Improved *Cryptosporidium parvum* oocyst propagation using dexamethasone suppressed CF-1 mice

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Abstract

This study evaluates *Cryptosporidium parvum* oocyst production in dexamethasone suppressed CF-1 and C57BL/6 mice. Both models can yield 1 x 10^9 total oocysts over a 20 day production period; however, only 20 CF-1 mice are required to reliably achieve this goal compared to 40 C57BL/6 mice. Although oocyst yields per mouse are similar for both mouse strains, the survival rate for CF-1 mice is higher, resulting in reduced lost production time per study when compared to the C57BL/6 mice. This study presents a more efficient and cost effective dexamethasone suppressed murine model of propagating high concentrations of *C. parvum* oocysts.

Key words: *Cryptosporidium parvum*, oocysts, mouse, immunosuppression, C57BL/6, CF-1

To conduct research using *Cryptosporidium parvum*, large numbers of oocysts are needed and so an efficient model for the propagation of oocysts is required. Several animal models have been described in the literature ranging from neonatal calves or sheep to immunosuppressed rodents (Arrowood and Sterling, 1987; Brasseur et al., 1988; Petry et al., 1995; Yang et al., 2000). An *in vitro* cell free system has also been described and could be adapted for oocyst production; however, it has poor oocyst yield and is very difficult to perform. Therefore, an animal model remains the best option available to propagate these oocysts (Girouard et al., 2006; Hijjawi et al., 2004; von Oettingen et al., 2008).

Most mouse models using C57BL/6 mice immunosuppressed with glucocorticoids administered either orally or by injection have been the model of choice. Studies by Rasmussen and Healy reported that C57BL/6 mice had the highest oocyst shedding intensity as compared with DBA/2N, CBA, C3H/HeN, and BALB/cAnN mice and thus maybe the best strain for oocyst production (Rasmussen and Healey, 1992). Miller and Schaefer recently reported that CF-1 mice immunosuppressed with methylpredisolone can also be infected with *C. parvum* similar to C57BL/6 mice, but they did not report oocyst yields (Miller and Schaefer, 2006). The C57BL/6 mice are approximately three times more costly compared to CF-1 mice at the same ages. This study compares the oocyst yield per mouse and mortality for C57BL/6 and CF1 mice using an immunosuppression protocol previously described (Cicmanec and Reasoner, 1997). The overarching goal was to produce at least 1×10^9 *C. parvum* oocysts in a 20 day collection period using fewer and less costly CF-1 mice than the 40 C57BL/6 mice required to reliably achieve this goal.

C. parvum oocysts (Harley Moon strain) were originally obtained from Waterborne, Inc. (New Orleans, LA) and serially passed through mice. Female 3 to 4 week old CF-1 and C57BL/6 mice were acquired from Charles Rivers Laboratories (Wilmington, MA) and housed in groups of 10 under barrier conditions (sterile cages with 0.22 µm cage isolator filters, raised wire mesh floor, and housed in an animal isolator (NuAire model 602-400, Plymouth, MN)). They received irradiated Pico Lab mouse diet ad libitum (Brentwood, MO). Briefly, the animals were immunosuppressed with sterile water amended with 30 µg/ ml dexamethasone phosphate (Sigma, St. Louis, MO) on odd numbered days and 50 µg/ ml tetracycline (Sigma) on even numbered days throughout the production cycle. On the eighth day of this immunosuppression regimen, the oocyst inoculum was surface sterilized with 50 ppm hypochlorite for 10 min on ice and was neutralized with sodium thiosulfate prior to dosing the animals. Mice were each exposed to 5×10^4 sterilized oocysts by oral gavage using a 22 gauge feeding needle. Feces were collected every 36 hours starting on day 11 and continuing through at least day 25 of the immunosuppression regimen to collect all of the oocysts shed during this production period (Cicmanec and Reasoner, 1997). Each fecal collection was processed by sieving and discontinuous sucrose gradients as previously described (Arrowood and Donaldson, 1996).

The oocyst yield for each collection was determined by hemacytometer as described in Baker (1980), except only 1 chamber was counted. The oocyst yield per mouse for each collection was determined by dividing the total number of oocysts by the number of living mice at that point in the study. Table one shows data gathered from five production studies for each model. The CF-1 mice averaged $7.5 \times 10^6 \pm 2.1 \times 10^6$

oocysts/ mouse and $1.6 \times 10^9 \pm 6.6 \times 10^8$ total oocysts per production study, compared to averages of $1.2 \times 10^7 \pm 4.3 \times 10^6$ oocysts/ mouse and $2.2 \times 10^9 \pm 1.5 \times 10^9$ total oocysts respectively for the C57BL/6 mice. Total oocyst yield per production cycle or oocyst yield per mouse were not statistically significant using a Student's T-test analysis ($P > 0.05$). Surprisingly, the production of 1×10^9 oocysts required at least 40 C57/BL6 compared to only 20 CF-1 mice (Table 1).

The lost production percentages for both animal models were determined for the same five studies. For all calculations, the production period was number of days after exposure to *C. parvum* oocysts. The lost production percentage was the ratio of lost mouse production days (L) divided the number of potential mouse production days (P) times 100%; where L is the sum of the total number of lost mouse days during the production study due to animal mortality, and P is the number of mice times the number of production days. For example a 3 mouse 3 day study, in which 2 mice died on day 2, L would be the 4 (2+2) and P would be 9 (3*3) and, the lost production percentage would be 44% (4/9*100%). The lost production percentage averaged 7% (± 5.6 SD) for the CF-1 mice compared to 25% (± 9.4 SD) for the C57BL/6 and these two models statistically are significantly different ($P = 0.0068$ by Student's T-test) (Figure 1). Because larger numbers of oocysts were required for studies ongoing at that time, the production studies were often extended, therefore, both the average number of days and the lost production percentages to produce 1×10^9 *C. parvum* oocysts was determined for both models. The CF-1 mice on average required 14 ± 2 days with a lost production percentage of 3% ± 3 compared to 12 ± 5 days and 18% ± 7 for the C57BL/6 mice, respectively. The lost production percentage averages are significantly different ($P = 0.01456$) by the Student's

T-test while the production days are not significantly different (Table 1). Assuming the number of oocysts shed per animal remains constant throughout the collection period, a higher lost production percentage results in fewer oocysts being produced by the remaining mice compared to a model with a lower lost production percentage.

These results revealed that CF-1 mice achieved similar oocyst yields with much lower lost production percentages than observed with C57BL/6 mice. The lower lost production percentage allowed the use of fewer animals to reliably achieve the total oocyst goal. It is not known why the 3-4 week old CF-1 mice have a lower lost production percentage when compared the same aged C57B/6 mice, but the CF-1 mice weigh approximately 5 g more than the C57BL/6, 19-23 g compared to 13-17 g. It is possible that the larger CF-1 mice are better able to withstand the immunosuppression; however, larger and older C57BL/6 mice were not evaluated because of the increased price of the mice at that size. In addition to improved survival during immunosuppression and *C. parvum* infection, the CF-1 mice also appeared to be healthier in that they had less ruffled fur and had weight gain or slower weight loss when compared to the C57BL/6 mice (data not shown). Historically, some propagation studies using C57BL/6 mice have failed to produce oocysts because of high mortality (data not shown). In conclusion, CF-1 mice immunosuppressed with dexamethasone amended drinking water is a reliable model for propagating *C. parvum* oocysts using fewer and less costly mice when compared to the C57BL/6 mice.

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