"IN VITRO" EXCYSTATION OF *CYSTOISOSPORA FELIS* (WENYON, 1923) FRENKEL, 1977 (APICOMPLEXA: SARCOCYSTIDAE)

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SUMMARY: The action of different pretreatment of sodium hypochlorite (NaClO) concentrations, as well as the effect of bovine sodium taurocholate, bovine bile and iso-octyl phenoxy-polyethoxy-ethanol (Triton X-100), were analysed to *Cystoisospora felis* sporozoites excystation. The best way to obtain good yelds of free sporozoites was determined. The best NaClO concentration used as a pretreatment to sporocysts liberation was of 5.5%. Following NaClO, tryptic digests associated to sodium taurocholate showed high efficiency (80% of excystation in 2 hours). The association of trypsin with bovine bile lead to the best results (90% excystation in 2 hours).

KEY WORDS: *Cystoisospora felis*, sporozoites, excystation.

INTRODUCTION

Cystoisospora felis is a protozoan parasite which affects bovines, rabbits, dogs, rodents and cats (BRÖSIGKE *et alii*, 1982). The infection is transmitted by accidental ingestion of oocysts as well as by viscera containing extraintestinal hypnozoites (DUBEY, 1979). Actually "*in vitro*" cultivation of *C. felis* will benefit the possibility of interventions to reverse the damaging aspects of events caused by these parasites (LOSS, 1992). Since coccidia species infecting livestock can be grown from sporozoites in cell cultures (SPEER, 1983), the conditions for obtaining good rates of excystation of sporozoites from sporocysts are upmost necessary (CAWTHORN *et alii*, 1986). The purpose of this study was to establish the best conditions of *C. felis* oocysts conservation, as well as the effects of different sodium-hypochlorite (NaClO) concentrations on the liberation of sporocysts and the activity of surfactants sodium-taurocholate, bovine-bile and iso-octyl phenoxy-polyethoxi-ethanol (Tryton X-100) associated or not to trypsin, for determining the best method of sporozoites excystation "*in vitro*".

MATERIALS AND METHODS

Oocysts of *C. felis* obtained from experimentally infected kittens were stored at 4°C in phosphate buffer saline (PBS) (8 gl^{-1}NaCl, 0.2 gl^{-1}KCL, 1.15 $gl^{-1}Na_2HPO_4$ and 0.2 $gl^{-1}KH_2PO_4$, pH 7.2) until 2 weeks. Oocysts were placed in 16 x 15 screwed capped sterile centrifuge tubes and concentrated by centrifugation (600 x g/20 minutes/ 4°C) in order to give 5 x 10^5 oocysts, which were ressuspended in 7 ml of different NaClO (Indústrias Químicas Ribeiro Ltda - Rio de Janeiro, Brasil) concentrations (5.46%, 2.73%, and 1.36%) and incubated during 30,60 or 120 minutes at 4°C. Sporocyst yelds were evaluated microscopically and their liberation rate determinated the number of free sporocysts in relation to the oocysts obtained after the different treatment in each preparation.

$$\text{Sporocysts} = \frac{\text{Free sporocysts}}{\text{Oocysts}} \times 100$$

Sporocysts were washed sequentially four times in cold PBS to remove traces of NaClO, and added 2.5% trypsin (SIGMA, 1:250) combinated to 0.75% taurocholic acid, 10% of bovine bile, or 1% solution of Triton X-100 (LKB, Sweden), in PBS, in order to give a final volume of 1 ml for each tube containing 0.5 x 10^5 sporocysts. All reagents were solubilized in cold PBS pH 7.2. Bovine bile was sterilized in a 0.22µ membrane filter (Millipore - USA) and stored at -20°C. All the mixtures were incubated equally at 37°C (water bath) during 6 hours. Aliquots were examined at 30 and 60 minutes intervals during the first 3 hours

incubation. Slides containing different mixtures were observed by bright field microscopy. The excystation rate was determinated by the formula:

$$ER = \frac{\text{Free sporozoites}}{\text{Free sporozoites} + \text{sporozoites inside sporocysts}} \times 100$$

Each result obtained was a mean value of four repetitions.

RESULTS AND DISCUSSION

The excystation process in *Cystoisospora felis* seems to be very similar to that described by SPEER (1983) for *C. canis*. The sporocyst wall showed rupture in a site between two of the four curved plates from the inner layer. With the excystation fluid (EF) action a splitting of the outer plate occurs with a break of the sporocyst wall. The sporozoites were liberated by splitting on the internal part of sporocyst (Figures 1 and 2).

Sporozoites of *C. felis* were excysted in the presence of different surfactants (taurocholic acid, Triton X-100, and bovine bile), associated or not with trypsin. Pretreatment of sporocysts with NaClO enhanced the sporozoites liberation.

Sporocystes obtained by treatment with NaClO showed a direct relationship between NaClO concentration and the rate obtained (Table 1). It may be done by the oxidation of the outer layer from the oocysts as previously demonstrated for *Eimeria* (ROBERTS *et alii*, 1970) and *Isospora* (SPEER, 1983). The best excystation was obtained when sporocysts were treated with 5.5% NaClO (Table 2).

Table 1 - *Cystoisospora felis* sporocysts rate[1] obtained according to oocysts treatment with different concentrations of NaClO, at 4°C

NaClO Concentration % (V/V)	Free sporocysts % at different time interval (in minutes)		
	30	60	120
0	0	5 ± 1.10	16 ± 2.00
1.4	20 ± 2.20	25 ± 2.50	30 ± 2.70
2.7	21 ± 2.30	45 ± 3.35	60 ± 3.90
5.5	70 ± 4.10	98 ± 4.70	ND[2]

[1] The values presented are the mean of four repetitions.
[2] ND = not determined.

Table 2 - Excystation rate of sporozoites of *Cryptoisospora felis* from oocysts submited to different fluids at 37°C, following pretreatments with 2.7% (A) or 5.5 (B) NaClO[1]

Excystation fluid[3]	Excystation rate (%)[2]/time (hours)					
	A			B		
	1	2	3	1	2	3
Trp.	8.55 ± 1.00	12.38 ± 1.00	14.90 ± 1.00	4.00 ± 0.70	10.00 ± 1.00	11.20 ± 1.10
Trp.+Taur.	9.52 ± 1.00	13.33 ± 1.00	19.80 ± 1.50	20.00 ± 1.40	80.00 ± 1.00	ND[4]
Taur.	13.50 ± 1.20	17.80 ± 1.40	16.70 ± 1.40	21.00 ± 4.60	56.00 ± 2.50	67.00 ± 2.70
Trp.+Bi.	14.50 ± 1.30	18.90 ± 1,40	62.40 ± 2.60	35.00 ± 2.00	90.00 ± 3.20	ND
Bi.	14.30 ± 1.30	16.50 ± 1.40	37.90 ± 2.00	46.00 ± 2.20	73.00 ± 2.80	ND
Trp.+TX	5.00 ± 0.70	12.10 ± 1.20	LYSIS	5.00 ± 0,74	LYSIS	ND
TX	2.10 ± 0.50	1.70 ± 0.40	LYSIS	10.00 ± 1.00	LYSIS	ND
TX+Bi.	1.70 ± 0.40	1.80 ± 0.40	LYSIS	20.00 ± 1.50	26.00 ± 1.60	LYSIS
TX+Taur.	3.50 ± 0.60	2.00 ± 0.50	LYSIS	30.00 ± 1.50	23.00 ± 1.60	LYSIS
Bi.+Taur.	4.00 ± 0.70	12.00 ± 1.20	15.40 ± 1.00	25.00 ± 1.70	20.00 ± 1.60	ND
Trp.+Bi.+Taur.+ TX	10.60 ± 1.00	10.40 ± 1.10	LYSIS	66.00 ± 2.70	LYSYS	ND
Trp.+Bi.+Taur.	10.00 ± 1.00	23.10 ± 1.60	35.00 ± 2.00	7.60 ± 0.49	71.00 ± 3.00	ND
Trp.+Taur.+TX	1.60 ± 0.40	7.60 ± 0.90	LYSIS	LYSIS	ND	ND
Bi.+Taur.+TX	1.00 ± 0.30	2.00 ± 0.50	0	10.00 ± 1.00	LYSIS	LYSIS
Control	0	2.00 ± 0.50	1.00 ± 0.30	2.00 ± 0.50	7.30 ± 0.90	15.00 ± 1.00

[1] Pretreatment with NaClO during (A) = 120 minutes at 4°C and (B) = 30 min./4°C.
[2] Percentual of excystation.
[3] Trp. = trypsin; Taur. = taurocholic acid; Bi. = bovine bile; TX = Triton X-100.
[4] ND = not determinated.

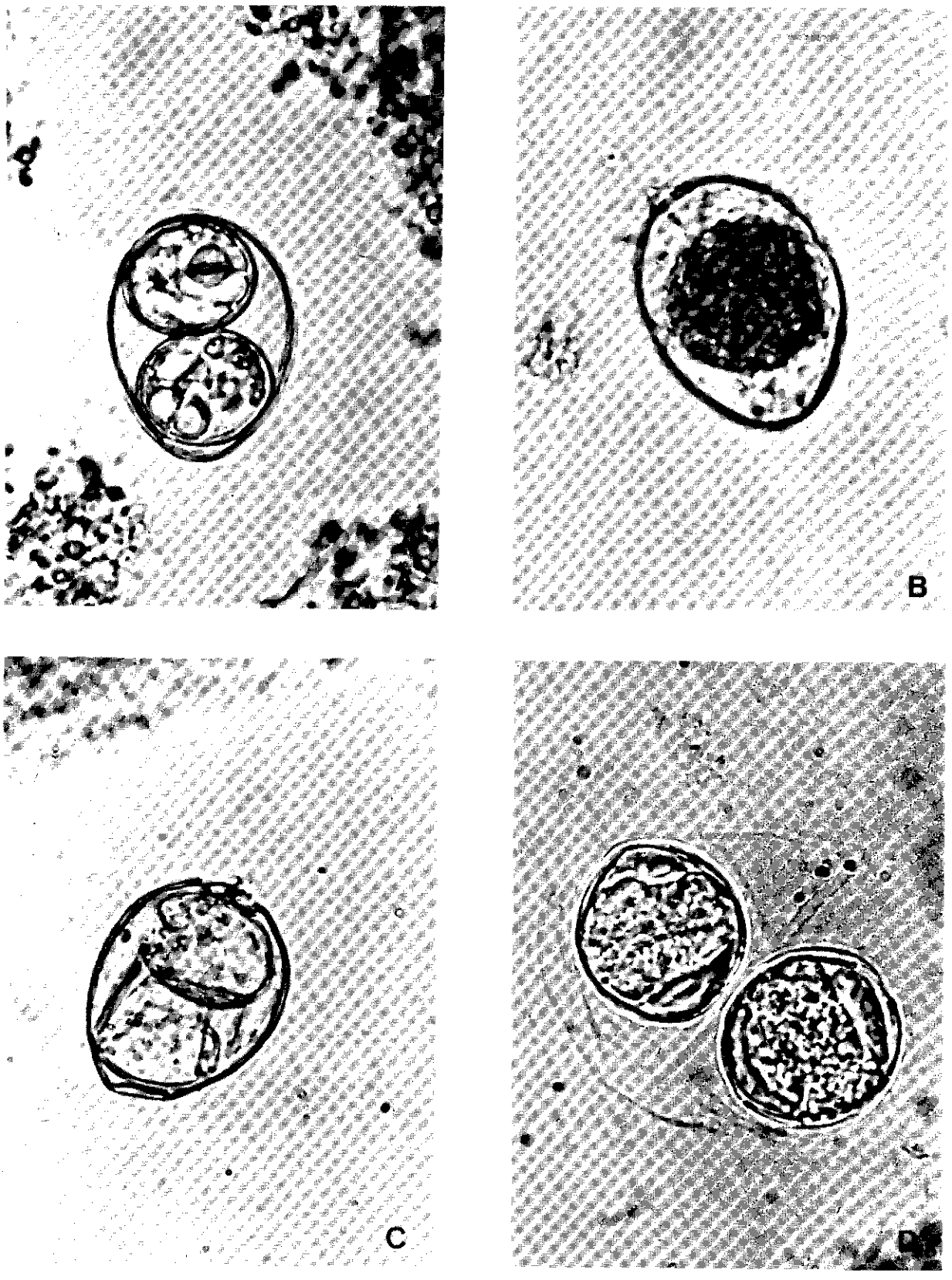

Fig. 1 - *Cystoisospora felis*. Sporulated (A) and unsporulated (B) oocysts in saturated sugar solution. Oocysts with fractures after treatment with 5.5% NaClO (C and D). 1250X.

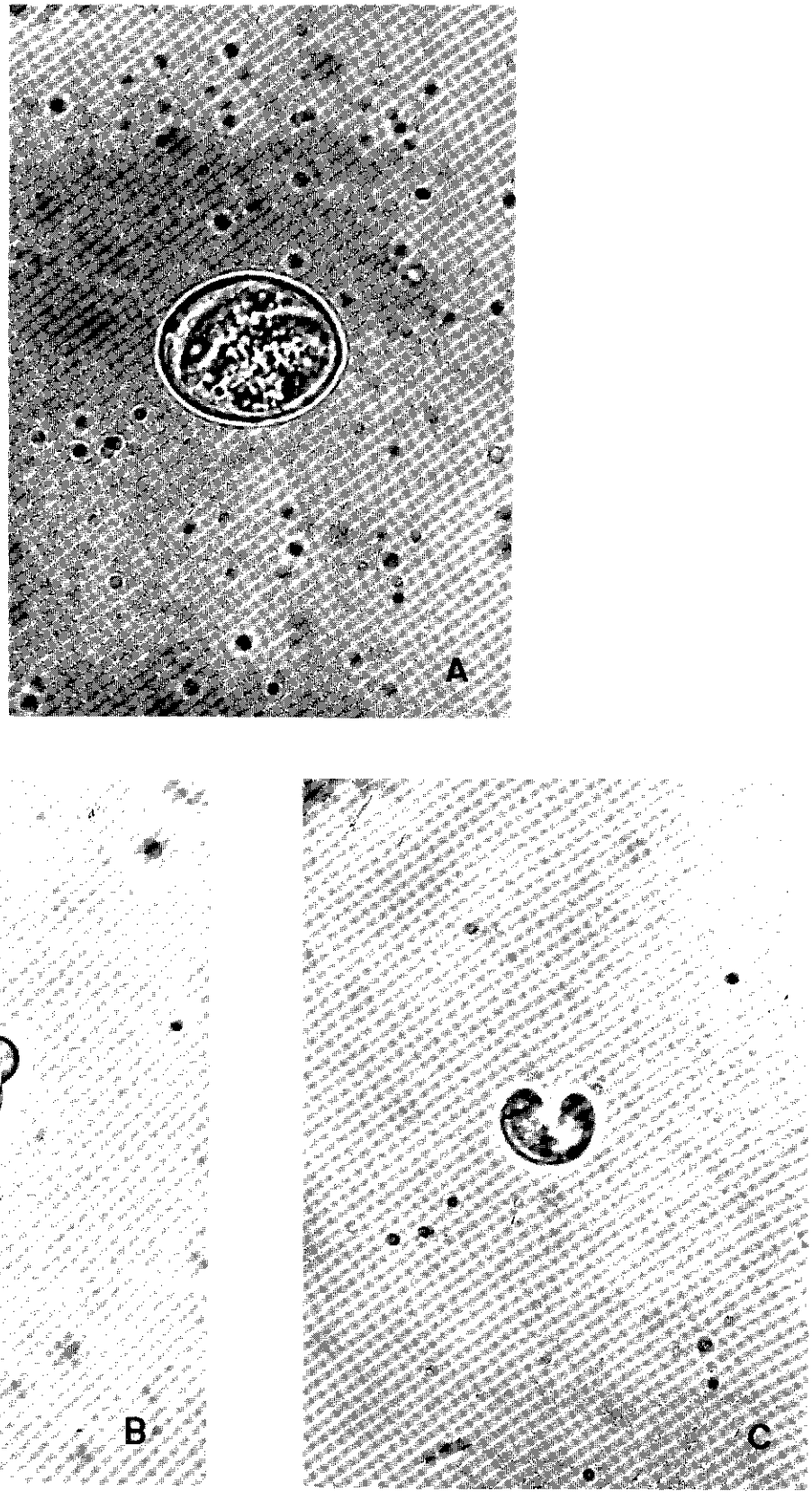

Fig. 2 - *Cystoisospora felis*. Sporocyst freed after treatment with 5.52 NaClO (A); sporozoite during desencystation (B), and free (C), after treatment with Trypsin-bile solution. 1250X.

As demonstrated to *Cryptosporidium* (REDUKER & SPEER, 1985), it was observed some excystation without addition of EF. This aspect seems to be relevant since this characteristic was not demonstrated for *Sarcocystis* (CAWTHORN *et alii*, 1986).

Taurocholic acid showed a positive surfactant action, stimulating the excystation process. Triton X-100, a very well known surfactant was not so good, causing death of all sporozoites.

Following the 5.5% NaClO treatment, the association of trypsin with taurocholic acid was highly efficient (80% of excystation in 2 hours) giving rise to results as good as those obtained when trypsin was associated to bovine bile (90% of excystation in 2 hours).

The greatest activity of trypsin-bile association may be related to other different enzymes in the bile, once the bile itself, without the association of trypsin, gave 73% of excystation in 2 hours of incubation (Table 2). The association of trypsin with taurocholic acid and bovine bile showed the same action as did bovine bile alone.

Excystation obtained with 5,5% NaClO pretreatment gave rise to more consistent results, showing that incubation with EF consisting of 2.5% trypsin plus 10% bovine bile is the best way for getting *C. felis* sporozoites excystation (Table 2).

It is important to point out that the storage conditions are determinant for the excystation of *C. felis*, as well as other coccidia (CAWTHORN *et alii*, 1986). Potassium bichromate ($K_2Cr_2O_7$), for example, has a deleterious effect on *Sarcocystis cruzi* yelding to a decrease of 60% in the excystation rate, after 2 weeks-storage. In the present experiment, oocysts maintained in 2.5% $K_2Cr_2O_7$ gave rise to, at least, 30% excystation. Storage in 1% NaClO also affected oocysts, giving low rates of excystation (40% to 50%). The optimum storage condition was in a sterile saline solution, free from calcium and magnesium, and with of antibiotics and fungistatics.

SUMÁRIO

A ação de diferentes concentrações de hipoclorito de sódio, assim como a ação de diferentes surfactantes: taurocolato de sódio, bile bovina crua e Triton X-100, foram utilizados visando o melhor rendimento na concentração de esporozoítas livres. O tratamento de oocistos com NaClO a 5,5% foi a maneira mais eficiente de promover a liberação de esporocistos. A digestão trípica associada ao taurocolato de sódio, quando aplicada aos esporocistos isolados mostrou alta eficiência (80% de excistação em 2 horas). A associação de tripsina e bile bovina levou aos melhores resultados (90% de excistação em 2 horas).

PALAVRAS-CHAVE: *Cystoisospora felis*, esporozoítas, excistação.

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