VERIFYING THE EFFECTIVENESS OF WATER TREATMENT FOR TOXOPLASMA. TENDER REFERENCE: CR/2016/09

Research Summary

Oocysts of the protozoan parasite *Toxoplasma gondii* (*T. gondii*) are shed in the faeces of cats and are known to contaminate the environment. If ingested, these oocyst can cause subsequent infection within animals and humans, with one third of the human population predicted to be infected. Over the last few years the parasite has become recognised as an emerging issue, as data has shown that *T. gondii* is in the top 3 food-borne pathogens worldwide due to its impact on Disability Adjusted Life Years (DALYS). *T. gondii* can cause a wide spectrum of clinical disease in humans, with the main risk groups being pregnant women who become infected for the first time during pregnancy and people who may be immunocompromised due to infectious disease or treatments for organ transplants or cancer. Congenital infection can be devastating, ranging from abortion or still birth to defects in the new born such as hydrocephalus (fluid in the brain), convulsions and retinochoroiditis (inflammation of the eye, which can cause blindness). Some babies may be born clinically healthy but develop symptoms later in life, particularly ocular disease.

In 2015 a scientific study was published describing the prevalence of DNA from the protozoan parasite *T. gondii* in drinking water sources in Scotland (Wells *et al*., 2015). The study described an overall prevalence of 8.8% (124/1411) from 147 water catchments in Scotland. However, as this was based on the molecular detection of *T. gondii*, further confirmation was necessary to determine if the DNA identified was derived from *Toxoplasma* oocysts.

The overall aim of the tender was to develop/refine a method to detect the presence of *T. gondii* oocysts from drinking water in order to confirm if oocysts are being removed effectively at Scottish Water treatments plants. In addition, this would address whether oocysts are present in Scottish raw and final waters, the effectiveness of individual water treatment plants and whether any oocysts detected were viable and therefore present any public health risk.

The current study involved close links with Scottish Water and all samples were obtained from Scottish Water, not just for testing, but also for the development of a concentration methodology. These samples were post *Cryptosporidium* immuno-magnetic separation (IMS) water samples from both raw (un-treated water) and final water (drinking water). Early into the development of the concentration methodologies, it became apparent that post *Cryptosporidium* IMS water samples that were dirty (turbid), whether originating from raw or final water, were problematic. Samples could appear dirty due to the concentration and filtration process carried out at Scottish Water laboratories, where 1000 L of water is concentrated to 10 ml. This process can also concentrate any particulate matter in the water, making them appear more turbid. The methodology development mainly focused on trying to "clean up" these dirty samples to enable them to be screened by microscopy, as well as ensuring that the method remained sensitive enough to detect low numbers of parasite oocysts. Of the nine methodologies tested, centrifugation of clear and cloudy spiked samples proved to be a successful quick and straight forward method for concentrating *Toxoplasma* oocysts, with as low as one oocyst being detected using this process. Overall, auto fluorescence provided the best results for visualisation the parasite oocysts. Despite repeated attempts, dirty raw and dirty final concentrated samples could not be screened due to their turbidity.

Overall, 201 post concentrated water samples (final water n=194, raw water n=6, unknown n=1) from 10 catchments were screened by microscopy. *T. gondii* like bodies (TLB's) were identified in 27 samples from 8 catchments, however, following DNA extraction and a *T. gondii* specific qPCR (a semi-quantitative detection technique to estimate the quantity of parasite DNA present) of these TLB's scraped from slides, only one sample was found to contain *Toxoplasma* from one catchment. The PCR product from this positive sample was sent for DNA sequencing which further confirmed the presence of *T. gondii*. The qPCR positive result had a high Cq value (36.8), and the final concentration calculated was very low (1.000 pg), equating to less than one oocyst. The oocyst was identified from a final water sample that was processed for routine *Cryptosporidium* testing at Scottish Water on 28th January 2018. The oocyst appeared to be sporulated, which is the known infectious/viable stage of the oocyst, however its appearance was not typical of a fresh sporulated oocyst. It is likely that the oocyst had been in the environment for some time

and had therefore been exposed to harsh environmental conditions. It is therefore not surprising that its morphology differed to an oocyst stored in optimum conditions in the laboratory. It was not possible to conclude whether the identified *T. gondii* oocyst was viable. However, given that a *T. gondii* oocyst must first sporulate to become infective, it is likely that, at one point, this oocyst was indeed viable.

Following the isolation of this single oocyst, Scottish Water carried out a site specific investigation into environmental conditions, water quality and water treatment works performance at the time of and prior to the period this sample was collected. The treatment plant was working effectively and Scottish Water were not able to identify the possible source of this *T. gondii* oocyst, or its route through the water treatment process.

As the majority of the concentrated final water samples, which could be screened by microscopy, were either clean or cloudy in appearance, there is possibly an underestimation to the number of oocysts which may be present. Although attempts were made to clean and concentrate oocysts from dirty/turbid raw and final water samples, the methodology still requires improvements. Ideally, a method similar to that used for concentrating *Cryptosporidium* oocysts, using immunomagnetic separation (IMS) along with a specific monoclonal antibody should be developed to improve detection of *T. gondii* oocysts in these turbid samples. However, within the current study, a DNA spiking experiment was conducted. The results of this experiment have shown that when *T. gondii* DNA is detected using the specific qPCR, the DNA is highly likely to have originated from an oocyst, other than DNA "floating" into a water catchment. This result could also have implications for the previously published study (Wells *et al*., 2015), as any *T. gondii* DNA detected most likely means that *T.gondii* oocysts have been present.

In light of this result, additional analysis was performed on raw (dirty) concentrated post *Cryptosporidium* IMS samples from the catchment the oocyst was identified in. As these were highly turbid/dirty samples, it was not possible to visualise them by microscopy and each sample was tested for the presence of *T. gondii* DNA using a *T. gondii* specific qPCR. Of the 31 samples which were tested by qPCR, 4 (12.9%) were identified to contain *T. gondii* DNA.

In summary, after visualising 201 concentrated post *Cryptosporidium* IMS samples for *T. gondii* oocysts between the periods of 26th September 2017 to 6th February 2018 across 10 different Scottish water catchments only one *T. gondii* oocyst from one catchment was identified. Therefore, it can be concluded that although the water treatment process appears to be effective at removing oocysts across the remaining nine catchments there may also be an underrepresentation, as the most turbid/dirty concentrated final water samples could not be screened for oocysts and it is possible that dirty samples are more likely to have oocysts present.

Microscopy for oocyst visualisation is currently not the best method to screen water samples for the presence of *T. gondii* oocysts. The qPCR optimised at Moredun for the detection of *T. gondii* oocysts is a sensitive and specific technique which has been further confirmed during this study to detect DNA which has been derived from oocysts. Recommendations which would enhance any future studies involving microscopy would be to develop a suitable IMS technique using a *T. gondii* specific monoclonal antibody, similar to what is already in place for the *Cryptosporidium* IMS methodology. This would ensure that *T. gondii* oocysts could be isolated from dirty/turbid concentrated water samples. In addition, if it was possible to examine samples from different catchments over a full year, this would resolve the issue of trying to predict high rainfall events. Another improvement would be to develop a sensitive oocyst viability assay, ideally one which moves away from using animal models. As to fully assess the risk, the viability of any *T. gondii* oocysts must be determined. Finally it is not known how *T. gondii* oocysts spread within the environment and understanding this would help to predict how they behave and how to potentially prevent them entering the water supply.

Current regulations in Scotland regarding pathogen testing in water supplies only tests for the parasite *Cryptosporidium* (THE CRYPTOSPORIDIUM (SCOTTISH WATER) DIRECTIONS 2003***).*** Given the potentially very serious human health consequences of *T. gondii* infection and the current lack of data on *T. gondii* prevalence in Scottish water supplies, there is a requirement for further research as highlighted above, to answer questions and fill information gaps to inform policy of *T. gondii* risk to public health through contaminated drinking water.