryptosporidium parvum* and *C. serpentis* oocysts were similar in molecular weights with 20-, 33- and 85-KDa bands of *C. felis* sporulated oocysts (TILLEY et al., 1990). However, these data cannot reflect similarities among oocyst walls of these coccidia. It is known that oocysts/sporozoites of *T. gondii* have two protein bands with approximated molecular weights of 25 and 67 KDa that is absent in tachyzoites (KASPER; WARE, 1985). Possibly, *C. felis* oocyst/sporozoite 25- and 64-KDa proteins can share some similarities with those of *T. gondii* oocysts/sporozoites, based upon taxonomic relationship among *Cystoisospora* species and *T. gondii* (SMITH, 1981; CARRENO et al., 1998). Omata et al. (1991b) identified a 22-KDa protein band using immunoblotting with *C. felis* sporozoites as antigen and serum of cats experimentally infected *per os* with *C. felis* sporulated oocysts and *T. gondii* cysts or sporulated oocysts. This band was considered by them specific for *C. felis* infection in cats.

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