Megasonic elution of waterborne protozoa enhances recovery rates

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Disciplines
Parasitology
Water And Environment

Keywords
Cryptosporidium
Waterborne Pathogens
Sample Processing
Filtration
Megasonic

Type of Observation
Standalone

Type of Link
Standard Data

Submitted Sep 5, 2017
Published Jan 23, 2018

Triple Blind Peer Review
The handling editor, the reviewers, and the authors are all blinded during the review process.

Full Open Access
Supported by the Velux Foundation, the University of Zurich, and the EPFL School of Life Sciences.

Abstract

Waterborne pathogens represent a major concern for human and animal health making monitoring of water essential to prevent outbreaks. Sample preparation is critical to assess a spatio-temporally representative volume of water and identify pathogens present at low concentrations, with filtration being the commonly adopted approach. Numerous different filter types and operational strategies have been investigated to consistently improve the low recovery rates of pathogens, with work now investigating creation of automated sampling systems.

Previous work has often focused on chemical strategies for maximising recovery rates during the elution from the filter. However, novel physical methods, like the use of megasonic sonication offer great potential for effective pathogen removal from filters. Compared to ultrasound assisted agitation, megasonic sonication, which operates at a higher excitation energy frequency, offers a gentler and more thorough process for elution with lower risk of pathogen damage during the process. Megasonic exposure of *Cryptosporidium* oocysts has been demonstrated to preserve their viability. This mode of elution enables the downstream identification of pathogen infectivity since viability and species information cannot be extracted from damaged or destroyed pathogens.

Here we investigate the use of megasonic elution to improve the recovery rates of *Cryptosporidium* in two different filtration set-ups: firstly dead-end filtration using a Rexeed filter and secondly, tangential flow filtration using a Fresenius filter. The results demonstrate that recovery rates are increased by around 50% for both set-ups highlighting the potential of megasonic elution in this application.

Introduction

Cryptosporidium is a particularly problematic waterborne pathogen due to resistance to chlorination, low infectious dose and impressive longevity in its environment [1]. Additionally, detection is challenging as recovery rates are often low and the protozoan cannot be cultured in the lab making effective sample processing for concentration essential [2].

Recently, the use of ultrasonic elution was demonstrated to result in a significant enhancement of recovery rate for *Cryptosporidium* [3]. However, a few minutes of continuous ultrasound was also shown to kill *Cryptosporidium* oocysts with more than 90% oocyst deactivation [4]. The integrated ultrasound filtration system described by Al-Sabi was limited to 5 s of ultrasound application as longer exposures significantly impacted viability [3]. Deactivation removes the ability to determine viability status of detected pathogens. Additionally, DNA degradation could be incompatible with the molecular tools for species determination.

In contrast, through the minimisation of the time required for bubble growth and the resulting low cavitation energy, megasonic sonication offers a way to elute undamaged and potentially viable oocysts from filters and membranes. This approach was recently applied *Cryptosporidium* in the Filta-Max® system [5] [6] as utilised in standard methods like US EPA1623.1 [7], ISO 15553:2006 [8] and the UK Environment Agency (2010) Microbiology of Drinking Water: Part 14 [9]. This work has also demonstrated the viability of *Cryptosporidium* even after 120 min of megasonic exposure, with an excystation assay reporting a 96% excystation rate with a sporoziote/shell ratio of 2.26 (compared to 97% and 2.4 for the control) [5].

Here, we explore the use of megasonic sonication for pathogen elution with different types of filter in an automated filtration system and suggest how the incorporation of megasonic transducers could enhance the performance of the set-up. Protozoan pathogen recovery rates from the filtration stages of the detection protocol are typically low; therefore, the key focus in this study was to determine whether megasonic elution would lead to an increase in recovery rates.

Objective

The aim was to investigate whether megasonic elution could increase the recovery rates of *Cryptosporidium* using filters included in automated filtration systems.

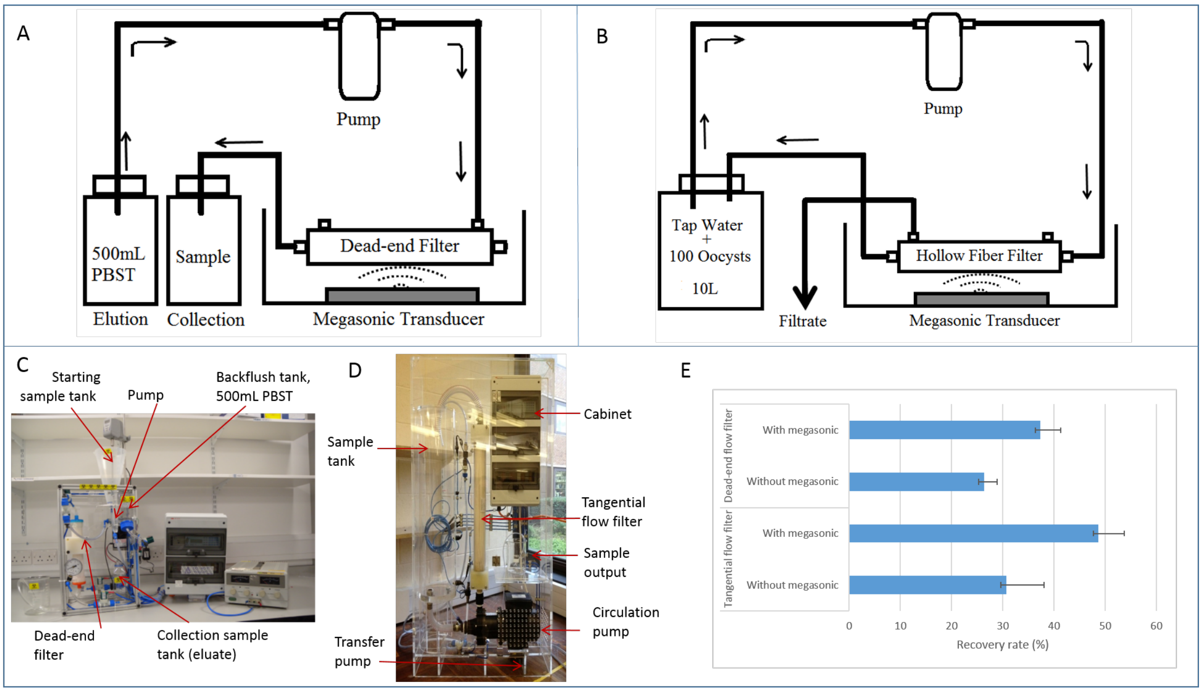

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Figure Legend

Figure 1. Filtration set-ups and pathogen recovery rate results.

(A) Schematic of the dead-end filtration set-up. Initially, the water sample is pumped through the filter from the sample starting tank (shown in C), and subsequently, valves are switched to pump the backflush solution through the filter into the sample collection bottle. As this elution stage incorporated megasonic transduction only this part is illustrated.

(B) Schematic of the tangential flow filtration set-up.

(C) Image of the dead-end filtration set-up.

(D) Image of the tangential flow set-up.

(E) Recovery rate results, obtained in triplicate after spiking tap water with 100 oocysts and counting the number in the final volume (performed at Scottish Water in accordance with standard protocols e.g. The UK Environment Agency (2010) Microbiology of Drinking Water: Part 14).

Results & Discussion

Experiments were undertaken using two different systems to explore the potential of megasonic elution with different filter types. A dead-end filtration system was used with the Rexeed 25AX filter and a tangential flow set-up was used with a Fresenius FX1000 filter. These filters were selected since they have been utilised in automated set-ups as well as on the basis of data, i.e. previous demonstration of good filtration performance with *Cryptosporidium* oocysts, from previous literature [10]. In the dead-end operation, the sample is first passed through the filter and pathogens captured before the filter is back-flushed to elute pathogens from the filter. Figure 1A illustrates schematically the second stage of this process, i.e. the elution step where megasonic was incorporated.

The dead-end set-up is fully automated (without megasonic agitation) and details will be published elsewhere. Figure 1B shows the functional operation of the tangential flow system in which clean water is extracted through the filter from a recirculating flow. The tangential flow system was part of a fully automated system to replace the *Cryptosporidium* regulatory monitoring procedure described earlier, developed by the Company Shaw Water. Images of the filter systems are shown in figures 1C and 1D; for the megasonic experiments, the filter was placed inside a waterbath with the megasonic transducer. For this study no chemical filter pre-treatments or elution buffers were utilised focusing solely on the impact of the physical elution method. Standard operating procedures were compared with the use of the megasonic transducer (during the back-flush phase for the dead-end filter and throughout operation of the tangential flow filter).

Recovery rate results are shown in figure 1E. For the dead-end filtration system with the Rexeed filter recovery rates were 26% in the standard procedure and this increased to 37% with the use of megasonic elution. A similar effect was observed in the tangential flow set-up where recovery rates increased from 31% to 49% when adding in the megasonic sonication. Overall, these recovery rates are in a similar range to previously reported data, although some work with chemical pre-treatments and different backflushing solutions showed better recovery rates than the without megasonic 26% and 31% found here, suggesting optimisation of selected chemicals is important as well as physical methods like the megasonic elution. However, importantly, both increases were statistically significant with a student T-value of 4.064 and 3.077, for the tangential flow and the dead-end flow filters, respectively, both of which are larger than the T-test value of 2.776 using a 95% confidence interval. This suggests that systems monitoring waterborne protozoa should consider the inclusion of megasonic elution. Previous work did not demonstrate such a large enhancement of recovery rates with the Filta-Max filter and rather advantages were observed, and emphasised, in terms of automated and more effective elution into smaller volumes [5]. Different filter types should also be explored to discover the best set-up before fully automated systems incorporating megasonic transducers are developed.

Conclusions

Megasonic elution delivers a significant enhancement in recovery rates of *Cryptosporidium* from automated filtration set-ups.

Limitations

Recovery rates, without megasonic, for both processes are lower than some in the literature, which is likely to be attributable to the lack of chemical pre-treatments employed. A combination of physical, e.g. megasonic elution, and chemical, e.g. particular pre-treatment and backflushing solutions, methods is likely to provide the best outcome.

Further studies should fully integrate megasonic transducers into automated filtration set-ups as well as explore the impact of megasonic operating parameters on recovery rates. The performance should also be investigated with a wider range of waterborne pathogens, further filter types and incorporated with chemical pre-treatments to maximize recovery rates.

Additional Information

Methods and Supplementary Material

Please see https://sciencematters.io/articles/201712000007.

Funding Statement

All authors would like to acknowledge funding from the EU "Aquavalens" (grant number 311846, theme KBBE-2012-2.5-01).

Acknowledgements

The authors express their thanks to Ben Horton (Moredun Scientific), Susan Lee and James Green (Scottish Water) for their time, assistance and help, which contributed to the development of this article. Their contribution to the assessment of the recovery rates and provision of oocysts are also gratefully acknowledged.

Ethics Statement

Not Applicable.

Citations

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