1. Executive summary

This report forms the third and final output of the Research Project Viruses in Groundwater Ref: DWI 70/2/325. The aim of this research project is to establish whether there is a potential risk of contamination of groundwater, abstracted for human consumption in England and Wales, by viruses emanating from sewage or other pollution sources.

The report presents the findings of a one year monitoring programme into the occurrence of viruses at sites across a range of aquifer types and land uses in England. The report also summarises the findings of a literature review presenting current published research into occurrence of enteric viruses in groundwater in the UK and internationally; and a questionnaire survey of 20 water companies on their virus monitoring capability and historic data records.. These previous outputs are included as Appendix 1 and 2 respectively.

1.1 Literature review

The literature review summarised current published research into the prevalence and measured quantities of enteric viruses in groundwater from studies previously conducted in England and Wales and internationally. The following conclusions were reached:

Transport of viruses in the subsurface is controlled by sorption to clay particles or organic colloids and inactivation influenced by moisture, pH, and temperature. Survival is greatest where there are limited predatory populations and in cool conditions as well as in the dark. Virus behaviour is likely to be site-specific. Some viruses, such as adenovirus, can persist in groundwater and in general they survive for longer than bacteria.

The unsaturated zone can play an important role in retarding viruses due, amongst other factors, to sorption at the air-water interface. However, vulnerability of the aquifer can be increased during high recharge events due to bypass flow and by mobilisation of sorbed viruses. Karst features, fissures and abandoned wells can provide rapid routes for virus movement to aquifers. The principal aquifers of England can exhibit both intergranular and fissure flow. There is relatively little systematic evidence for virus occurrence in groundwater in England. Most of the existing data is derived from site investigations and is mainly for the Chalk and Permo-Triassic sandstone aquifers.

Neither bacteria, such as *Escherichia. coli*, nor phages, may provide a surrogate for enteric virus transport and evidence for their application for water contamination is contradictory. Indicators could be sewage-related emerging contaminants, such as acesulphame or ibuprofen. Phages have been used as tracers in England but work has not been focussed on studying them in relation to other pathogens; rather for the identification of rapid pathways. It remains difficult to identify surrogates for enteric viruses in groundwater as risk settings may be different from more-widely studied pathogens, such as coliforms or cryptosporidium.

1.2 Questionnaire survey

The questionnaire survey canvassed a total of 20 water companies and sought information on groundwater abstractions, historic and current groundwater monitoring data and details of water safety planning for viruses. 17 of the 20 responded. Overall, the survey indicated that routine viral monitoring is not being undertaken, and therefore validation of any viral risk where included in drinking water safety plans is not being undertaken. Historically, monitoring was undertaken in the 1980s but the data is predominantly unavailable. One off special research investigations into the presence of viruses in groundwater have been undertaken over the period 1990 through to present day.

1.3 Groundwater monitoring study

The field sampling programme comprised a selection of eight ground water abstraction points representing the major aquifers of England and Wales but also covering a mix of rural and urban catchments and mains and private sewerage systems. The eight sites selected were distributed across the country and were focused on the four aquifers that are most utilised for water supply, i.e. the Cretaceous Chalk, Permo-Triassic Sandstone, the Jurassic limestone and Carboniferous limestone. Four rounds of sampling were undertaken between February 2019 and January 2020 focusing on distinct periods of differing microbial risk and hydrological conditions.

Samples of approximately 1000 litres were obtained using a bespoke sampling rig and concentrated by filtering through hollow fibre Asahi Polysulfone filters. Quantitative Polymerase Chain Reaction (qPCR) was used to detect viral RNA. Samples for bacterial analysis were also taken at each site on each sampling occasion. Bacterial index organisms were assessed as predictors of virus presence/absence.

Two thirds or 66% of all the samples taken (29 in total) did not show the presence of any of the target virus RNA. However, virus RNA was detected in 34% of samples, and 17% of samples tested positive for multiple viruses. Furthermore, the risk varies considerably between seasons, with eight of the ten virus RNA positive samples occurring in rounds R3 (Oct/Nov 2019) and R4 (Jan 2020). Hepatitis A was the most frequently detected virus RNA (24% samples, 75% sites), followed by Norovirus GI (14% samples, 38% sites), Hepatitis E (10% samples, 38% sites), and single detections of Human Adenovirus F and Norovirus GII. There were no positive detections of either Enterovirus or Rotavirus A RNA. Hepatitis E co-occurred with Hepatitis A on all occasions, although there were no consistent patterns of co-occurrence amongst other viruses.

Sites 1 to 7 tested positive for virus RNA on at least a single occasion demonstrating that viruses were present in all four aquifers, beneath both rural and urban land uses, and across a range of microbial risk settings. Site 8 was the only location testing negative for virus RNA throughout the study. Hepatitis A was the only virus detected in all four aquifers. Virus RNA was most prevalent during sampling rounds in October 2019 (R3) and January 2020 (R4) which coincide with the groundwater recharge season.

All sites with previous evidence of total coliforms and *Escherichia coli* tested positive for virus RNA on at least one sampling round. These sites all tested positive for total coliforms and all but Site 7 tested positive for *E. coli* between February 2019 and January 2020. Site 8 with no previous evidence of any coliforms showed no evidence of virus contamination.

1.4 Risk assessment and quantification

A source pathway receptor risk assessment model similar to that developed for Cryptosporidium in the Bouchier Report (1998) is proposed to meet the wholesomeness requirement outlined in Regulation 4 of the Water Supply (Water Quality) Regulations (HMSO 2016) and the Water Supply (Water Quality) Regulations 2018 (Wales) (HMSO 2018). Viral sources are generally linked to human faecal contamination, although some, such as Norovirus GII also has animal (zoonotic) origins. The most likely route for faecal contaminants to enter the environment and groundwater is via sewage collection systems and sewage treatment works discharges. The depth and connectivity between the unsaturated zone and the groundwater beneath is important in the retardation of viruses. A deep unsaturated zone affords more protection than a shallow one, whilst fractures, karst features, man-made soakaways and mines increase vulnerability of the aquifer by reducing travel time. The physical borehole construction and operation of a groundwater abstraction and treatment site is the last step in the pathway aspect of the risk assessment. The receptor is defined as the raw water as it enters the groundwater abstraction point, with treatment before distribution considered the final barrier to protect customers' health.

A conceptual source pathway receptor risk model is discussed. Assuming numerical risk values are assigned to each element within the model, water companies could rank sources within their operational area based on viral risk. The basic use of the risk methodology would permit the identification of groundwater sources most at risk and this single step forward would aid in the expansion of additional targeted monitoring programmes to provide a more extensive data base on which to base and conclude more definitive actions as required.

1.5 Water treatment processes

Regulation of the treatment of abstracted water is implemented through the Water Supply (Water Quality) Regulations 2016 (as amended) (HMSO 2016) in England and the Water Supply (Water Quality) Regulations (Wales) (HMSO 2018). Water treatment processes such as. coagulation, filtration, sedimentation, aeration, ozonation are not normally deployed at groundwater abstraction sites. Removal of pathogenic organisms is achieved through the disinfection processes.

Disinfection with chlorine is the most widely used method for large public water supplies, and is commonly the only treatment applied to some groundwater supplies. Ultra-violet (UV), the most widely used alternative to chlorination, whilst commonly used for private groundwater supplies its use is becoming more widespread in public supplies. UV provides the added the benefit of potentially both virus and cryptosporidium treatment. Other disinfection approaches include the use of ozone (O_3), and injection of hydrogen peroxide (H_2O_2),

Changes in raw water quality such as variable turbidity levels (UKWIR 2016), can affect the disinfection process. Generally, the poorest water quality occurs due to rainfall and recharge occurrence in winter months, which also aligns with the understanding that viruses are able to survive in this environment whereas other pathogens do not. Thus, the virus risk and treatment challenge to manage virus concentrations occurs at the same time as poorer water quality.

1.6 Conclusions

There have been relatively few well-documented published accounts of pathogenic viruses in groundwater supplies with most of the work being undertaken in the USA in the early 2000s. UK data is primarily derived from site investigations rather than monitoring data at abstraction points and is mainly for the Chalk and Permo-Triassic sandstone aquifers. Monitoring was undertaken by Thames Water in the 1970's and 1980's, but the programme was discontinued due to changes in regulatory requirements.

Virus RNA was recorded in all major aquifers across the 4 sampling rounds, and in both rural and urban settings. Their presence at sites with low, as well as high permeability protective cover is likely to reflect the nature of rapid, preferential flow paths in both the unsaturated and saturated zone. The absence of virus RNA, or any indicator organisms, at Site 8 which is located in an urban setting, with no protective geology over the aquifer, and a shallow water table provides further evidence that it is the absence of fractures, rather than the depth of the aquifer which has the strongest influence on the presence or absence of viruses.

Based on the results of this study, the qPCR method provides an effective method of detecting and quantifying viral RNA. However, it provides no indication of the viability, and therefore whether viruses are infectious whilst present in the aquifer. There were also no consistent patterns of co-occurrence of any viruses, although Hepatitis E co-occurred with Hepatitis A on all occasions in which they were detected. A viral cell culture method may offer a supplementary means of investigating both viability and co-occurrence. However, this requires specialist laboratory facilities and is not suitable for all viruses.

The occurrence of virus RNA in the samples was closely associated with groundwater recharge. 8 out of 10 detections were made during rounds R3 and R4 in Oct/Nov 2019 and Jan 2020 respectively, during the main groundwater recharge season.

Hepatitis A, E and Norovirus GI were the most commonly detected viruses. The presence of Hepatitis is unexpected given its low and apparently declining occurrence in the population. This may be due to infections being asymptomatic, and therefore unreported; coupled with the high number of Hepatitis A virus particles shed by infected individuals. The virus also has an enhanced mobility through the unsaturated zone. The presence of Hepatitis E and Norovirus GI is more predictable due to their prevalence within the UK population, and in the case of Norovirus, in groundwater sources.

There was no clear relationship between the occurrence of viruses and other environmental indicator organisms such as total coliforms and E. coli when considered for an individual sample. This supports the WHO position that "Generally, faecal bacteria have lower persistence in the environment compared to viruses and protozoa and are more sensitive to common disinfectants. Faecal indicators (bacteria) found in water therefore do not necessarily correlate well with the presence of viral and protozoal pathogens." (WHO, 2019). However, this study found that the relationship is stronger if the data is compared at a site level over a longer period. This is supported by the findings by Fout *et al.* (2017) in their meta-analysis data covering 746 boreholes that total coliforms and *E. coli* had a specificity of 88 and 97%, respectively, for the prediction of viruses by molecular methods. It suggests that they have merit for evaluating whether a site is at-risk or not of virus contamination. However, the relative value of using microbial indicators and other associated organic compounds, is less relevant now given the ease of use of virus qPCR methods.

Although the findings of the field study have led to valuable observations on the occurrence of virus RNA the data are limited and do not permit the production of a detailed quantitative risk assessment of water supplies in England. In addition, routine monitoring and laboratory analyses at a wider scale is expensive and therefore not expected to become common practice unless specific regulations requiring this are introduced. The source pathway receptor model used for drinking water safety plans under the Water Supply (Water Quality) Regulations 2016 (HMSO 2016) and the Water Supply (Water Quality) Regulations 2018 (Wales) (HMSO 2018) could be adapted for assessing the potential risk of groundwater contamination by viruses.

Although the risk assessment methods used for other sources of microbial contamination rely on substantial data to predict consumer risk, the basic use of the risk methodology would permit the identification of groundwater abstraction sites most at risk. A potential approach is presented in which the presence of potential viral sources is first assessed. For sites where a viral source is present each of the possible pathways to the aquifer are assigned a numerical weighting relative to their risk level, thus allowing potential sources of viral contamination within a catchment to be ranked. This step would aid in identifying vulnerable sources; assist in prioritising sites for targeted monitoring programmes, thus providing a more extensive data base on which to base and conclude more definitive actions.

The review of the current UK monitoring regime concluded that there is a gap in respect of viruses in the quality monitoring regimes. It has become obvious from this work that no current virus monitoring is being conducted and that the only laboratory capacity exists in the private sector, Government bodies and Universities. This leaves a gap in the ability of operators to demonstrate compliance with the wholesomeness test as required by Regulation 4 of the Water Supply (Water Quality) Regulations 2016.

1.7 Suggested next steps

It is suggested that monitoring capacity and a monitoring programme is established to support and validate risks assessments and could incorporate the following 4 principles:

1. Reviews of drinking water safety plans should incorporate an assessment of the potential sources and pathways that may exist for viruses to enter groundwater and arrive at groundwater abstractions.
2. A regular review of all sources to identify which are at the highest risk of viruses being present. For sources where an initial viral risk to groundwater is identified, a more detailed risk assessment could be undertaken to determine which sources are at the highest risk and where monitoring is most appropriate to provide useful confirmatory data
3. Where confirmatory monitoring is undertaken the frequency and viruses considered should reflect the behaviour of the pathway and magnitude of the sources of virus identified over an annual water cycle.
4. Where a risk exists minimum treatment requirements should meet the disinfection recommendations of DWI (2018) or WHO (2011) whichever is the most appropriate given the viral challenge. The WHO evidence refers to a maximum contact time (CT) of 30 minutes with a free chlorine residual of 1.0 mg/l and pH of less than 8.

Suggested next steps for further study include:

1. A wider monitoring programme focusing on aspects identified in the risk assessment including the range of viral sources in urban and rural settings; risk of transport through the various aquifer types; the influence of antecedent conditions, particularly heavy rainfall on viral presence; and environmental indicators and index organisms under a range of conditions.

   It is suggested that the programme cover all aquifer types in England and Wales and include a range of control sites with little or no known history of viral contamination. Although qPCR is considered the best analytical technique for detecting viral RNA, supplementary methods should be considered to determine the viability of viruses. This could consist of a targeted viral culture trial.

2. Refining the risk assessment method including development of a numerical method for weighting sources and pathways at both site and aquifer scale based on the findings of the monitoring programme.

3. Undertaking desk based treatability tests to be conducted to consider tolerance and susceptibility to England and Wales based disinfection processes.

4. Reviewing groundwater policy with respect to the definition of Source Protection Zone 1 taking account of existing and emerging research into saturated zone groundwater flow travel time for viruses.