HYPNOZOITES OF *Cystoisospora felis* (WENYON, 1923) FRENKEL, 1977 (APICOMPLEXA: CYSTOISOSPORINAE) IN SWINE (*Sus scrofa domesticus*) VISCERAS: A NEW INTERMEDIATE HOST*

PATRÍCIA S. MELO[1]; PAULO R. DE CARVALHO FILHO[2]; FRANCISCO CARLOS R. DE OLIVEIRA[3]; WALTER FLAUSINO[4]; CARLOS W.G. LOPES[4]

ABSTRACT. MELO, P.S.; CARVALHO FILHO, P.R. DE; OLIVEIRA, F.C.R. DE; FLAUSINO, W.; LOPES, C.W.G. **Hypnozoites of *Cystoisospora felis* (Wenyon, 1923) Frenkel, 1977 (Apicomplexa: Cystoisosporinae) in swine (*Sus scrofa domesticus*) visceras: a new intermediated host.** [Hipnozoítas de *Cystoisospora felis* (Wenyon, 1923) Frenkel, 1977 (Apicomplexa: Cystoisosporinae) em vísceras de suínos: um novo hospedeiro intermediário.] *Revista Brasileira de Parasitologia Veterinária*, v. 12, n. 3, p. 103-107, 2003. Curso de Pós-Graduação em Ciências Veterinárias, Universidade Federal Rural do Rio de Janeiro, Km 7 da BR 465, Seropédica, RJ 23890-000, Brazil. E-mail: *paulorcf@ufrrj.br*

This research aimed at to evaluate the possibility of swines to become infected by *Cystoisospora felis*. Eight Large White weaned pigs were divided into two groups: infected and control, each one with four animals. Animals from infected group were orally inoculated with 3.5 x 10^5 *C. felis* sporulated oocysts. One animal from each group were euthanasied on 3rd, 7th, 14th and 33rd days after infection (DAI). During the necropsies, the large and the small intestines, Payer's patches, mesenteric lymph nodes, spleen and liver were taken, weighed, ground and submitted to the peptic tissue digestion technique. Hypnozoites were quantified in each viscera and their length and width were measured. Except for the large intestine, *C. felis* hypnozoites were found in mentioned visceras. Hypnozoite length and width varied during the experimental interval and in general their dimensions were larger on 7th DAI. Beyond it, hypnozoites had their cytoplasms full of granules which are supposed to be amilopectin, demonstrating that in swines, the parasite could change their evolutive stage from sporozoites to hypnozoites, reason for to consider this animal an intermediate host of *C. felis*.

KEY WORDS*:* Pigs; visceras; hypnozoites; *Cystoisospora felis;* peptic digestion.

RESUMO

Este estudo objetivou avaliar a infecção de suínos por *Cystoisospora felis*. Para isto, oito leitões da raça Large White recém-desmamados foram divididos em dois grupos: controle e infectado, cada qual com quatro animais. Os leitões do grupo infectado foram inoculados com 3,5 x 10^5 oocistos esporulados de *C. felis*. Um a um, os animais dos dois grupos foram eutanasiados nos dias 3, 7, 14 e 33 após a infecção (DAI). Durante a necropsia, os intestinos delgado e grosso, placas de Peyer, linfonodos mesentéricos, baço e fígado foram separados, pesados, triturados e submetidos à técnica de digestão péptica de tecidos. Os hipnozoítas foram quantificados em cada víscera e medidos a partir do comprimento e da largura. Com exceção do intestino grosso, hipnozoítas de *C. felis* foram encontrados em todas as vísceras estudadas. Quanto às medidas, o comprimento e a largura variaram durante todo o intervalo experimental e em geral, eles se apresentaram maiores no 7° DAI (p=0,01). Além disto, os hipnozoítas observados apresentaram o citoplasma granulado o que vem a se supor ser grânulos de amilopectina, demonstrando que o parasito poderia mudar a sua fase evolutiva de esporozoíta para hipnozoíta, razão

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[1]Coordenadoria de Defesa Sanitária Animal / SEAAPI-RJ. E-mail: *patriciaseibel@superig.com.br*

[2]Curso de Pós-Graduação em Ciências Veterinárias, Universidade Federal Rural do Rio de Janeiro, Km 7 da BR 465, 23890-000 Seropédica, RJ, Brasil; bolsista do CNPq. E-mail: *paulorcf@ufrrj.br*

[3]Laboratório de Sanidade Animal, CCTA, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, RJ, Brasil. E-mail: *foliveira@uenf.br*

[4]Departamento de Parasitologia Animal/IV, UFRRJ, Km 7 da BR 465, Seropédica, RJ 23890-000, Brasil. E-mail: *flausino@ufrrj.br*; *lopescwg@ufrrj.br*

para considerar o suíno como um hospedeiro intermediário de *C. felis.*

PALAVRAS-CHAVE*:* suínos, vísceras, hipnozoítas, *Cystoisospora felis*, digestão péptica

INTRODUCTION

Cystoisospora felis belongs to the family Sarcocystidae and to the subfamily Cystoisosporinae because its biological and morphological characteristics, life cycle and evolutive stages, such as the ability to produce monozoic cysts in intermediate and definitive hosts and having fractures on the sporocyst wall from sporulated oocysts (SMITH, 1981).

Domestic and wild cats are the definitive hosts of this coccidium (HITCHCOCK, 1955; AMARAL et al., 1966; PATTON; RABINOWITZ, 1994) and several warm blood animals are considered intermediate hosts, such as: mice, rats, hamsters (FRENKEL; DUBEY, 1972; LOSS; LOPES, 1992; FREIRE; LOPES, 1996), dogs (DUBEY, 1975), birds (LINDSAY; BLAGBURN, 1994), cattle (FAYER; FRENKEL, 1979), rabbits (COSTA; LOPES, 1998) and broiler chickens (MASSAD et al., 2002).

It is already known that some livestock species such as swines, cattle, rabbits and poultry can harbor cysts of many protozoa species in their organs and muscles and many of them are zoonosis. But there is no study evaluating the effects of *C. felis* infection in swines.

This paper aimed at to verify whether swines can be considered experimental intermediate hosts of *C. felis* by using a modified tissue peptic digestion technique in order to search for hypnozoites in digested visceras and to evaluate whether these animals could also show clinical signs of this infection.

MATERIALS AND METHODS

Animals

Eight Large White weaned pigs were obtained from the Sector of Swine Breeding at the Universidade Federal Rural do Rio de Janeiro in the State of Rio de Janeiro. They were divided into two groups, each one with four animals. Each pig of the first group were orally infected with a purified solution containing 3.5 x 10^5 *C. felis* sporulated oocysts (SOUZA; LOPES, 1984). The second one received NaCl solution at 0.9% and they remained as control. All pigs had their rectal temperatures daily taken. One by one, a piglet from both groups were successively euthanasied by using endovenous injection of sodium thiopental and posted on 3rd, 7th, 14th and 33rd days after infection (DAI). At necropsy, small and large intestines, Peyer's patches, mesenteric lymph nodes, liver and spleen were removed. Visceras were individually weighed, ground, blended and submitted to the peptic digestion. During the experimental period, pigs had food and water *ad libitum*.

Peptic digestion of visceras

The technique used in this experiment was modified from Dubey's tissue digestion method (DUBEY, 1998; OLIVEIRA et al., 2001). All visceras were individually ground using a Mix Braun Handblender MR310® and therefore, 10g-aliquot obtained from each total mixed visceras was separated and submitted to tissue digestion. To each tissue sample it was added 200 ml of acid pepsin (Sigma, St. Louis - USA, pepsin 2.6g; NaCl 5.0g; HCl 7.0 ml; H_2O q.s.p. 500 ml; pH 1.10 - 1.20) with assay activity 1:3,000 at 37°C and shaked for 60 minutes in a shaker chamber (Câmara Modelo 346, FANEM, São Paulo, Brazil). After digestion, the tissue-pepsin solution was filtrated throughout a double-layered gauze and centrifuged at 1,200 x *g* for 10 minutes in 50 ml-plastic tubes. Pellets were suspended in 10 ml of PBS pH 7.2 (0,025 M) and were neutralized with 8 ml of 1.2% $NaHCO_2$ pH 8.3 solution. The neutralized solution was centrifuged again just like before but at this time, the pellets were resuspended in 2.5% gluteraldehyde (volume around 0.5 and 1.0 ml).

Estimation of hypnozoites in swine visceras

An aliquot of 10 µl of digested viscera in 2.5% gluteraldehyde suspension were used to count hypnozoites between slide and coverglass on an light microscope using the following formula: "**H = n . 100 . v**", being "**H**" the number of hypnozoites harbored in 10g of digested viscera; "**n**" the number of hypnozoites counted in 10 µl of digested viscera suspension; "**100**" the correction factor to 1000 µl and "**v**" the aliquot volume.

Measurement of hypnozoites

The measurement of hypnozoites resuspended in 2.5% gluteraldehyde were done by using a K-15X PZO micrometric ocular in a optical microscope and for this, it was used 10 µl of digested tissue suspension. Length and width of five hypnozoites found in each sample were measured.

Statistical analysis

Hypnozoite measurements were evaluated using the Multiple Comparison Tukey-Kramer statistic test. This test was done by using the program Graph Pad Instat™, Copyright 1990-1994, Graph Pad Software. Comparisons were done within a same day of necropsy using measurements from different visceras and using hypnozoite length and width from similar visceras on different days of necropsy.

RESULTS AND DISCUSSION

No clinical signs were observed either on infected piglets or on control ones, during the experiment.

At the necropsy, all animals presented no alteration on their carcasses. However, the infected animal euthanasied on 3rd DAI had its Peyer's patches strongly swollen (Figure 1) and the infected swine euthanasied on 33rd DAI had hypertrophic mesenteric lymph nodes.

All animals from control group had no *C. felis* hypnozoites in the studied visceras while in the infected piglets, the parasite was found in similar organs. On 3rd DAI, hypnozoites were found in the small intestine, mesenteric lymph nodes and

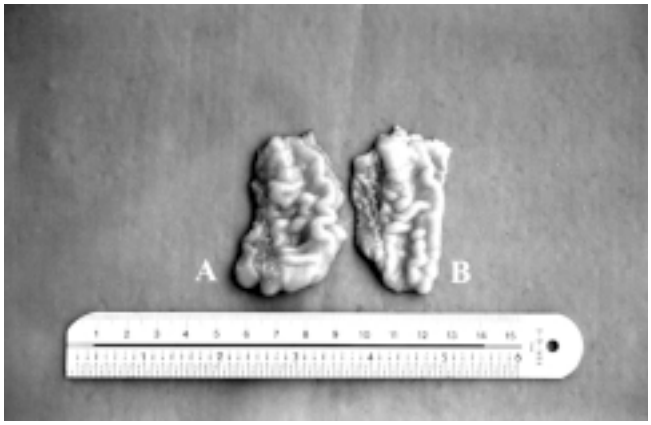

Figure1. Ileum segment of piglet infected with 3,5 x 10^5 *Cystoisospora felis* sporulated oocysts and euthanasied on 3rd day after infection showing Peyer's patches swelling (A). The control animal, euthanasied on the same day, had similar small intestine segment with normal characteristics (B).

The quantity of hypnozoites isolated from each day of necropsy presented no similar characteristics as observed by Costa and Lopes (1998) in rabbits. On 3rd DAI (Table 1), it was isolated 5.0 x 10^3 hypnozoites, on 7th DAI, 1.8 x 10^3 hypnozoites and on 14th and 33rd DAI, it were isolated respectively 4.8 x 10^3 and 9.0 x 10^3 hypnozoites from piglet visceras. The small intestine, mesenteric lymph nodes and spleen were highly attractive for *C. felis* hypnozoites on the first days of experiment. However, the liver became highly harbored by hypnozoites on 33rd DAI.

It is difficult to explain the reasons for this distribution among these visceras. It can be observed that spleen become harbored by hypnozoites earlier than the liver. It could be logical that liver and spleen were reached by this parasite at the same time because both receive blood from Aorta branches through hepatic and splenic arteries, respectively. The liver could be reached earlier than spleen because the reception of blood drained from the intestines by the hepatic portal vein

Table 1. Estimative of *Cystoisospora felis* hypnozoites harbored in visceras of swines experimentally infected with 3.5 x 10^5 sporulated oocysts.

Days after infection	Visceras						Total
	Small intestine	Large intestine	Mesenteri lymph nodes	Peyer's patches	Liver	Spleen	
3rd	2,000	0	1,500	*	0	1,500	5,000
7th	150	0	188	175	560	740	1,813
14th	340	0	920	175	875	2,520	4,830
33rd	1,500	0	500	750	1,500	4,800	9,050

* Not analyzed.

Table 2. Means of length measures of *Cystoisospora felis* hypnozoites harbored in visceras of swines infected with 3.5 x 10^5 sporulated oocysts.

Days after infection	Visceras (means ± standard deviation)#				
	Small intestine	Mesenteric	Peyer's patches	Liver	Spleen
3rd	17.18 ± $0.15^{b,A}$	14.21 ± $0,37^{a,A}$	*	N	14.29 ± $0.32^{a,A}$
7th	17.42 ± $0.95^{a,A}$	17.23 ± $1.01^{a,B}$	15.50 ± $0.4^{b,A}$	18.16 ± $0.41^{a,B}$	17.57 ± $0.32^{a,B}$
14th	14.70 ± $0.34^{a,b,B}$	13.62 ± $0.3^{b,A}$	15.46 ± $0.15^{a,c,A}$	15.34 ± $0.23^{c,A}$	16.99 ± $0.76^{c,B}$
33rd	15.52 ± $0.19^{a,B}$	16.32± $0.41^{a,B}$	15.66 ± $0.40^{a,A}$	15.90 ± $0.69^{a,A}$	14.83 ± $0.33^{a,A}$

#One hundred hypnozoites measured, five for cell.

^{A,B} In columns, different capital letters mean statistic difference with p£0,01.

^{a,b,c} In lines, different minuscule letters mean statistic difference with p£0,01.

* Not analyzed.

^{N} Organ negative for hypnozoites.

spleen, as much as on the following days of experiment. On 3rd DAI, Peyer's patches aliquot had to be despised because this sample was lost during the laboratorial procedures. Liver became harbored by hypnozoites on 7th DAI and hypnozoites were also found in this viscera on 14th and 33rd DAI. Only the large intestine was negative for *C. felis* hypnozoites all over the study.

(GETTY, 1986; SAAR; GETTY, 1986). But for some reason the spleen became harbored by hypnozoites at first.

Cystoisospora felis seems to have its biological cycle more accelerated than *C. rivolta*. This can be plausible because *C. felis* can be highly isolated before 10th DAI (FREIRE; LOPES, 1996) and *C. rivolta* used to be highly isolated between 7th and 28th DAI (BRÖSIGKE et al., 1982).

Table 3. Means of width measures of *Cystoisospora felis* hypnozoites harbored in visceras of swines infected with 3.5 x 10^5 sporulated oocysts.

Days after infection	Visceras (means ± standard deviation)#				
	Small intestine	Mesenteric	Peyer's patches	Liver	Spleen
3rd	5.2 ± 0.16^{a,A}	4.10 ± 0.32^{b,A}	*	N	5.52 ± 0.46^{a,A}
7th	5.46 ± 0.50^{a,A}	5.25 ± 0.43^{a,B}	5.54 ± 0.26^{a,A}	5.90 ± 0.26^{a,A}	5.46 ± 0.36^{a,A}
14th	4.93 ± 0.64^{a,A}	5.09 ± 0.27^{a,B}	5.06 ± 0.19^{a,A}	5.02 ± 0.31^{a,B}	5.6 ± 0.27^{a,A}
33rd	5.06 ± 0.28^{a,A}	5.02 ± 0.25^{a,B}	5.41 ± 0.31^{a,A}	4.91 ± 0.32^{a,B}	4.82 ± 0.13^{a,A}

#One hundred hypnozoites measured, five for cell.

^{A,B} In columns, different capital letters mean statistic difference with p≤0,01.

^{a,b} In lines, different minuscule letters mean statistic difference with p≤0,01.

* Organ not analyzed.

^{N} Organ negative for hypnozoites.

The present paper cannot help to confirm this hypothesis because the higher quantity of hypnozoites was isolated on the 33rd DAI.

About the hypnozoite measurements taken at the present work (Tables 2 and 3), it was possible to evidence that hypnozoite length was more influenced by differences among organs and days of necropsy than its width. In general, the length means increased on 3rd and 7th DAI and decreased on 14th and 33rd DAI. One reason for that could be the accumulative process that the hypnozoite begins, when a zoite gets into a cell and forms a monozoic cyst. On the following days, the ability to store nutrients seems to decrease and the parasite gets smaller. When it is compared different visceras on a same day, it is difficult to observe a repetition characteristic, however liver had longer and thicker hypnozoites on 7th DAI but these differences were not statistically significant on the same day. Even so, it is coherent that visceras with higher nutrient reservation could provide better conditions to hypnozoite development. This comment can be coincident with observation of hypnozoites full of granulations, which are supposed to be amilopectin granules, isolated from swine visceras, especially from 7th and 14th DAI. Electron-dense granules, similar to amilopectin, in *C. felis* zoites were found by Mehlhorn and Markus (1976) in mesenteric lymph nodes of mice infected with sporulated oocysts.

Freire and Lopes (1996), and Costa and Lopes (1998) measured *C. felis* hypnozoites harbored in mesenteric lymph nodes of mice and rabbits respectively. No longer similar, results found in rabbits seem to resemble with the present results more than measurements from mice. It is possible that *C. felis* has more adaptability to rodents, providing conditions to increase hypnozoite development, than to rabbits and to swines.

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