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Short communication

Use of Percoll for the infection of cells in vitro with  
*Cryptosporidium parvum* oocysts

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Abstract

A method for the infection of non-adherent THP-1 cells and adherent MDBK cells with *Cryptosporidium parvum* oocysts using isotonic Percoll solutions was developed. Excystation was maximal after 2 h, but toxicity increased with the oocyst/cell ratio and the incubation time. The infection rates did not increase with the oocyst/cell ratio and both cell types were equally parasitized.

**Keywords:** *Cryptosporidium parvum*; Culture; In vitro; MDBK; Percoll; THP-1

At present, there is still no ideal method available for the in vitro cultivation of the enteric protozoan *Cryptosporidium parvum*. We previously developed a technique using non-adherent monocytic cells (Lawton et al., 1996) that displays many advantages over the use of monolayers. However, the infection of cells involves either direct contact of the cells with oocysts or addition of purified free sporozoites for various periods of time. When *C. parvum* is cultured in adherent monolayers, high numbers of oocysts are used in order to achieve a correct invasion (Upton et al., 1994a,b; Woods et al., 1995; Griffiths et al., 1998; Theodos et al., 1998; You et al., 1998). Our original protocol, which involved mixing oocysts and THP-1 cells in the culture wells

for at least 4 h (Lawton et al., 1996), resulted in many oocysts and sporozoites remaining in suspension while the host cells quickly sedimented, unless periodically resuspended. Thus, to optimize infection, we designed a protocol ensuring that both excysting oocysts and host cells were in close contact, thereby reducing the amount of oocysts needed and the invasion time.

The myelomonocytic cell line THP-1 (ECACC 88081201; Sophia Antipolis, France) was continuously maintained as previously described (Lawton et al., 1996). Madin Darby Bovine Kidney cells (MDBK; ATCC 6071, Rockville, MD) were grown as monolayers in 75-cm<sup>2</sup> Falcon culture flasks (Becton Dickinson, Meylan, France) in the same conditions, except that they were detached with trypsin-EDTA solution (Sigma, L'Isle d'Abeau, France) for routine passaging and prior to the experiments. *C. parvum* oocysts were passaged in neonatal (age 24–48 h) male calves (INRA, Nouzilly, France) and

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83 purified as previously described (Lawton et al., 1996)  
84 except that the pooled fecal pellet was allowed to  
85 sediment twice in 25-ml cold tap water in order to  
86 remove larger debris. Sterile isotonic Percoll was  
87 prepared by mixing one part of  $10 \times$  Hanks bal-  
88 anced salts solution (HBSS; Sigma H-1641) with  
89 nine parts Percoll (Sigma P-4937) and stored at  $4^\circ\text{C}$ .  
90 Solutions of 30% and 60% were made by diluting  
91 the isotonic Percoll with RPMI-1640 and growth  
92 medium, respectively. The solutions were stored at  
93  $4^\circ\text{C}$  and prewarmed at  $37^\circ\text{C}$  before use.

94 1–4 h prior to infection, the cells were collected  
95 and pelleted for 4 min at  $160 \times g$ . They were sus-  
96 pended in 5-ml growth medium and kept in two  
97 wells of a 24-well Falcon microplate (Becton-Dick-  
98 inson; Meylan, France) (mean concentration:  $6.9 \pm$   
99  $2.2 \times 10^6 \text{ ml}^{-1}$ ) in the incubator until required. The  
100 MDBK cells were periodically resuspended to mini-  
101 mize their adherence to the bottom of the wells.

102 The oocysts (approximately  $15\text{--}50 \times 10^6$ ) were  
103 processed, as previously described (Lawton et al.,  
104 1996), and were mixed with the cell suspension from  
105 one well (oocyst to cell ratio was 1:1–3:1). The  
106 suspension was carefully layered on top of 2.5 ml of  
107 the 60% Percoll solution and centrifuged for 10 min  
108 at  $1500 \times g$  in a 15-ml Falcon centrifuge tube. Ex-  
109 cystation was allowed to proceed for 1.5–3 h at  $37^\circ\text{C}$   
110 in the parasite–cell layer at the interface between the  
111 Percoll solution and the medium. This layer was then  
112 retrieved with a Pasteur pipette and washed once in  
113 RPMI-1640 for 9 min at  $600 \times g$ . After dilution with  
114 the cells from the remaining well, the parasitized  
115 cells were layered on top of a 30% Percoll solution  
116 and centrifuged at  $1500 \times g$  for 10 min. The pellet  
117 was subsequently washed once at  $600 \times g$  and thrice  
118 at  $160 \times g$  as above and distributed at an approxi-  
119 mate density of  $6 \times 10^6 \text{ cells ml}^{-1}$  under reduced  
120 oxygen in a candle jar for 24 h. When MDBK cells  
121 were used, the 30% Percoll step was omitted. After  
122 invasion, the parasitized cells were mixed with the  
123 remaining cells, washed thrice at  $160 \times g$ , dis-  
124 tributed in six-well microplates fitted with  $22 \times 22$ -  
125 mm glass coverslips and allowed to settle and ad-  
126 here.

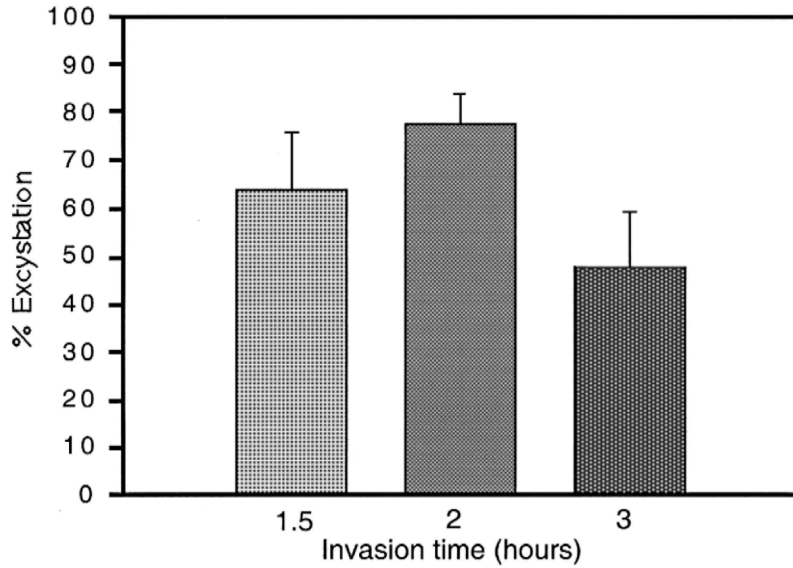
127 After 24 h, the percentage of THP-1 cells infected  
128 with *C. parvum* was assessed on thin smears, fixed  
129 in 100% methanol and stained with 20% Giemsa and  
1% Alcian blue, as previously described (Lawton et

130 al., 1996). The parasite growth in the adherent MDBK  
131 cells was also evaluated 24 h post-infection, after the  
132 removal of the coverslips from the bottom of the  
133 wells. When needed, the cell suspension was diluted  
134 1:10 in saline and counted in a Malassez cell. Toxicity  
135 was assessed by Trypan blue dye exclusion. The  
136 excystation yield was evaluated by counting the re-  
137 maining unexcysted oocysts after infection of the  
138 cells. The Student's *t*-test was used to assess the  
139 statistical significance of the results.  
140

141 The excystation yield depends on the viability of  
142 the oocysts and on the duration of incubation at  
143  $37^\circ\text{C}$ . According to Robertson et al. (1993), maxi-  
144 mum excystation occurs after 4 h, but the maximum  
145 sporozoite yield is achieved after 30 min. Upton et  
146 al. (1994b) observed a maximum between 90 and  
147 120 min, and Joe et al. (1998) reported that the  
148 attachment of sporozoites to Caco-2 cells reached a  
149 maximum after 2 h of incubation. Whatever the  
150 oocyst/cell ratio, the percentage of excystation in-  
151 creased when the invasion duration was increased  
152 from 1.5 to 2 h, then significantly decreased ( $p <$   
153 0.05) at 3 h (Fig. 1). This might be due to the  
154 acidification of the medium (pH 6.5) and/or close  
155 contact with toxic molecules. However, the damage  
156 to the THP-1 cells was significantly more important  
157 ( $p < 0.05$ ) as the oocyst/cell ratio increased ( $6.1 \pm$   
158  $2.5\%$ ,  $19.2 \pm 5.1\%$  and  $39.2 \pm 4.3\%$  dead cells at  
159 oocyst/cell ratios of 1:1, 2:1 and 3:1, respectively).

160 The infection rates at 24 h p.i. were independent  
161 from the oocyst/cell ratio, with about 4.5% of the  
162 host cells harboring developing parasites. The infec-  
163 tion rates of MDBK cells were comparable to those  
164 of THP-1 cells (Fig. 2). The initial percentages of  
165 infected cells depend on the availability of host cells  
166 and on the invasiveness of the sporozoites. Whatever  
167 the cells used in our experiments, monocytic or  
168 epithelial, the invasion rates were lower than ex-  
169 pected. Several authors have reported comparable  
170 infection rates (Slifko et al., 1997; Chen and LaRusso,  
171 2000). This may also be compared to experiments  
172 with human volunteers (Chappell et al., 1996) where  
173 a minimum of 30 oocysts was necessary to initiate  
174 an infection. It is thus possible that only a small  
175 fraction of sporozoites are able to successfully multi-  
176 ply into the host cells.

177 This technique provides maximum contact be-  
between host cells and parasites on short periods of



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180 Fig. 1. Efficiency of excystation as a function of incubation time. The maximum excystation yield was achieved after 2 h. THP-1 cells and an 1:1 oocyst/cell ratio were used. Results from three different experiments.

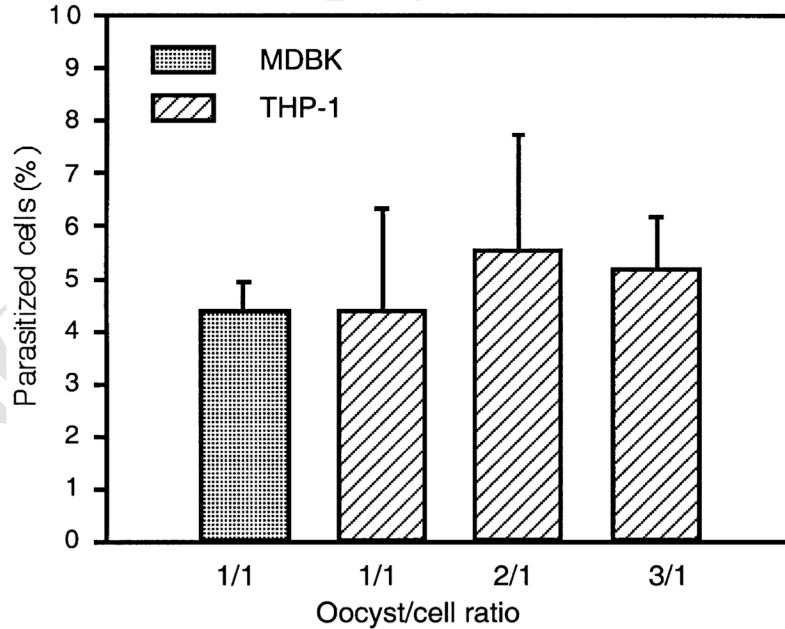
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182 time at an acceptable oocyst/cell ratio. Accordingly, our current standard procedure uses an incubation

time of 1.5 h at a 1:1 oocyst/cells ratio. The subsequent use of 30% Percoll also allows the elimination

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187 Fig. 2. Percentage of parasitized cells 24 h p.i., with an incubation time of 1.5 h. The mean number of cells harboring developing parasites slightly increased with the amount of oocysts added to the host cells. The infection rates of THP-1 cells and MDBK cells were similar with the same oocyst/cell ratio. Results from three different experiments.

- 189  
 190 of most of the cell debris and of the oocysts shells,  
 191 as previously reported (Nesterenko and Upton, 1996)  
 192 and may also be used in the course of cultivation in  
 193 order to eliminate dead cells, which can accumulate  
 194 during these rather stringent culture conditions.  
 195 However, this step is omitted with MDBK cells  
 196 because these cells have a wider size heterogeneity,  
 197 resulting in significant amounts remaining at the  
 198 surface of the 30% Percoll solution. The present  
 199 technique designed for non-adherent cells is applica-  
 200 ble to epithelial cells and allows the use of the same  
 201 parasitized adherent cell suspension and its even  
 202 distribution in culture wells for drug testing.
- 203  
 204 **References**  
 205  
 206 Chappell, C.L., Okhuysen, P.C., Sterling, C.R., Dupont, H.L.,  
 207 1996. *Cryptosporidium parvum*: intensity of infection and  
 208 oocyst excretion patterns in healthy volunteers. *J. Infect. Dis.*  
 209 173, 232–236.  
 210 Chen, X.M., LaRusso, N.F., 2000. Mechanisms of attachment and  
 211 internalization of *Cryptosporidium parvum* to biliary and in-  
 212 testinal epithelial cells. *Gastroenterology* 118, 368–379.  
 213 Griffiths, J.K., Balakrishnan, R., Widmer, G., Tzipori, S., 1998.  
 214 Paromomycin and geneticin inhibit intracellular *Cryptosporid-*  
 215 *ium parvum* without trafficking through the host cell cyto-  
 216 plasm: implications for drug delivery. *Infect. Immun.* 66,  
 217 3874–3883.  
 218 Joe, A., Verdon, R., Tzipori, S., Keusch, G.T., Ward, H., 1998.  
 219 Attachment of *Cryptosporidium parvum* sporozoites to human  
 255 intestinal epithelial cells. *Infect. Immun.* 66, 3429–3432.
- Lawton, Ph., Naciri, M., Mancassola, R., Pétavy, A.-F., 1996. 220  
 Cultivation of *Cryptosporidium parvum* in a non-adherent 221  
 human monocytic cell line. *J. Microbiol. Methods* 27, 165– 222  
 173. 223  
 224  
 Nesterenko, M.V., Upton, S.J., 1996. A rapid microcentrifuge 225  
 procedure for purification of *Cryptosporidium* sporozoites. *J.* 226  
*Microbiol. Methods* 25, 87–89. 227  
 Robertson, L.J., Campbell, A.T., Smith, H.V., 1993. In vitro 228  
 excystation of *Cryptosporidium parvum*. *Parasitology* 106, 229  
 13–19. 230  
 Slifko, T.R., Friedman, D., Rose, J.B., Jakubowski, W., 1997. An 231  
 in vitro method for detecting infectious *Cryptosporidium* 232  
 oocysts with cell culture. *Appl. Environ. Microbiol.* 63, 3669– 233  
 3675. 234  
 Theodos, C.M., Griffiths, J.K., D’Onfro, J., Fairfield, A., Tzipori, 235  
 S., 1998. Efficacy of nitazoxanide against *Cryptosporidium* 236  
*parvum* in cell culture and in animal models. *Antimicrob.* 237  
*Agents Chemother.* 42, 1959–1965. 238  
 Upton, S.J., Tilley, M., Brillhart, D.B., 1994a. Comparative devel- 239  
 opment of *Cryptosporidium parvum* (Apicomplexa) in 11 240  
 continuous host cell lines. *FEMS Microbiol. Lett.* 118, 233– 241  
 236. 242  
 Upton, S.J., Tilley, M., Nesterenko, M.V., Brillhart, D.B., 1994b. 243  
 A simple and reliable method of producing in vitro infections 244  
 of *Cryptosporidium parvum* (Apicomplexa). *FEMS Microbiol.* 245  
*Lett.* 118, 45–50. 246  
 Woods, K.M., Nesterenko, M.V., Upton, S.J., 1995. Development 247  
 of a microtitre ELISA to quantify development of *Cryp-* 248  
*tosporidium parvum* in vitro. *FEMS Microbiol. Lett.* 128, 249  
 89–94. 250  
 You, X., Schinazi, R.F., Arrowood, M.J., Lejkowski, M., Juo- 251  
 dawkis, A.S., Mead, J.R., 1998. In-vitro activities of paro- 252  
 momycin and lasalocid evaluated in combination against 253  
*Cryptosporidium parvum*. *J. Antimicrob. Chemother.* 41, 293– 254  
 296. 255