Transport and influential parameters of *Cryptosporidium* from soil to surface water with preferential flow

Tao Yuan

School of Architectural Decoration,
Jiangsu Vocational Institute of Architectural Technology,
Xuzhou, 221000, China
Email: yuantaocumt@126.com

Sen Cheng, Yadong Kong and Ping Lu*

School of Environmental Science and Spatial Informatics,
China University of Mining and Technology,
Xuzhou, 221116, China
Email: chengsen9711@163.com
Email: 57246776@qq.com
Email: lupingcumt@126.com
*Corresponding author

Abstract: *Cryptosporidium* outbreaks in surface water are the main route of the human infection cryptosporidiosis; however, *Cryptosporidium* transport from soil to surface water is not clear. In this paper, two transport paths were simulated to determine the transport behaviour and mechanism of *Cryptosporidium* oocyst substitutes in a soil-water medium (i.e., root path with preferential flow and root-free path without preferential flow) through laboratory experiments. Fluorescent polystyrene microspheres were used as substitutes for *Cryptosporidium* oocysts. A 50 cm slope model was used to simulate the two-dimensional transport of *Cryptosporidium* oocyst substitutes under rainfall conditions. The results showed that the preferential flow formed by plant roots enhanced the transport of *Cryptosporidium* oocysts. Soil physicochemical properties affected the transport of *Cryptosporidium* oocyst substitutes, and the results indicated that a high sodium ion intensity and organic matter content in soil inhibited the transport of *Cryptosporidium* oocyst substitutes; low soil pH values enhanced the adsorption by plant roots, thereby inhibiting the transport of *Cryptosporidium* oocyst substitutes.

Keywords: *Cryptosporidium* substitutes; transport mechanisms in environment; influencing factors; public health.

Reference to this paper should be made as follows: Yuan, T., Cheng, S., Kong, Y. and Lu, P. (2023) 'Transport and influential parameters of *Cryptosporidium* from soil to surface water with preferential flow', *Int. J. Environment and Pollution*, Vol. 72, No. 1, pp.29–39.

Biographical notes: Tao Yuan, Associate Professor, Dean of School of Architectural Decoration, Jiangsu Vocational Institute of Architectural Technology.

Sen Cheng is an Engineer specialised in environmental engineering.

Yadong Kong is a government employee specialised in environmental management.

Ping Lu, Associate Professor, specialised in environmental research.

1 Introduction

Cryptosporidium is a protozoan pathogenic microorganism and an intestinal parasite that parasitises the gastrointestinal tract of humans and animals and causes cryptosporidiosis. Very few *Cryptosporidium* oocysts can cause infection, and the minimum infectious dose in adults is less than 30 oocysts (Chappell et al., 1999). Among the global outbreaks of digestive diseases, diarrhoea caused by *Cryptosporidium* ranks first in terms of parasitic diarrhoea. In more than 90 countries including Australia and those in North America, Central and South America, Asia, Africa and Europe, cases of *Cryptosporidium* have been reported in more than 300 regions (Bitto and Aldras, 2009; Lu et al., 2013). China has reported many cases of *Cryptosporidium* infections since 1987 (Han et al., 1998), followed by reports of human infections in Nanjing, Xuzhou, Anhui, Inner Mongolia, Fujian, Shandong, Hunan and Zhejiang (Xu, 2005). However, due to the different degrees of attention in different countries, the actual number of outbreaks is much higher than that in the reported data (Sunderland et al., 2007). Most infections are caused by contact with recreational water, such as swimming pools and lakes where people dive or swim (Lu et al., 2013). Therefore, it is important to understand the transport mechanism of *Cryptosporidium* from the soil to the surrounding recreational water bodies.

Human and animal waste, garbage, etc. are the main sources of *Cryptosporidium* in the environment. Pathogenic microorganisms accumulate in soil (McDonald et al., 1982; Jamieson et al., 2004). The main transport of pathogenic microorganisms, such as *Cryptosporidium,* includes diffusion, erosion, convective transport, and active movement. (Anderson et al., 1998; Bhattarai et al., 2011; Bradford et al., 2013; Davies et al., 2004; Dorner et al., 2006; Krometis et al., 2007;Sterk et al., 2013; Tate et al., 2000; Trask et al., 2004). The influencing factors of surface transport include rainfall intensity, rainfall time, rainfall pattern, soil type and other factors (Sun, 2016; Xu, 2016). According to a previous study, a lower soil pH and high sodium ion intensity can increase the attachment of *Cryptosporidium* onto soil and holly roots (Yuan et al., 2019). Surface runoff and underground flow are closely related. Research on the transport of *Cryptosporidium* from soil to surface water mainly focuses on surface runoff, and there is limited research on preferential flow as the dominant transport mechanism and its influencing factors.

In the natural soil medium, due to its heterogeneity and the presence of large pores such as dry cracks, plant roots, and wormholes, underground soil preferential flow plays an important role in the underground transport of pathogenic microorganisms (Fox et al., 2010; Niu et al., 2006). Studies have proven that the transport of *Cryptosporidium* in the vertical direction of the soil through finger flow in the preferential flow is more significant than that in other flows (Christophe et al., 2004). The transport of *Cryptosporidium* in soil is affected by many factors, which are related not only to rainfall intensity, plant density and pollutant size (Ferguson et al., 2007; Tate et al., 2004; Trask et al., 2004) but also closely to the surface properties of *Cryptosporidium*, dissolved organic carbon and soil mineral composition (Mohanram et al., 2012). Previous studies have mostly focused on one-dimensional transport (or vertical direction transport) of *Cryptosporidium* in the soil. In reality, two-dimensional transport is ubiquitous and involves horizontal and vertical transport. However, there is limited research on the transport of *Cryptosporidium* with two-dimensional preferential flow. Therefore, this paper focuses on the transport characteristics and influencing factors of *Cryptosporidium* with two-dimensional preferential flow.

To understand the transport of *Cryptosporidium* from surface soil to surrounding surface water bodies, experiments were conducted to simulate the transport of *Cryptosporidium* oocyst substitutes with two-dimensional preferential flow. The results will determine the factors that impact the transport of *Cryptosporidium* oocyst substitutes.

2 Materials and methods

2.1 Materials

2.1.1 Cryptosporidium oocyst substitutes

Based on information in a previous study (Amburgey, 2002; Amburgey et al., 2004; Dai and Hozalski, 2003; Lu and Amburgey, 2016; Lu et al., 2017a, 2017b), polystyrene fluorescent microspheres have been used by multiple researchers instead of *Cryptosporidium,* and these microspheres were used in this study. Microspheres with a diameter of 4.5 µm were used as the substitute since microspheres are virtually identical to *Cryptosporidium* oocysts in size, shape, density, and surface charge in pool water (Fluorsebrite™ Carboxylate YG 4.5 micron microspheres, Cat. #16592, 4.5 µm, std.dev. 0.246 µm, Polysciences, Inc., Warrington, Pennsylvania, USA) (Amburgey, 2002; Amburgey et al., 2004; Dai and Hozalski, 2003). The stock suspension microsphere concentration was 4.37×10^{11} #/L. A total of 10^7 microspheres were used in each of these experiments.

2.1.2 Experimental soil and plants

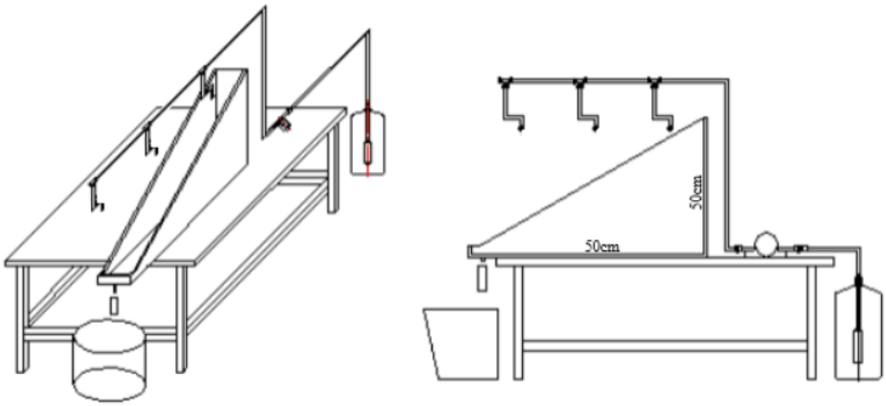
Quartz sand was used for the experiments. Since holly trees are widely grown throughout China, holly roots were used to simulate the preferential flow of soil formed by plant roots.

2.2 Experimental methods

2.2.1 Transport experiments

According to rainfall classification, the rainfall intensity of a rainstorm, which is 55 mm/12 h, was selected as the rainfall intensity in this study (Kong, 2017). The experiments used a 50 cm slope model (the experimental device is shown in Figure 1). According to the soil slope in the study area, 30° was selected as the soil slope in the study to simulate the most favourable way for *Cryptosporidium* to be transported from the soil to the surface water under natural conditions.

Figure 1 Schematic diagram of the two-dimensional experimental device (see online version for colours)



The experiments involved a root group and root-free group (that is, whether there is a preferential flow). The root group was a transport simulation experiment with a 25 cm holly tree root (length 25 cm, diameter 2 cm), which was cut off and buried in the soil, at a slope of 30° to completely cover it with the soil and have it at a slope. The root-free group was a transport simulation experiment without holly tree roots, and the experimental conditions were the same as those of the root group except for the absence of plant roots. The soil column was 45 cm (height) × 50 cm (length) × 5 cm (width).

The 10^7 *Cryptosporidium* oocyst substitutes were added at the top of the soil slope. Then, the simulated rainfall device was turned on, and tap water was used (Kong, 2017). At 0 min, the first drop of water reached the bottom of the device. Water drop samples were collected at 1 min, 2 min, 3 min, 5 min, 7 min, 9 min, 12 min, 15 min, 20 min, 25 min, and 30 min. Duplicate samples were collected and analysed. After sampling, 1 mL of the sample was taken to make a slide for microscopic examination under a fluorescence microscope. The mobility (i.e., the ratio of the transported number of oocyst substitutes to the initial dosage) was calculated (Lu et al., 2017a, 2017b).

2.2.2 Influential factors affecting the transport experiments

The influential factors of the root group experiments were soil sodium ion strength, soil pH, and the initial concentration of the added substitutes. The rainfall was simulated at the top of the slope (the rainfall intensity was 55 mm/12 h), and 1 mL of *Cryptosporidium* oocyst substitutes was added at the top of the slope (initial concentration was 10^7 microspheres/mL). Samples were collected at 1 min, 2 min, 3 min, 5 min, 7 min`, 9 min, 12 min, 15 min, 20 min, 25 min, and 30 min. After sampling, a 1 mL sample was taken with a pipette to make a slide for fluorescence microscope inspection, and the mobility was calculated.

- *Soil sodium ion*

Sodium chloride was used to control the soil sodium ion intensity. The variable gradient was set to 0.1 mol/kg, 0.2 mol/kg, and 0.3 mol/kg. The weighed sodium chloride was dissolved in water and stirred until uniform. The sodium chloride solution was added to the weighed soil and mixed evenly. After the soil moisture content reached 90%, it was added to the slope model at 30°.

- *pH value*

The experiment used a 1 mol/L sodium hydroxide solution and 1 mol/L dilute hydrochloric acid solution to control the pH value. The prepared solution was added to the weighed soil; after mixing, 10 g of soil was taken, 25 mL of water was added; and the mixture was shaken for 5 min. After standing for 1 h, the pH of the supernatant was measured with a pH metre to determine the pH value of the soil. The pH variable gradient was set to 5, 7, and 9.

- *Initial concentration of Cryptosporidium oocyst substitutes*

The changes in the transport of the different concentrations of *Cryptosporidium* oocyst substitutes were studied experimentally. One millilitre of *Cryptosporidium* oocysts was added at the top of the slope. The initial concentration variable gradient was 10^7 microspheres/mL, 10^6 microspheres/mL, and 10^5 microspheres/mL.

2.2.3 Enumeration of Cryptosporidium oocyst substitutes

The samples were mixed by vortexing and hand shaking for at least two min each before analysis. Samples were passed through 3.0 μm pore size polycarbonate filters (Product #K30CP02500, GE Osmonics, Minnetonka, Minnesota, USA). Each polycarbonate filter was mounted on a glass microscope slide with a polyvinyl alcohol-DABCO solution, covered with a glass cover slip (25-mm square, No. 1.5, Corning, Inc., Corning, New York, USA), and counted under an epifluorescence microscope at a magnification of 100X (Zeiss Standard 25 microscope, Carl Zeiss MicroImaging, LLC, Thornwood, New York, USA) (Lu, 2012).

3 Results

3.1 Transport of Cryptosporidium oocyst substitutes

Figure 2 shows the migration curve of *Cryptosporidium* oocyst substitutes through the column with and without preferential flow under rainfall conditions. The results show that the number of *Cryptosporidium* oocyst substitutes that migrated decreased over time and eventually stabilised. The migration of *Cryptosporidium* oocyst substitutes was more obvious in the early stage of rainfall. The number of migrated *Cryptosporidium* oocyst substitutes in the root group was generally greater than that in the root-free group.

3.2 Transport with preferential flow

Figure 3 shows the transport of *Cryptosporidium* oocyst substitutes over time in the root group. As shown in Figure 3, in the early stage of rainfall, a large number of *Cryptosporidium* oocyst substitutes were transported through surface runoff and root preferential flow. With increasing rainfall duration, the transport of *Cryptosporidium* oocyst substitutes decreased rapidly. At the same time, as the sodium ion intensity in the soil increased from 0.1 mol/kg to 0.3 mol/kg, the number of transported *Cryptosporidium* oocyst substitutes consequently decreased. The results showed that the increase in sodium ion intensity inhibited the transport of *Cryptosporidium* oocysts.

Figure 2 Transport of *cryptosporidium* oocyst-sized microspheres

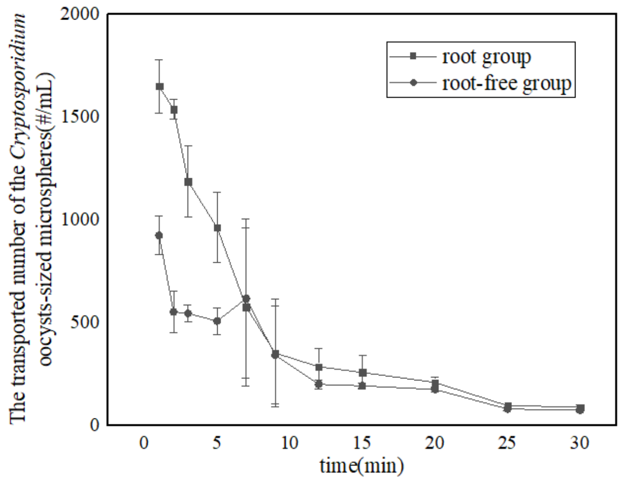


Figure 3 Transport of *Cryptosporidium* oocyst-sized microspheres over time under different sodium ion intensity conditions

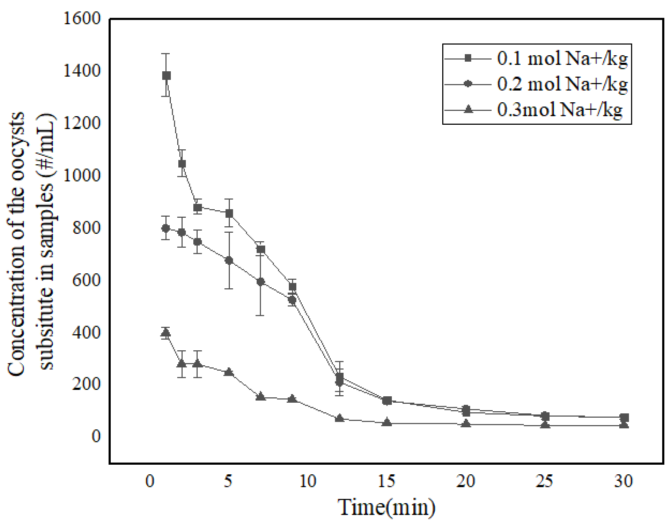


Figure 4 shows the transport of *Cryptosporidium* oocyst substitutes in the soil with roots at different pH values. As shown in Figure 4, the trend in the two-dimensional transport of *Cryptosporidium* oocyst substitutes through soil preferential flow with varied pH values over the rainfall duration was the same as that of other variable groups, and the trend decreased sharply with increasing rainfall duration and eventually stabilised. At the beginning of the rainfall, when the pH value was 9, the transported number of *Cryptosporidium* oocyst substitutes was relatively high and was greater than that at pH values of 5 and 7, and when the pH value was 5, the number transported was relatively small.

Figure 4 Transport of *Cryptosporidium* oocyst-sized microspheres over time under different soil pH values

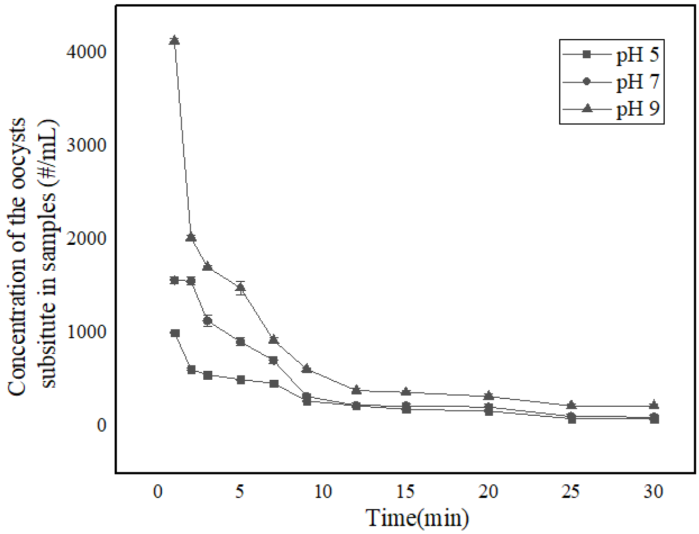


Figure 5 Transport of *Cryptosporidium* oocyst-sized microspheres over time with different initial concentrations of the substitutes

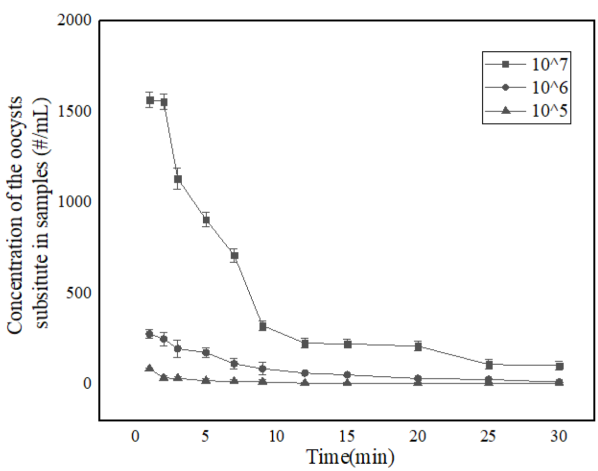


Figure 5 shows the initial concentrations of the substitutes affecting the transport of *Cryptosporidium* oocysts through the plant roots over time. As shown in Figure 5, although the *concentrations* of the added *Cryptosporidium* oocyst substitutes were different, the transport of *Cryptosporidium* oocyst substitutes with rainfall duration had the same trend. The transport of each variable group decreased sharply over the rainfall duration and eventually stabilised. In addition, as the initial concentration of *Cryptosporidium* oocyst substitutes in the soil decreased, the number of transported

Cryptosporidium oocyst substitutes significantly decreased. When the initial concentration of the substitutes was reduced from 10^7 microspheres/mL to 10^5 microspheres/mL, the transported number of *Cryptosporidium* oocyst substitutes declined.

4 Discussion

The transport of *Cryptosporidium* oocyst substitutes under rainfall conditions showed that the amount of transported *Cryptosporidium* oocyst substitutes in the root group was generally greater than that in the root-free group, and that in the initial stage of rainfall was more obvious.

At the very beginning of the rainfall, the transport of *Cryptosporidium* oocyst substitutes reached maximum values. The potential reason for this result was that the thick plant roots formed a large-aperture root preferential flow in the soil. Under high-intensity rainfall conditions, *Cryptosporidium* oocyst substitutes can be transported through preferential flow of large-aperture roots. Brush et al. (1999) and Harter et al. (2000) found that *Cryptosporidium* oocysts were transported rapidly through the soil during percolation experiments on packed columns, and the experimental results fully confirmed that *Cryptosporidium* can be transported downwards. In addition, Christophe et al. (2004) used a 50 cm packed column to simulate a one-dimensional transport experiment of *Cryptosporidium* through large-pore preferential flow under rainfall conditions. It was found that macroporous preferential flow significantly promoted the transport of *Cryptosporidium* oocysts. The number of *Cryptosporidium* oocysts that are transported through the preferential flow of macropores is significant, exceeding the order of magnitude of the adult infective dose, which fully confirms that the large-aperture preferential flow of plant roots can promote the transport of *Cryptosporidium* oocyst substitutes. This scenario is consistent with the results of this experiment, and the preferential flow also slightly promoted the transport of *Cryptosporidium* oocyst substitutes.

According to the colloidal DLVO theory, an increase in sodium ion intensity leads to compression of the double layer of colloids, which will stabilise the colloid. Furthermore, in this experiment, the increased sodium ion intensity enhanced the adsorption of *Cryptosporidium* by the roots and soil, thereby inhibiting the transport of *Cryptosporidium* through the roots. Yu et al. (2013) showed that increasing the ionic intensity of the solution can significantly increase the removal of colloids passing through a plant system, which is also consistent with the experimental results.

According to adsorption experiments (Kong, 2017), a lower pH value promotes the adsorption of *Cryptosporidium* oocyst substitutes by plant roots, which could support transport behaviour under different pH values in this work.

The transport of *Cryptosporidium* oocyst substitutes increased with the increased number of initial oocyst substitutes. The potential cause was that the adsorption of *Cryptosporidium* oocyst substitutes by soil particles decreased as the concentration of substitutes increased. A previous study also showed that the number of oocysts passing through a column increased with increasing oocysts (Lu and Amburgey, 2016).

5 Conclusions

In this study, polystyrene fluorescent microspheres were used as substitutes for *Cryptosporidium* oocysts, and holly roots were selected to simulate the preferential flow of plant root formation. The most favourable transport mode of *Cryptosporidium* oocyst substitutes was simulated from sloping soil to a lake over natural conditions (i.e., transport experiments under rainstorm conditions and a 30° slope). The main results are the following:

- The preferential flow of plant root formation enhances the number of transported *Cryptosporidium* oocyst substitutes.
- The transport of *Cryptosporidium* oocyst substitutes is inhibited when the soil has a higher sodium ion intensity.
- The transport of *Cryptosporidium* oocyst substitutes is inhibited when the soil has a lower pH.

In summary, the preferential flow of plant root formation had a better transport effect on *Cryptosporidium* oocyst substitutes under experimental conditions. Sodium ion strength plays an important role in the transport mechanism of *Cryptosporidium* oocyst substitutes from soil.

Acknowledgement

This project is supported by the Foundation of Excellent Young Teacher of the Qinglan Project in Jiangsu Colleges and Universities.

References

Amburgey, J.E. (2002) *Improving Filtration for Removal of Cryptosporidium Oocysts and Particles From Drinking Water*, Doctor's thesis, Georgia Institute of Technology, Atlanta, USA.

Amburgey, J.E., Amirtharajah, A., Brouchaert, B. and Spivey, N.C. (2004) 'Effect of washwater chemistry and delayed start on filter ripening', *J. Am Water Works*, Vol. 96, pp.97–110.

Anderson, M.A., Stewart, M.H., Yates, M.V. and Gerba, C.P. (1998) 'Modeling the impact of body-contact recreation on pathogen concentrations in a source drinking water reservoir', *Water Research*, Vol. 32, pp.3293–3306.

Bhattarai, R., Kalita, P., Trask, J. and Kuhlenschmidt, M.S. (2011) 'Development of a physically-based model for transport of *Cryptosporidium* parvum in overland flow', *Environmental Modelling and Software*, Vol. 26, pp.1289–1297.

Bitto, A. and Aldras, A. (2009) 'Prevalence of giardia and cryptosporidium in muskrats in Northeastern Pennsylvania and New Jersey', *Journal of Environmental Health*, Vol. 71, pp.20–26.

Bradford, S.A., Morales, V.L., Zhang, W., Harvey, R.W., Packman, A.I., Mohanram, A. and Weity, C. (2013) 'Transport and fate of microbial pathogens in agricultural settings', *Critical Reviews in Environmental Science and Technology*, Vol. 43, pp.775–893.

Brush, C.F., Ghiorse, W.C., Anguish, L.J., Parlange, J.Y. and Grimes, H.G. (1999) 'Transport of *Cryptosporidium* parvum oocysts through saturated columns', *Journal of Environmental Quality*, Vol. 28, pp.809–815.

Chappell, C.L., Okhuysen, P.C., Sterling, C.R., Wang, C., Akubowski, J.W. and Dupont, H.L. (1999) 'Infectivity of *Cryptosporidium* parvum healthy adults with pre-existing anti-C. parvum serum immunoglobulin', *American Journal of Tropical Medicine and Hygiene*, Vol. 80, pp.157–164.

Christophe, J., Darnault, G., Steenhuis, T.S., Garnier, P., Kim, Y.J., Jenkins, M.B., Ghiorse, W.C., Baveye, P.C. and Parlange, J.Y. (2004) 'Preferential flow and transport of cryptosporidium parvum oocysts through the vadose zone: experiments and modeling', *Vadose Zone Journal*, Vol. 3, pp.262–270.

Dai, X. and Hozalski, R.M. (2003) 'Evaluation of microspheres as subsitutes for cryptosporidium parvum oocysts in filtration experiment', *Environmental Science and Technology*, Vol. 37, pp.1037–1042.

Davies, C.M., Ferguson, C.M., Kaucner, C., Krogh, M., Altavilla, N., Deere, D. and Ashbolt, N.J. (2004) 'Dispersion and transport of cryptosporidium oocysts from fecal pats under simulated rainfall events', *Appl. Environ. Microbiol.*, Vol. 70, pp.1151–1159.

Dorner, S.M., Anderson, W.B., Smechanismson, R., Kouwen, M.N. and Huck, P.M. (2006) 'Hydrologic modeling of pathogen fate and transport', *Environment Science and Technology*, Vol. 40, pp.4746–4753.

Ferguson, C.M., Davies, C.M., Kaucner, C., Krogh, M., Rodehutskors, J., Deere, D.A. and Ashbolt, N.J. (2007) 'Field scale quantification of microbial transport from bovine faeces under simulated rainfall events', *Journal Water Health*, Vol. 5, pp.83–95.

Fox, G.A., Muñoz-Carpena, R. and Sabbagh, G.J. (2010) 'Influence of flow concentration on parameter importance and prediction uncertainty of pesticide trapping by vegetative filter strips', *Journal of Hydrology*, Vol. 384, pp.164–173.

Han, F., Tan, W. and Zhou, X. (1998) 'Human cryptosporidiosis in Nanjing area: report of 2 cases', *Jiangsu Medical Journal*, Vol. 12, pp.692–703 (in Chinese).

Harter, T., Wagner, S. and Atwill, E.R. (2000) 'Colloid transport and filtration of *Cryptosporidium Parvum* in sandy soils and aquifer sediments', *Environment Science and Technology*, Vol. 34, pp.62–70.

Kong, Y. (2017) *Plant Roots Controlled CryptosporidiumOocysts Subsitute Transport and the Mechanism between the Soil-Water*, Thesis, China University of Mining and Technology.

Krometis, L.A.H., Characklis, G.W., Simmons, O.D., Dilts, M.J., Likirdopulos, C.A. and Sobsey, M.D. (2007) 'Intra-storm variability in microbial partitioning and microbial loading rates', *Water Research*, Vol. 41, pp.506–516.

Lu, P. (2012) *Enhanced Removal of Cryptosporidium Parvum Oocysts and Cryptosporidium-Sized Microspheres from Recreational Water*, Doctor's thesis, University of North Carolina at Charlotte, USA.

Lu, P. and Amburgey, J.E. (2016) 'A pilot-scale study of cryptosporidium-sized microsphere removals from swimming pools via sand filtration', *J. Water Health*, pp.109–120.

Lu, P., Amburgey, J.E., Hill, V.R., Murphy, J.L., Schneeberger, C. and Arrowood, M.J. (2017a) 'A full-scale study of cryptosporidium parvum oocyst and cryptosporidium-sized microsphere removals from swimming pools via sand filtration', *Water Qual. Res.*, Vol. 52, pp.18–25.

Lu, P., Amburgey, J.E., Hill, V.R., Murphy, J.L., Schneeberger, C., Arrowood, M.J. and Yuan, T. (2017b) 'Removals of cryptosporidium parvum oocysts and cryptosporidium-sized polystyrene microspheres from swimming pool water by diatomaceous earth filtration and perlite-sand filtration', *J. Water Health*, Vol. 15, pp.374–384.

Lu, P., Yuan, T., Feng, Q., Xu, A. and Li, J. (2013) 'Review of swimming-associated cryptosporidiosis and *Cryptosporidium* oocysts removals from swimming pools', *Water Quality Research Journal of Canada*, Vol. 48, pp.30–39.

McDonald, A., Kay, T. and Jenkins, A. (1982) 'Generation of faecal and total coliform surges by stream flow manipulation in the absence of normal hydrometeorological stimuli', *Applied Environmental Microbiology*, Vol. 44, pp.292–300.

Mohanram, A., Ray, C., Metge, D.W., Barbe, R.L.B., Ryan, J.N. and Harvey, R.W. (2012) 'Effect of dissolved organic carbon on the transport and attachment behaviors of *Cryptosporidium Parvum* oocysts and carboxylate-modified microspheres advected through temperate humic and tropical volcanic agricultural soil', *Environment Science Technology*, Vol. 46, pp.2088–2094.

Niu, J., Xu, X. and Zhang, Z. (2006) 'The present and future research on preferential flow', *Acta Ecologica Sinica*, Vol. 16, pp.231–243 (in Chinese).

Sterk, A., Schijven, J., Nijs, T.D. and Husman, A.M.D.R. (2013) 'Direct and indirect effects of climate change on the risk of infection by water-transmitted pathogens', *Environment Science Technology*, Vol. 47, pp.12648–12660.

Sun, H. (2016) *Effects of Soil Physical and Chemical Properties on the Transport of Cryptosporidium with Surface Runoff*, Master's thesis, China University of Mining and Technology, Xuzhou, China (in Chinese).

Sunderland, D., Graczyk, T.K., Tamang, L. and Breysse, P.N. (2007) 'Impact of bathers on levels of *Cryptosporidium* parvum oocysts and giardia lamblia cysts in recreational beach waters', *Water Research*, Vol. 41, pp.3483–3489.

Tate, K.W., Atwill, E.R., George, M.R., McDougald, M.K. and Larsen, R.E. (2000) *Cryptosporidium* parvum transport from cattle faecal deposits on California rangelands', *Journal of Range Management*, Vol. 53, pp.295–299.

Tate, K.W., Pereira, M.D.C. and Atwill, E.R. (2004) 'Efficacy of vegetated buffer strips for retaining *Cryptosporidium Parvum*', *Journal of Environmental Quality*, Vol. 33, pp.2243–2251.

Trask, J.R., Kalita, P.K., Kuhlenschmidt, M.S., Smith, R.D. and Funk, T.L. (2004) 'Overland and near-surface transport of *Cryptosporidium Parvum* from vegetated and nonvegetated surfaces', *Journal of Environmental Quality*, Vol. 33, pp.984–993.

United States National Research Council (2008) *Urban Stormwater Management in the United States*, Washington DC, USA.

Xu, L. (2005) *Study on Epidemiology of Cryptosporidium Parvum Infection in Anhui Province and Expression of Cellular Immune Function in Person Infected by Cryptosporidium Parvum*, Master's thesis, Anhui University of Science and Technology, Huainan, China (in Chinese).

Xu, Y. (2016) *Study on the Effect of Runoff Conditions on the Transport of Cryptosporidium.* Master's thesis, China University of Mining and Technology, Xuzhou, China (in Chinese).

Yu, C.R., Munoz-Carpena, R., Gao, B. and Perez-Ovilla, O. (2013) 'Effects of ionic strength, particle size, flow rate, and vegetation type on colloid transport through a dense vegetation saturated soil system: experiments and modeling', *Journal of Hydrology*, Vol. 499, pp.316–323.

Yuan, T., Lu, P. and Li, Z. (2019) 'Attachment of cryptosporidium-sized microspheres by holly plant roots', *Nature Environment and Pollution Technology*, Vol. 18, pp.629–632.