# Physical Straining of *Cryptosporidium parvum* Oocysts through Saturated Soils

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**Abstract**
The colloid-sized *C. parvum* oocysts may be physically strained through porous media during transport. However, most of studies on *C. parvum* oocysts were performed with a constant flux (CF) controlled by a peristaltic pump, in which the unsteady flux caused by the physical straining mechanism was ignored. In this study, we first compared two systems in which oocysts transport through homogenous pure sand or soils either by a CF or by a constant pressure head (CP). The results showed oocysts solution flux ($J_o$) reduced remarkably in a CP system, but not in a CF system. More oocysts from the effluents in CF system were observed than in CP system, indicating that oocysts breakthrough was overestimated in CF system. Using the CP system, 6 intact soils typical of Ireland were further investigated. One soil had oocyst breakthrough earlier than $Br^-$, and $J_o$ was nearly identical to $Br^-$ flux ($J_B$) due to macropore flow. For the other five soils oocyst breakthrough was later than $Br^-$, and $J_o$ tended to decrease with pore volume. The decrease of $J_B$ was not found during the experiments. The unsteady-state flux of oocysts solution put forward a new challenge for us how to simulate the colloid-sized pathogen transport in soils.

**Key Words**
Breakthrough curve, *Cryptosporidium*, Physical straining, Transport

## Introduction

*Cryptosporidium parvum* is a zoonotic protozoan parasite that can infect the intestines of animals as well as humans (Fayer 2008). It poses a significant risk to public health through incidences of human cryptosporidiosis, and has become a global concern to water resource managers since the 1993 outbreak in Milwaukee, US. The main source of *C. parvum* oocysts is from infectious calves when agricultural water including runoff, infiltration and subsurface flow from dairies, calving house, silage and grazing lands may be loaded with high concentrations of oocysts. There is little knowledge of the transport of *Cryptosporidium* oocysts in agricultural ecosystems (Fayer 2004; Pachepsky *et al*. 2006).

Some studies have examined the transport and retention behavior of *Cryptosporidium* oocysts in repacked sand columns under a steady-state flux controlled by a peristaltic pump (Brush *et al.* 1999; Harter *et al.* 2000; Logan *et al.* 2001; Bradford and Bettahar 2005). They are more interested in how oocysts are filtered through the homogeneous porous media either by the particle size or solution chemical properties (Bettahar 2005, Brush *et al.*1999; Harter *et al.* 2000; Logan *et al*.. 2001). Harter *et al*. (2000), and Logan *et al*. (2001) emphasized that decreasing the median sand size tended to produce lower effluent concentrations and greater oocyst retention near the column inlet. Bradford and Bettahar (2005) further postulated that three physical mechanisms of attachment, detachment and irreversible straining might be involved in the tailing and heterogeneous distribution of oocysts at depth. However, we hypothesize at the steady-state flux the colloid-sized oocysts may be forced to pass through the pores. The filtered oocysts will be as a result overestimated. On the other hand, the homogeneous porous media is not able to represent effects of structured soil on oocyst breakthrough behavior.

In Ireland *C. parvum* is a potentially significant zoonotic disease due to the large bovine population (~6 million cattle in 2008) and the dependence on surface water or shallow groundwater for drinking water supply. Agricultural soils are typically glacially derived and mostly classified by association as Podzols, Cambisols, Luvisols and Gleysols from FAO world reference base for soil resources. There is a wide range of texture, structure and other properties, the influence of which cannot be properly established using disturbed soil, therefore the objectives of this research were: (i) to prove the physical straining of oocysts through porous media; and (ii) to investigate the role of undisturbed soil structure in oocysts transport and retention.

## Materials and Methods

*Undisturbed soil cores and pure sand columns*

Nine typical grassland undisturbed soil cores (20 cm high, 10 cm diameter) from Ireland with a limited range of soil texture and wider range of soil organic C were used (Table 1). A composite disturbed sample from five points around the same fields was taken, air-dried and ground to <2 mm for determining soil properties. Two pure sand sizes and one seized <2 mm CG soil were prepared homogeneous porous media. The three homogeneous columns were used to compare oocysts breakthrough between constant flux (CF) and constant pressure head (CP) systems. In CF system the flux was determined by the flux of $Br^-$ solution in CP system.

**Table 1. Some selective soil properties of nine soils present in permanent pastures in Ireland**

| Soil code | Silt | Clay | SOC¶ | CEC¶ | Total pore | Macropore¶ | $K_s$¶ | pH |
|---|---|---|---|---|---|---|---|---|
| | g/kg | | | mmol/kg | $cm^3\ cm^{-3}$ | | cm/day | [-] |
| S02 | 397 | 159 | 27.1 | 169 | 0.477 | 0.109 | $2.94\times10^3$ | 6.08 |
| S01 | 440 | 187 | 40.8 | 202 | 0.571 | 0.060 | $7.00\times10^2$ | 5.72 |
| CG | 431 | 212 | 44.0 | 187 | 0.533 | 0.096 | $4.42\times10^2$ | 5.32 |
| EG | 424 | 221 | 48.4 | 212 | 0.596 | 0.039 | $9.26\times10^1$ | 6.57 |
| OG | 364 | 162 | 30.0 | 151 | 0.525 | 0.036 | $7.30\times10^1$ | 5.6 |
| JSG | 217 | 116 | 16.3 | 92 | 0.419 | 0.008 | $2.16\times10^1$ | 6.33 |
| KG | 513 | 102 | 90.5 | 259 | ND | ND | $<1.0\times10^0$ | 6.3 |
| RG | 444 | 162 | 37.5 | 163 | ND | ND | $<1.0\times10^0$ | 6.56 |
| CCG | 496 | 217 | 72.3 | 225 | ND | ND | $<1.0\times10^0$ | 5.29 |

¶SOC, soil organic carbon, CEC, Cation exchange capacity, Macropore, >60 μm diameter, $K_s$, saturated hydraulic conductivity of distilled water; ND, not determined.

*Cryptosporidium parvum* oocysts were purchased from Creative Science Company, UK. These oocysts were purified from manure of experimentally infected calves by sucrose and Percoll gradient centrifugation and water washes. They were stored in 0.1% PSB (phosphate buffered saline) solution at 4 $^oC$ before use. The number of oocysts in the stock solution was about 5.0 x $10^8$ oocysts/ml. The oocyst staining protocol was used after following the instruction of the Dynabeads® anti-*Cryptosporidium* kit (Invitrogen, Norway).

*Column Experiment*

The columns were firstly saturated from the bottom with distilled water for an overnight. Then about 1-2 pore volume of 0.1 M NaBr solution was run through the column from top to bottom, followed by another 1-2 pore volume of $2.0\times10^3$ oocysts/ml solution. The oocysts reservoir was placed on a stirrer to keep the oocyst homogeneously mixed during the experiment. The breakthough of $Br^-$ and oocysts solutions was pushed through the column under a 22 cm CP between the inlet and the outlet or under a CF controlled by a peristaltic pump. The homogenous pure sand and disturbed soil were performed in the both systems, while the undisturbed soils were only performed in CF system. The effluent was collected from the outlet periodically and the time (Δt) and volume (ΔV) were recorded. The flux of distilled water ($J_w$), $Br^-$ solution ($J_B$) or oocyst solution ($J_o$) was calculated by the following equation.

$$J_i = \frac{\Delta V}{A\Delta t} \quad (1)$$

where A is the cross area of the soil column (78.5 $cm^2$), and *i* is the flow phase of distilled water, $Br^-$ solution or oocysts solution.

## Results and Discussion

*Comparison of CF and CP systems*

Figure 1 presents the relative concentration ($C_i/C_0$) of $Br^-$ and oocyst in the effluents as a function of pore volume. For the coarse sand column, the colloid-sized oocysts breakthrough curve is nearly identical to the ionic $Br^-$ curve either in CF system or in CP system. That means the physical straining is negligible in the coarse sand. For the fine sand column, the breakthrough curve of oocysts is consistent with the $Br^-$ in CF system, whereas the filtration of oocysts was lower than the $Br^-$ in CP system. For the disturbed soil, only few oocysts passed through columns in CF system, and even less in CP system. The flux of oocysts was at

steady-state, identical to $Br^-$ in the coarse and fine sand columns (Figure 2). However, in the disturbed soil column, the flux of oocysts was decreased in the entire period due to the blockage of pores by oocysts. This physical function may be caused mainly by the oocysts preferentially attached to soil particles rather than oocysts themselves. These results clearly prove that the physical staining of colloid-sized oocysts through porous media may be readily ignored in CF system.

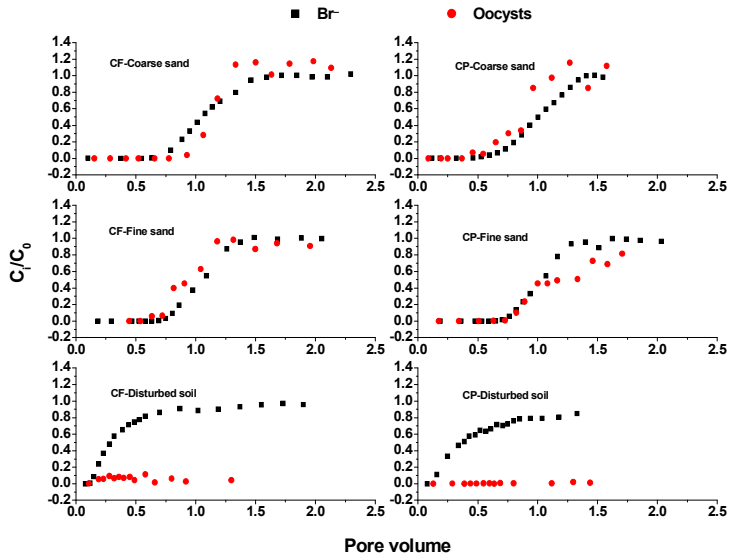


**Figure 1. Breakthrough curves of $Br^-$ and oocysts in homogenous columns.**

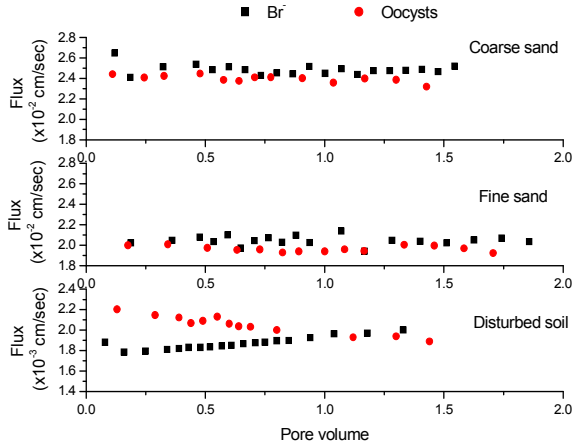


**Figure 2. Flux of $Br^-$ and oocysts through homogeneous columns in CP system.**

*Structured soils*

The $Br^-$ and *Cryptosporidium* oocyst effluent concentration curves and the related $J_B$ and $J_o$ values of the six soils used for the soil column experiments show the relative effluent concentrations ($C_i/C_0$) as a function of pore volumes (Figure 3). $Br^-$ concentration increased with pore volume up to the initial concentration. The oocyst breakthrough curve was different to the $Br^-$ curve. The oocyst concentration increased at the beginning of the experiment and then decreased to some extent with the exception of EG, in which oocyst concentration always increased but remained at very low concentrations during the first pore volume. For the S02 soil the oocyst breakthrough curve was earlier than $Br^-$, while $J_o$ was nearly identical to $J_B$ during the whole experiment. In the other five soils, the oocyst breakthrough curves were all later than $Br^-$ and their $J_o$ tended to decrease with pore volume. The change in $J_B$ was minimal. The time to start of decrease in $J_o$ tended to coincide with the decrease of oocyst concentration. The reduction of $J_o$ indicated that the CP system could keep ionic $Br^-$ under steady state flow but failed for colloid-sized oocysts. As a result, the oocyst concentration of the effluent eventually decreased when the oocyst flow was reduced. The decrease in oocyst flow probably means that physical straining and trapping of oocysts in the soil pores was occurring.

The decrease in oocyst concentration with an increase of pore size indicated that oocysts were physically strained or trapped in smaller pores. With the blockage of pores by oocysts, the flux of oocysts decreased as shown in Figure 3. The pores smaller than the oocysts could not transfer oocysts with a fixed pressure head

(22 cm in this case), but if using a peristaltic pump some oocysts might be forced to pass through colloid-sized pores as discussed previously. The results suggest that the boundary of mobile and immobile pores sometimes used in soil hydrology, or even in colloid-sized *Cryptosporidium* transport , depends on whether the experiment conducted uses a constant head (giving the potential for a change in effective pore size which cannot be readily modelled using classical theory) or a constant flux that might 'force' oocysts through pores that would not normally conduct them. Oocyst removal by six structured soils was calculated to range from 0.076 up to 0.864 and it was more closely related to macroporosity than to total porosity. The oocyst fraction either in the effluent or in the pores was significantly related with macroporosity and hydraulic conductivity ($P<0.05$), which plays a negative role in the removal fraction.

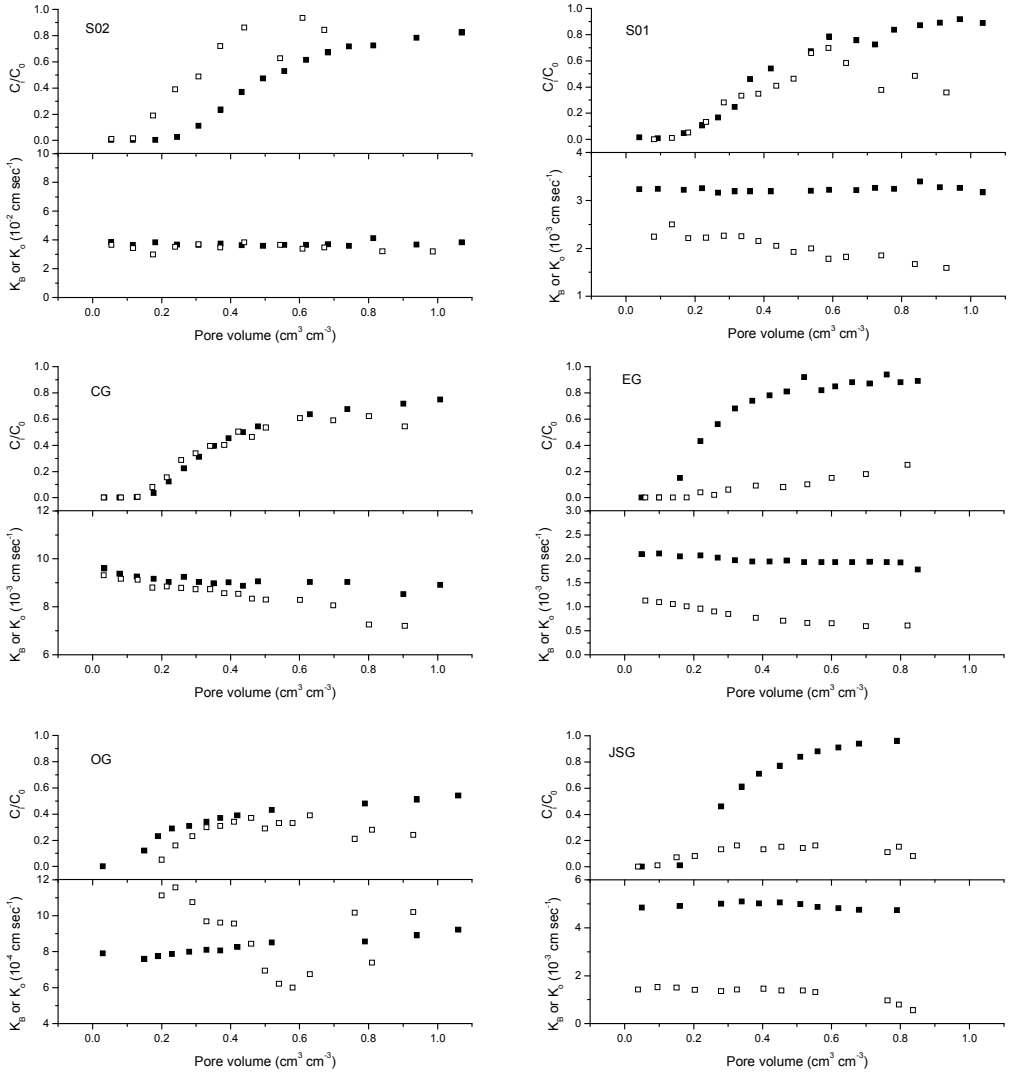


**Figure 3. Breakthrough curves and saturated hydraulic conductivity of $Br^-$ (solid squares) and oocysts (open squares) in structured soil cores.**

## Conclusions

For colloid-sized *Cryptosporidium parvum* oocysts transport through soils or porous media, the filtration of oocysts could be overestimated in the steady-state flux system. Use of a constant pressure head system the flux of oocysts solution decreased and the reduction of its concentration coincided in the effluent as a result of physical straining. These results provide an additional evidence to demonstrate that straining is one of important physical mechanisms regulating colloid-sized oocyst transport through structured soils.

## Major References

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