

SHORT COMMUNICATION

HYPNOZOITES OF *Cystoisospora felis* (WENYON, 1923) FRENKEL, 1977 (APICOMPLEXA: CYSTOISOSPORINAE) ISOLATED FROM PIGLETS EXPERIMENTALLY INFECTED*

PATRICIA S. MELO¹, PAULO R. DE CARVALHO FILHO², CARLOS W. G. LOPES³,
WALTER FLAUSINO³, FRANCISCO C. R. DE OLIVEIRA⁴

ABSTRACT: MELO, P.S.; CARVALHO FILHO, P.R. DE; LOPES, C.W.G.; FLAUSINO, W.; OLIVEIRA, F.C.R. DE. **Hypnozoites of *Cystoisospora felis* (Wenyon, 1923) Frenkel, 1977 (Apicomplexa: Cystoisosporinae) isolated from piglets experimentally infected.** [Hipnozoítas de *Cystoisospora felis* (Wenyon, 1923) Frenkel, 1977 (Apicomplexa: Cystoisosporinae) isolados de suínos experimentalmente infectados.] *Revista Brasileira de Parasitologia Veterinária* v. 12, n. 2, p. 82-84, 2003. Departamento de Parasitologia Animal/IV – UFRRJ – Km 7 da BR 465, Seropédica, RJ 23890-000, Brazil. E-mail: lopesw@ufrrj.br

Four Large White weaned pigs were orally infected with 3.5×10^5 *Cystoisospora felis* oocysts. One by one, they were successively euthanased and posted on 3rd, 7th, 14th and 33rd days after infection (DAI) and posted. During the necropsies, small and large intestines, Peyer's patches, mesenteric lymph nodes, spleen and liver were removed, weighed, blended and submitted to the peptic tissue digestion technique. Each digested tissue sample was analyzed to verify the presence of hypnozoites harbored in the studied viscera. Only the large intestine was negative for hypnozoites. They were easily found in the small intestine, mesenteric lymph nodes and spleen. The presence of amilopectin granules in *C. felis* hypnozoites is an evidence of development in swines.

KEY WORDS: Piglets, hypnozoites, *Cystoisospora felis*, peptic tissue digestion.

RESUMO

Quatro leitões recém-desmamados da raça Large White foram infectados oralmente com $3,5 \times 10^5$ oocistos de *C. felis*. Um a um, eles foram eutanasiados e necropsiados no 3º, 7º, 14º e 33º dias após infecção (DAI) e necropsiados. Durante a necropsia, os intestinos delgado e grosso, as placas de Peyer, os linfonodos mesentéricos, o baço e o fígado foram pesados,

triturados e submetidos à técnica de digestão péptica de tecidos. Cada amostra de víscera digerida foi analisada com a finalidade de se verificar a presença de hipnozoítas nelas albergados. Apenas não se observou hipnozoítas de *C. felis* no intestino grosso e eles foram mais facilmente encontrados no intestino delgado, linfonodos mesentéricos e no baço. A presença de grânulos de amilopectina no protoplasma dos hipnozoítas é uma evidência de seu desenvolvimento em suínos, razão pela qual, este pode ser considerado um hospedeiro intermediário deste parasito.

PALAVRAS-CHAVE: leitões, hipnozoítas, *Cystoisospora felis*, digestão péptica de tecidos.

Cystoisospora felis is able to infect several hosts (BOCH et al., 1981) and also can cause damage to the animal health (LOSS; LOPES, 1992; FREIRE; LOPES, 1995). Domestic cats and wild felids such as cougar (*Puma concolor*)

*Supported by CNPq - Brazil.

¹Coordenadoria de Defesa Sanitária Animal / SEAAPI - RJ E-mail: patriciasel@openlink.com.br

²Curso de Pós-Graduação em Ciências Veterinárias da Universidade Federal Rural do Rio de Janeiro e bolsista do CNPq - Brasil E-mail: paulorcf@ufrrj.br

³Departamento de Parasitologia Animal / IV – UFRRJ – BR 465 Km 7 CEP 23890-000 - Seropédica, RJ, Brasil E-mail: lopesw@ufrrj.br

⁴Laboratório de Sanidade Animal, CCTA, UENF, Campos dos Goytacazes, RJ, Brasil E-mail: foliveira@uenf.br

Table 1. Presence of *Cystoisospora felis* hypnozoites in piglet viscera.

Organs	Days after inoculation			
	3 rd	7 th	14 th	33 rd
Small intestine	+	+	+	+
Large intestine	-	-	-	-
Peyer's patches	LS	+	+	+
Mesenteric lymph nodes	+	+	+	+
Liver	-	+	+	+
Spleen	+	+	+	+

(+) positive; (-) negative; (LS) lost sample.

(AMARAL et al., 1966), leopards (*Panthera pardus*), tigers (*P. tigris*), clouded leopards (*Neofelis nebulosa*) and Asian golden cats (*Felis temmincki*) (PATTON; RABINOWITZ, 1994) can act as definitive hosts, possibiliting the occurrence of this coccidiosis in natural environment with no human changes. Mice, rats, hamsters (FRENKEL; DUBEY, 1972), dogs (DUBEY, 1975), birds (LINDSAY; BLAGBURN, 1994), cattle (FAYER; FRENKEL, 1979), rabbits (COSTA; LOPES, 1998) and broiler chicken (MASSAD et al., 2002) are considered intermediate hosts of *Cystoisospora* species. The distribution of hypnozoites among several viscera, which were studied, suggests major tropism for mesenteric lymph nodes, spleen, liver and Peyer's patches (FRENKEL; DUBEY, 1972; BRÖSIGKE et al., 1982; FREIRE; LOPES, 1996; COSTA; LOPES, 1998). The tissue enzymatic digestion has been broadly used (MARKUS, 1978; FAYER; FRENKEL, 1979; FREIRE; LOPES, 1996). In addition, Dubey (1998) included at this technique sodium hydroxide and sodium bicarbonate for neutralizing the hydrochloric acid. In this experiment, the ability of *C. felis* to infect swine viscera after feeding sporulated oocysts of this coccidium was evaluated as well as to describe the presence of hypnozoites among the following sites: mesenteric lymph nodes, Peyer's patches, liver, spleen, small and large intestines at a 33-day after infection (DAI). For this reason, four Large White weaned pigs were taken and each piglet was orally infected with 3.5×10^5 *C. felis* sporulated oocysts prepared according to Souza e Lopes (1984).

One by one, they were successively euthanased by using endovenous injection of sodium thiopental and posted on 3rd, 7th, 14th and 33rd DAI. At the necropsy table, small and large intestines, Peyer's patches, mesenteric lymph nodes, liver and spleen were taken. These viscera were individually weighed, triturated and submitted to the peptic digestion. The technique used in this study was modified by Oliveira et al. (2001) from Dubey's tissue digestion technique (DUBEY, 1998). The digested tissue sample suspended in PBS pH 7.2 were used to verify the presence of *C. felis* hypnozoites sought between slide and coverglass on light microscope, in each viscera and on different DAI. The small intestine, mesenteric lymph nodes and spleen were positive for hypnozoites on 3rd, 7th, 14th and

33rd DAI. The liver was negative on 3rd DAI only. Peyer's patches were positive on 7th, 14th and 33rd DAI but it is uncertain whether 3rd DAI was positive or not because this sample had to be despised. Hypnozoites isolated from piglet viscera had their cytoplasm full of granules, which were supposed to be amilopectin. Freire e Lopes (1995); Costa e Lopes (1998) and Oliveira et al. (2001) also identified these granules in the cytoplasm of hypnozoites harbored in mice and rabbits. They concluded that these granules were accumulated during the expoliative process and it is characterized developmental type in the intermediate host. For this reason, swines could be considered another *C. felis* intermediate host.

REFERENCES

- AMARAL, V.; AMARO, R. G.; BIRGEL, E. H. Ocorrência da *Isospora felis* Wenyon, 1923, em suçuarana (*Puma concolor*). *Revista da Sociedade Paulista de Medicina Veterinária*, v. 4, n. 1, p. 25-28, 1966.
- BOCH, J.; GOBEL, E.; HEINE, J.; ERBER, M. *Isospora*-infektionen bei hund und katze. *Berliner und Munchener Tierärztliche Wochenschrift*, v. 94, n. 19, p. 384-391, 1981.
- BRÖSIGKE, S.; HEINE, J.; BOCH, J. Der nachweis extraintestinalen entwicklungstadien (Dormozoitien) in experimentall mit *Cystoisospora rivolta* oozysten infierten maue. *Klontier Praxis*, v. 27, n. 1, p. 25-34, 1982.
- COSTA, P. S. da; LOPES, C. W. G. Avaliação do parasitismo por *Cystoisospora felis* (Wenyon, 1923) Frenkel, 1977 (Apicomplexa: Cystoisosporinae) em coelhos do tipo carne. *Revista Brasileira de Parasitologia Veterinária*, v. 7, n. 1, p. 15-19, 1998.
- DUBEY, J. P. Refinement of pepsin digestion method for isolation of *Toxoplasma gondii* from infected tissue. *Veterinary Parasitology*, v. 74, n. 1, p. 75-77, 1998.
- DUBEY, J. P. Experimental *Isospora canis* and *I. felis* infection in mice, cats and dogs. *Journal of Protozoology*, v. 22, n. 3, p. 416-417, 1975.
- FAYER, R.; FRENKEL, J. K. Comparative infectivity for calves of oocysts of feline coccidia: *Besnoitia*, *Hammondia*, *Cystoisospora*, *Sarcocystis* and *Toxoplasma*. *Journal of Parasitology*, v. 65, n. 5, p. 756-762, 1979.
- FREIRE, R. B.; LOPES, C. W. G. Distribuição de hipnozoítas de *Cystoisospora felis* (Wenyon, 1923) Frenkel, 1977 (Apicomplexa: Sarcocystidae) em camundongos albinos experimentalmente infectados. *Revista Brasileira de Parasitologia Veterinária*, v. 5, n. 1, p. 23-28, 1996.
- FREIRE, R. B.; LOPES, C. W. G. Infecção experimental em camundongos neonatos com esporozoítas de *Cystoisospora felis* (Wenyon, 1923) Frenkel, 1977 (Apicomplexa: Sarcocystidae). *Revista Brasileira de Ciência Veterinária*, v. 2, n. 1, p. 33-34, 1995.
- FRENKEL, J. K.; DUBEY, J. P. Rodents as vectors for feline Coccidia, *Isospora felis* and *Isospora rivolta*. *Journal of Infectious Disease*, v. 125, n. 1, p. 69-72, 1972.
- LINDSAY, D. S.; BLAGBURN, B. L. Biology of mammalian

- Isospora*. *Parasitology Today*, v. 10, n. 6, p. 214-220, 1994.
- LOSS, Z. G.; LOPES, C. W. G. Alguns aspectos clínicos da infecção por *Cystoisospora felis* (Wenyon, 1926) Frenkel, 1977 (Apicomplexa: Cystoisosporinae) em gatos. *Arquivos da Universidade Federal Rural do Rio de Janeiro*, v. 15, n. 1, p. 79-84, 1992.
- MARKUS, M. B. Terminology for invasive stages of protozoa of the subphylum Apicomplexa (Sporozoa). *South African Journal of Science*, v. 74, n. 2, p.105-106, 1978.
- MASSAD, F. V., OLIVEIRA, F. C. R., ALBUQUERQUE, G. R., LOPES, C. W. G. Hipnozoítas de *Cystoisospora ohioensis* (Dubey, 1975) Frenkel, 1977 (Apicomplexa: Cystoisosporinae) em frangos. *Revista Brasileira de Ciência Veterinária*, v. 10, n. 1, p. 57-58, 2003.
- OLIVEIRA, F. C. R. de; ALBUQUERQUE, G. R.; LOPES, C. W. G.; MUNHOZ, A. D.; MASSAD, F. V. Hipnozoítas de *Cystoisospora ohioensis* (Dubey, 1975) Frenkel, 1977 (Apicomplexa: Cystoisosporinae) recuperados de órgãos de camundongos através da digestão péptica. *Revista Brasileira de Parasitologia Veterinária*, v. 10, n. 1, p. 29-35, 2001.
- PATTON, S.; RABINOWITZ, A. R. Parasites of wild felidae in Thailand: a coprological survey. *Journal of Wildlife Diseases*, v. 30, n. 3. p. 472-475, 1994.
- SOUZA, W. J. S. de; LOPES, C. W. G. Separation and purification of *Hammondia heydorni* (Apicomplexa: Sarcocystidae) oocysts from dogs faeces by modified Vetterling's method. *Arquivos da Universidade Federal Rural do Rio de Janeiro*, v. 7, n. 2, p. 161-164, 1984.

Received on April 24, 2003.

Accepted for publication on August 4, 2003.