RAPID COMMUNICATION

Fulminant Cryptosporidiosis Associated with Digestive

Adenocarcinoma in SCID Mice Infected with *C. parvum* TUM1

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Abstract

We demonstrated recently that *Cryptosporidium parvum* IOWA strain induces in situ ileo-caecal adenocarcinoma in an animal model. Herein, the ability of another *C. parvum* strain and other *Cryptosporidium* species to induce digestive neoplasia in dexamethasone-treated SCID mice was explored. SCID mice infected with *C. parvum* TUM1 developed a fulminant cryptosporidiosis associated with intramucosal adenocarcinoma, which is considered an early histological sign of invasive cancer. Both, evidence of a role of *C. parvum* in adenocarcinoma induction and the extended prevalence of cryptosporidiosis worldwide, suggest that the risk of *C. parvum* induced gastro-intestinal cancer in humans should be assessed.

Keywords: *Cryptosporidium parvum* TUM1, fulminant infection, intestinal adenocarcinoma, intraepithelial neoplasia, dysplasia, SCID mice

1 The genus *Cryptosporidium* comprises several species and genotypes which infect a wide range
2 of hosts, including humans (Xiao, 2010). These parasites are ubiquitous, and the usual lack of
3 morphological differentiation between the species has made difficult to track down sources of
4 human and animal infections. Molecular typing has shown that the main agents of human
5 cryptosporidiosis are *Cryptosporidium hominis* and *C. parvum*. However, *C. meleagridis*, *C. felis*,
6 *C. canis*, *C. suis*, *C. muris*, and several infraspecific *Cryptosporidium* genotypes have also been
7 reported in humans (Xiao, 2010). Humans and animals with cryptosporidiosis can exhibit diverse
8 expression of disease severity with regard to either host susceptibility to infection or virulence of
9 the parasite isolate (Okhuysen and Chappell, 2002). To investigate these biological divergences and
10 to contribute to the understanding of the dynamics of the infection, we developed a reproducible
11 animal model of chronic cryptosporidiosis using dexamethasone (Dex)-treated adult SCID (severe
12 combined immunodeficiency) mice (Certad et al., 2007; Certad et al., 2010). Using such a model,
13 oocyst shedding, clinical impact, location of the infection, associated histopathological changes in
14 the gastrointestinal tract and virulence could be assessed. Unexpectedly, we found that *C. parvum*
15 IOWA strain was able to induce the development of intestinal adenomas with areas of
16 intraepithelial neoplasia in chronically infected SCID mice (Certad et al., 2007; Certad et al., 2010).
17 This important observation stimulated our interest and led us to perform a series of in vivo
18 experiments to determine whether non-IOWA strains of *C. parvum* or other *Cryptosporidium*
19 species are able to both propagate and induce gut neoplasia in the Dex-treated SCID mouse model.

20 *Cryptosporidium* oocysts of different species and strains were obtained from sources indicated in
21 Table I. Particularly, oocysts of *C. molnari* (the sole non mammalian species tested) were obtained
22 from mucosal scrapings of gilthead sea bream stomachs heavily infected by the parasite, as
23 previously described (Alvarez-Pellitero and Sitja-Bobadilla, 2002). Oocyst viability before
24 inoculation was assessed (Certad et al., 2007; Certad et al., 2010) and absence of bacteria or fungi

1 was assured by testing the oocyst suspensions on Plate Count Agar and on Sabouraud plates (37°C,
2 one week).

3 CB17-SCID mice between six and eight weeks of age were obtained from a colony bred and
4 regularly controlled for assessing microbial or parasitological infections, including *Helicobacter*
5 infection, at the Pasteur Institute of Lille (France). Animals were maintained under aseptic
6 conditions in an isolator with standard laboratory food and water ad libitum. Mice were housed and
7 cared following the European guidelines (Council directives on the protection of animals for
8 experimental and other scientific purposes. J. Off. Communautés Européennes, 86/609/EEC, 1986,
9 December 18, L358).

10 SCID mice were housed in covered cages by groups. Infective doses were prepared and were
11 inoculated as indicated in Table I by oral-gastric gavages (18-20 gauge feeding tubes). When
12 needed, SCID mice received 4 mg/L of dexamethasone sodium phosphate (Dex) (Merck, Lyon,
13 France) via the drinking water (Certad et al., 2007; Certad et al., 2010) (Table 1). In all the
14 experiments there were control groups comprised of 4 animals inoculated only with PBS without or
15 with Dex administration. On days 20, 35, 45 and 60 post-infection (PI) or when signs of imminent
16 death appeared mice were euthanatized by cervical dislocation.

17 Fecal specimens were collected from the first day PI until the end of the experiment as
18 previously (Certad et al., 2010). The feces were homogenized by extensive vortexing. Oocysts
19 were concentrated by Dynabeads anti-*Cryptosporidium* kit (Invitrogen, Cergy-Pontoise, France)
20 (Certad et al., 2010). The oocyst number per milligram of feces from all mice in each cage was
21 obtained (Certad et al., 2010). The mouse number in each cage decreased from 4 to 3, to 2, and
22 finally to 1 after sequential euthanasia or death. An immunochromatographic HEM-Sign-3 test
23 (V6063, Servibio, Meudon, France) was used to detect faecal occult blood in experiment 1 (Table
24 1).

Stomach, liver, duodenum, samples of proximal, medium and distal parts of jejunum, ileo-caecal region, and colon were removed from each mice, fixed in 10% buffered formalin, and processed using standard histological techniques and embedded in paraffin. Sections of 5-mm thick were stained with hematoxylin and eosin (H&E). Several stained sections of each solid organ and many longitudinal and transversal sections of gastrointestinal organs were examined using a Leica DMRB microscope equipped with a Leica digital camera connected to an Imaging Research MCID analysis system (MCID software, Cambridge, UK).

All immunohistochemistry was performed on deparaffinized and rehydrated 5 mm sections using the BenchMaerk XTstaining module. Following instructions of suppliers a rabbit polyclonal antibody to laminin (dilution 1: 15) (PU078-UP, BioGenex, Netherlands) was employed as a marker of the basement membrane integrity; a mouse monoclonal antibody to cytokeratin (AM071-5M, Biogenex, Netherlands) was used to visualize epithelial cells; to stain muscle fibers monoclonal antibody anti alpha smooth muscle actin (dilution 1: 100) (M0851, Dako, Denmark) was used.

Parasite rates in digestive sections were scored as follows: 0, no parasites; 1+, small number of parasites focally distributed; 2+ moderate number of parasites widely distributed; 3+, abundant presence of parasites widely distributed throughout the section (Certad et al., 2010).

Pathological changes were classified as previously, according to the “Nomenclature for Histologic Assessment of Intestinal Tumors in the Rodent”, and compared to the “Vienna classification of the epithelial neoplasia of the gastrointestinal tract for humans” (Certad et al., 2010). Lesions at different sites were scored as previously (Certad et al., 2010) with slight modifications Briefly: 0, no lesion; 1, inflammation and/or regenerative changes; 2, low grade intraepithelial neoplasia (LGIEN) or low grade dysplasia; 3, high grade intraepithelial neoplasia (HGIEN) or high grade dysplasia. In this category, adenoma with HGIEN, carcinoma in situ (limited to the epithelium) or intramucosal adenocarcinoma (invasion of the basal membrane of

glands) were also included; 4, suspicion of invasive adenocarcinoma or invasive adenocarcinoma (penetration of dysplastic glands through the muscularis mucosae with desmoplastic stromal response). The degree of severity of histological damage for each mouse was calculated by the sum of individual scores over the five organs as previously (Certad et al., 2010). In order to include spontaneous death as a factor of disease severity, each died mouse before planned euthanasia was assigned with a number of points equivalent to: $20 + ((60 - \text{day of death})/2)$ (where 20 corresponded to the maximum score of severity in animals euthanatized as planned, and 60 corresponded to the end time of the experiment).

An analysis of variance was conducted to account for the effects of relevant factors (inocula, Dex administration, day PI) and their interactions on average daily oocyst excretion or severity. Data analysis was performed with the statistical software S-PLUS 2000 (MathSoft, Seattle, WA, USA). Logarithmic transformations of oocyst excretion values were used. The amount of oocysts/mg of feces was estimated per day and per group of mice, with the number of mice per group decreasing over time. Significance was defined as $P \leq 0.05$.

An experiment was developed using *C. parvum* TUM1 isolated from a calf about 18 months prior to this study (Donna Akiyoshi, personal communication). Regardless of inoculum size, all groups of SCID mice infected with *C. parvum* TUM1 strain showed oocysts in the feces from day-1 PI until the end of the experiment. Oocyst shedding intensities are summarized in table 2. On the whole, the shedding intensity increased over time. After statistical analysis, the time PI had an impact in oocysts shedding ($P < 0.001$) (Dex-treated mice that died before planned euthanasia were excluded from the analysis).

Results reveal variations in relation with clinical manifestations and severity of the disease among groups of Dex untreated or treated animals. The group of Dex-treated animals exhibited diarrhea, in contrast to infected Dex-untreated mice. Diarrhea appeared at 4 weeks PI in animals infected with 10^7 oocysts, at 5 weeks PI in mice infected with 10^6 and at 6 weeks PI in mice

1 receiving 10^5 oocysts. Fecal blood in diarrheic stools was confirmed using the HEM-Sign-3 test. A
2 high spontaneous mortality of Dex- treated mice was recorded as early as the 34th day PI. Seven
3 mice out of 24 died unexpectedly (29%), and additional animals (mice 6 and 14) with evident signs
4 of clinical aggravation (bloody diarrhea, rough hair coat, hunched posture and lethargy) were
5 euthanatized. In these two mice, necropsy showed hemorrhagic caeca, with a dramatic increase in
6 size (Fig. 1-A).

7 Parasite distribution in digestive organs is shown in Table 2. Microscopic examination of tissue
8 sections showed heavy cryptosporidial colonization in the ileo-caecal region. Moderate number of
9 parasites widely distributed in other organs such as stomach, jejunum and colon was also observed.

10 The parasitic colonization level in tissues increased progressively toward the end of the
11 experiment as shown in Table 2.

12 Whatever the inoculum size, histological lesions characterized by different grades of dysplasia
13 were observed in the stomach (Fig.1-B), biliary tree, duodenum, ileo-caecal region (Fig. 1 C-F), and
14 colon, always associated with parasites, and a strong positive correlation between intensity of
15 parasites in the tissues and the development of neoplastic lesions was observed ($r = 0.97$, $P <$
16 0.001). Distribution of mice displaying lesions in different organs is summarized in Table 2. In
17 total, 16/17 (94%) mice had neoplastic lesions in one organ or more. Particularly, adenomas lined
18 by dysplastic glandular epithelium, containing high number of parasites were observed in the ileo-
19 caecal region of 11/11 (100%) SCID mice after day 35 PI. Ileo-caecal lesions reported severity
20 scores of 3 and 4, and were characterized by loss of cellular polarity, increased nuclear
21 polymorphism, increased number of mitosis and fusion of glands (Fig. 2). Laminin stain showed an
22 irregular and fragmented basement membrane (Fig. 1-C). Neoplastic epithelial cells at the corion
23 level were identified as focus of intramucosal adenocarcinoma by cytokeratin staining (Fig.1-D).
24 Invasion of the submucose was suspected in four mice (mice N° 4, 6, 13 and 14) in which an
25 interruption of the muscularis mucosae was observed using immunocytochemical staining for alpha

1 smooth muscle actin (Fig. 1-E, 1-F). Multifocal necrosis of the glands accompanied by
2 inflammatory cell infiltration was also observed.

3 The earliest apparition of intraepithelial neoplasia was recorded at day 20 in the ileo-caecal
4 region of Dex-treated mice infected with 10^5 - 10^7 , and in the stomach of one mouse infected with
5 10^6 oocysts not treated with Dex.

6 Variable colonization of the duodenum and intrahepatic bile ducts was found, and the presence
7 of parasites in these organs coincided with the presence of dysplastic changes. LGIEN or HGIEN in
8 the duodenum was observed in two Dex-treated mice. In the liver of 2/12 (17%) Dex-untreated
9 SCID mice inoculated with 10^7 oocysts, bile ducts were enlarged and lined by mild dysplastic cells.
10 At the histological examination of the jejunum a small number of parasites attached to the epithelial
11 cells and villus atrophy and crypt hyperplasia were found without significant differences between
12 groups of Dex-untreated or treated SCID mice. Jejunum infection was not associated with
13 dysplastic changes.

14 Analysis of variance of the whole dataset showed that day PI, administration of Dex and the
15 interaction between both, significantly influenced the severity of the infection ($P < 0.001$, $P <$
16 0.001 , $P = 0.006$, respectively). Neither parasites nor lesions were observed in Dex-treated control
17 animals.

18 Although a good oocyst viability was observed (>80%) before inoculation, neither *C. molnari*
19 nor *C. hominis* infections developed in Dex-treated or untreated SCID mice. All fecal samples of
20 mice infected with these two species were negative from the beginning of the study until the end.
21 The histological examination of the stomach, duodenum, jejunum, ileo-caecal region and colon
22 revealed absence of parasites or lesions. Neither *Cryptosporidium* development, nor histological
23 lesions were recorded in control groups.

24 The initial objective of this series of experiments was to set a reproducible animal model to
25 propagate *Cryptosporidium* isolates in order to characterize and compare their phenotypic features.

1 SCID mice treated with Dex were selected as experimental hosts. Using such a model good
2 propagation of at least two species of *Cryptosporidium* was obtained, and marked divergences
3 between *Cryptosporidium* species were revealed (Certad et al., 2007; Certad et al., 2010). Thus, the
4 present study strengthen previous observations (Certad et al., 2007; Certad et al., 2010) and showed
5 how this experimental model revealed more efficient than current rodent models to perpetuate and
6 amplify isolates of *C. muris* (Certad et al., 2007) and *C. parvum*.

7 Variations in the expression of the disease in terms of species, inoculum size or oocyst shedding
8 were found when the infectivity and pathogenicity of different species and strains of
9 *Cryptosporidium* were compared. In the present work *C. parvum* TUM1 strain developed a
10 fulminant infection entailing a high morbidity and mortality. The infection was rapidly established
11 and remained throughout the study. Additionally, histopathological examination revealed digestive
12 neoplasia as reported previously in *C. parvum* IOWA infected mice (Certad et al., 2007; Certad et
13 al., 2010). However, *C. parvum* TUM1 strain seems to induce more severe illness than *C. parvum*
14 IOWA (Certad et al., 2007; Certad et al., 2010). In SCID mice infected with *C. parvum* TUM1
15 treated or not with DEX, the intensity of oocyst shedding was about 18 folds higher than current
16 oocyst shedding of *C. parvum* IOWA infected animals (Certad et al., 2007), and persistent
17 infections were even lethal. As well, in mice infected with TUM1 strain there was an earlier onset
18 of neoplastic lesions detected with a very rapid progression to invasive cancer at day 20 PI (Fig. 1-
19 E, 1-F). In contrast, in our previous works in *C. parvum* IOWA infected mice, first dysplastic
20 lesions were found after day 35 PI (Certad et al., 2007; Certad et al., 2010) and signs of invasive
21 adenocarcinoma were observed after day 90 PI (unpublished data). Extraintestinal involvement
22 was also more pronounced in SCID mice infected with TUM1 isolate as shown by parasitic
23 involvement of the bile ducts presenting dysplastic changes.

24 Despite the dearth of molecular data, *C. molnari* was described as a new species located mostly
25 at the stomach in two marine fish species, *Sparus aurata* and *Dicentrarchus labrax* (Alvarez-

1 Pellitero and Sitja-Bobadilla, 2002). Being the stomach its predilection site of infection, we were
2 interested in assessing whether *C. molnari* could be related to other gastric *Cryptosporidium* species
3 such as *C. muris*, which propagates easily in our model (Certad et al., 2007) and in a wide spectrum
4 of hosts (Kvác et al., 2008). Nevertheless, in this study *C. molnari* was not infectious to SCID mice
5 treated with Dex. The failure of *C. molnari* to develop in this model suggests that this species is not
6 able to grow in the digestive tract of mammals.

7 On the other hand, the lack of development of *C. hominis* in SCID mice was an expected
8 observation since this species has been isolated almost exclusively from human hosts (Baishanbo et
9 al., 2005). However, we were interested in exploring the capacity of infection using our model since
10 isolates of this species have been successfully propagated in other experimental models (Baishanbo
11 et al., 2005).

12 Our results confirm that infection dynamics varies according to different species and strains of
13 *Cryptosporidium*. Some previous studies have shown that humans and other mammals with
14 cryptosporidiosis can exhibit diverse expression of disease severity depending on host susceptibility
15 or virulence of the parasite isolate (Okhuysen and Chappell, 2002). As inferred from experimental
16 infections and epidemiological data, the piscine *C. molnari* have been found more pathogenic for
17 gilthead sea bream than for sea bass (Alvarez-Pellitero and Sitja-Bobadilla, 2002). Additionally,
18 species of *Cryptosporidium* and sub-types families are linked to different clinical manifestations in
19 humans and have shown to have a different potential to cause outbreaks (Xiao, 2010).

20 A strong positive correlation between presence of parasites in the tissues and the development of
21 neoplastic lesions was observed. A similar correlation was found in our previous study (Certad et
22 al., 2010) strengthening a direct role of *C. parvum* in the development of dysplasia and
23 adenocarcinoma in SCID mice. Particularly, the severity of ileo-caecal lesions confirms the
24 correlation between elevated parasite loads and pathology (Certad et al., 2010). Previous studies
25 have shown that the terminal ileon is the center of *C. parvum* colonization (Mead et al., 1991a),

1 maybe explaining the highest parasite loads and primary neoplastic lesions in this area, which
2 spread gradually throughout the gastro-intestinal tract and towards extraintestinal tissues.

3 Dysplasia-related terminology is continuously changing according to schools of thought,
4 countries or other factors. However, the most serious lesions observed in *C. parvum*-infected SCID
5 mice clearly correlated with severe epithelial dysplasia as cytological anomalies were marked.
6 Additionally, intramucosal adenocarcinoma, considered as the earliest histopathologically
7 recognizable form of colorectal adenocarcinoma (MacDonald et al., 2009) was observed clearly
8 invading connective tissue stroma. Even more, some of these lesions were highly suggestive of
9 invasive adenocarcinoma, as long as features of malignancies were observed, and since
10 immunohistochemical staining using alpha smooth actin showed real permeation of the submucosa
11 (Fulcheri et al., 1988).

12 Most animals showed severe neoplastic changes. Only some mice showed minor dysplasia but
13 even in these cases, dysplastic changes induced by *C. parvum* can be considered as putative
14 precursors to digestive carcinoma (Luperchio and Schauer, 2001). Actually, colorectal
15 tumorigenesis is believed to involve a series of genetic changes leading to the initiation of the
16 neoplastic process and the progression from normal epithelium to carcinoma (Luperchio and
17 Schauer, 2001). Taken together, higher rates of oocyst shedding seem to be associated with an
18 earlier onset and more rapid evolution of neoplastic lesions. Lower dose groups could reach the
19 similar high intensity of shedding and a similar severity of lesions but they may require a longer
20 period.

21 In animals, the presence of *Cryptosporidium* in intestinal polyps of natural infected sheep
22 (Gregory et al., 1987), and in aural-pharyngeal pedunculated polyps of iguanas (Uhl et al., 2001)
23 was reported, but no signs of dysplasia or malignancy were observed. The occurrence of dysplastic
24 changes in bile ducts was previously reported in a model of IFN- γ knockout mice infected with
25 *Cryptosporidium* (Stephens et al., 1999). The authors of this study suggest that the response of these

1 mice to *Cryptosporidium* infection may model the initial steps towards the development of
2 cholangitis and bile duct related cancer in patients with immunodeficiency (Stephens et al., 1999).
3 In the present study, particularly notable were the dysplastic changes found in the biliary tree of
4 mice.

5 Concerning the human host, a recent study reported 18% of cryptosporidiosis prevalence among
6 55 patients with colorectal cancer in Poland (Sulzyc-Bielicka et al., 2007). This prevalence is higher
7 in comparison to the burden of cryptosporidiosis for the European general population (Semenza and
8 Nichols, 2007). In HIV-infected subjects known to be highly susceptible to *Cryptosporidium*
9 infections, the incidence of colorectal cancer was found 2.3-fold (1.8 to 2.9) higher than in the
10 general population (Patel et al., 2008).

11 In conclusion, *C. parvum* TUM1 strain has an aggressive development in SCID mice.
12 Additionally, the present work provides supplementary evidences of a direct role of *C. parvum* in
13 the induction of gastrointestinal cancer, and shows that the ability of the parasite to induce
14 neoplasia is not a strain dependent process. Furthermore, our data demonstrate that *C. parvum*
15 induced neoplasia is an invasive process that can evolve rapidly, at least in immunosuppressed hosts.
16 For these reasons we consider that the risk of gastrointestinal cancer in humans due to
17 *Cryptosporidium* needs to be assessed. In fact, mouse models of cryptosporidiosis mimic the
18 evolution of persistent cryptosporidiosis in immunodeficient patients (chronic diarrhea, extra-
19 intestinal dissemination, clinical deterioration) (Mead et al., 1991b). Research on the topic could be
20 worthwhile since the incidence of *Cryptosporidium* infection seems to increase not only among
21 immunosuppressed patients but also in the general population worldwide. For instance, in Trujillo
22 State, Venezuela, *Cryptosporidium* oocyst shedding was reported in 89% of healthy children
23 attending day-care centers (Miller et al., 2003). In the United States a dramatically increase of the
24 incidence in last years was recently reported, mainly due to a substantial raise in community
25 outbreaks (Yoder and Beach, 2010). Moreover, low infectious dose and the chlorine resistance of

1 oocysts make *Cryptosporidium* ideally suitable for transmission through drinking and recreational
2 water, food, person to person and animal to person contact, facilitating the expansion of the
3 infection in the community (Yoder and Beach, 2010).

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Table 1. Testing SCID mice susceptibility to *Cryptosporidium* species: experimental design

Experiment Nº	<i>Cryptosporidium</i> species	Strain, origin	Nº of mice							
			Inoculum size (number of oocysts) ^a							
			10 ⁵		10 ⁶		4x10 ⁶		10 ⁷	
			Dexamethasone							
-	+	-	+	-	+	-	+			
1	<i>C. parvum</i>	TUM1 ^b , Tufts Cummings School of Veterinary Medicine, (Dr. S. Tzipori)	4	4	4	4	ND	ND	4	4
2	<i>C. molnari</i>	Instituto de Acuicultura Torre la Sal (CSIC), Ribera de Cabanes, Castellón, Spain (Dr. P. Alvarez)	6	6	ND	ND	ND	ND	ND	ND
3	<i>C. hominis</i>	TU502, Tufts Cummings School of Veterinary Medicine, (Dr. S. Tzipori)	4	4	4	4	4	4	ND	ND

ND: not done

^aOocysts were inoculated in 200 µl of PBS.

^bIsolated from calf about 18 months prior to the present study.

Table 2. Development of *Cryptosporidium* species in SCID mice: associated lesions

Mous e N ^o	Dex ^a	Inoculum	Day PI	Log ₁₀ of ooc/mg feces (number of mice) ^b	Histopathological findings ^c										Death before planned euthanasia (Day PI)	Score ^e
					(Presence of <i>C. parvum</i> organisms) ^d											
					Stomach		Biliary tree		Duodenum		Ileo- caecal region		Colon			
1	No	10 ⁵	20	4,13 (4)	0	(0)	0	(0)	0	(0)	0	(+1)	0	(0)	-	0
2			35	5,69 (3)	2	(+2)	0	(0)	0	(0)	3	(+3)	2	(+2)	-	7
3			45	5,44 (2)	2	(+1)	0	(0)	0	(0)	3	(+3)	2	(+2)	-	7
4			60	5,83 (1)	2	(+2)	0	(0)	0	(0)	4	(+3)	3	(+3)	-	9
5	Yes	10 ⁵	20	5,13 (4)	0	(0)	0	(0)	0	(0)	3	(+3)	0	(+1)	-	3
6			35	6,08 (3)	2	(+2)	0	(0)	2	(+2)	4	(+3)	0	(0)	-	8
7			45	-	-	-	-	-	-	-	-	-	-	-	Yes (45)	27.5
8			60	-	-	-	-	-	-	-	-	-	-	-	Yes (45)	27.5
9	No	10 ⁶	20	4,37 (4)	2	(+1)	0	(0)	0	(0)	0	(+1)	0	(0)	-	2
10			35	5,79 (3)	0	(0)	0	(0)	0	(0)	3	(+2)	0	(0)	-	3
11			45	5,42 (2)	2	(+1)	0	(0)	0	(0)	3	(+3)	2	(+2)	-	7
12			60	6,00 (1)	2	(+2)	0	(0)	0	(0)	3	(+3)	3	(+3)	-	8
13	Yes	10 ⁶	20	5,53 (4)	0	(0)	0	(0)	1	(0)	4	(+3)	0	(0)		5
14			35	6,56 (3)	3	(+3)	0	(0)	3	(+3)	4	(+3)	0	(0)		10
15			45	-	-	-	-	-	-	-	-	-	-	-	Yes (34)	33
16			60	-	-	-	-	-	-	-	-	-	-	-	Yes (38)	31
17	No	10 ⁷	20	4,59 (4)	0	(0)	0	(0)	0	(0)	2	(+2)	0	(0)		2
18			35	5,76 (3)	0	(0)	0	(0)	0	(0)	3	(+3)	2	(+2)		5
19			45	6,11 (2)	2	(+2)	2	(+2)	0	(0)	3	(+3)	2	(+2)		9
20			60	6,04 (1)	2	(+2)	2	(+2)	0	(0)	3	(+3)	3	(+3)		10
21	Yes	10 ⁷	20	5,59 (4)	2	(+2)	0	(0)	1	(0)	2	(+2)	0	(0)		5
22			35	-	-	-	-	-	-	-	-	-	-	-	Yes (34)	33
23			45	-	-	-	-	-	-	-	-	-	-	-	Yes (38)	31
24			60	-	-	-	-	-	-	-	-	-	-	-	Yes (38)	31

Dex, Dexamethasone; PI, Post-Infection; ooc/mg, oocysts per milligram.

^a Dexamethasone (4 mg/l of drinking water) administration was started 2 weeks prior to inoculation and maintained during the whole experimentation.

^b The amount of oocysts was calculated according to the number of mice in each cage at each time point (showed in brackets). One mouse was euthanized at each of the indicated times.

^c Lesions at different sites were scored as follows: 0: No lesion, 1: Inflammation or regenerative changes, 2: Low grade Intraepithelial neoplasia, 3: High grade intraepithelial neoplasia or adenoma with high grade dysplasia, and 4: Suspicion of invasive adenocarcinoma or invasive adenocarcinoma.

^d In parentheses were added the results of the histological semi-quantitative assessment of *C. parvum* organisms in the host tissues (see 'Materials and Methods'). The presence of parasites in the tissues was scored as follows: 0, no parasites; 1+, small number of parasites focally distributed; 2+ moderate number of parasites widely distributed; 3+, abundant presence of parasites widely distributed throughout the tissue.

^e The degree of severity of histological damage for each mouse was calculated by the sum of individual scores over the five organs. In order to include the mortality as a factor of disease severity, each died mouse before planned euthanasia was assigned with a number of points equivalent to: $20 + ((60 - \text{day of death})/2)$ (where 20 corresponded to the maximum score of severity that can be reached by animals euthanized as planned, and 60 corresponded to the end time of the experiment). Control mice (uninfected Dex-treated SCID mice, and Dex-treated SCID mice inoculated with an inoculum from which *Cryptosporidium* oocysts were removed by filtration, see 'Materials and Methods') were euthanized at the same dates as experimental mice. None of the mice of control groups developed *Cryptosporidium* infection or histological gastrointestinal lesions.

1 **Figure legends**

2 **Fig. 1.** (A) Ileo-caecal region at necropsy of a Dex-treated SCID mouse infected with 10^5 oocysts of
3 *C. parvum* TUM1 (day-42 PI). The caecum is enlarged and hemorrhagic. (B) Histological section of
4 the stomach of a Dex-treated SCID mouse infected with 10^6 oocysts of *C. parvum* TUM1 (day-60
5 PI). Intraepithelial neoplasia of high grade is characterized by architectural distortion of glands and
6 loss of gland differentiation with epithelial atypias consisting of loss of normal polarity, nuclear
7 stratification, prominent nucleoli and irregularly scattered chromatin. Presence of parasites (arrow)
8 inside the glands is shown (Hematoxylin & Eosin) (Bar=50 μ m). (C) Histological section of a
9 polypoid adenoma of the ileo-caecal region of a Dex-treated SCID mouse infected with 10^6 oocysts
10 of *C. parvum* TUM1 (day-35 PI). Laminin stain shows an irregular and fragmented basement
11 membrane of the glands (arrows) (intramucosal carcinoma) (Bar=100 μ m). (D) Histological section
12 of a polypoid adenoma of the the ileo-caecal region of a Dex-treated SCID mouse infected with 10^6
13 oocysts of *C. parvum* TUM1 (day-35 PI). Cytokeratin stain shows invasion of neoplastic epithelial
14 cells into the lamina propria (arrows) (intramucosal carcinoma) (Bar=100 μ m). (E) Histological
15 section of the ileo-caecal region of a Dex-treated SCID mouse infected with 10^6 oocysts of *C.*
16 *parvum* TUM1 (day-20 PI). Neoplastic glands penetrate the muscularis mucosa (herniation vs
17 invasion) (Hematoxylin & Eosin) (Bar=200 μ m). (F) Histological section of the ileo-caecal region
18 of a Dex-treated SCID mouse infected with 10^6 oocysts of *C. parvum* TUM1 (at day-20 PI).
19 Interruption of the muscularis mucosae was observed using immunocytochemical staining for alpha
20 smooth muscle actin (Bar=200 μ m).

21 **Fig. 2.** Histological section of the ileo-caecal region of a Dex-treated SCID mouse infected with 10^6
22 oocysts of *C. parvum* TUM1 (euthanatized at day-35 PI). Intraepithelial neoplasia of high grade is
23 characterized by architectural distortion of glands and loss of gland differentiation with epithelial
24 atypias consisting of loss of normal polarity, nuclear stratification, prominent nucleoli and

- 1 irregularly scattered chromatin. Presence of parasites (arrow heads) inside the glands is shown
- 2 (Hematoxylin & Eosin) (Bar=20 μm).
- 3

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