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Transport pathways for viruses in a sandstone aquifer

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Abstract
Following the discovery of viable human enteric viruses at depth in a sandstone aquifer in the U.K., a set of tracer experiments was conducted between March 2004 and August 2005 at a test site in a similar sandstone aquifer, using bacteriophages as human virus surrogates. Initial tests showed a range of bacteriophages (PRD1, φX174, H40/1 and MS2) to be transported between two 50 m boreholes, but attempts to identify the transport pathway(s) by tracer tests in packered horizons between boreholes proved unsuccessful. A new inter-borehole tracer test with injection at one borehole and abstraction from a pumping borehole has now been designed with novel instrumentation to identify the lithological horizons transporting viruses. In addition to the detailed descriptions of core available, the array of boreholes has been subjected to extensive geophysical logging (resistivity, natural gamma, optical televiewer) and hydraulic testing (constant discharge tests, single-hole and cross-hole packer tests). On the basis of these data a detailed conceptualisation of the site has been constructed, with a number of hydraulically significant low permeability horizons clearly identified. The new instrumentation allows the viruses and a conservative tracer to be sampled at different horizons in the abstraction borehole while the hole is being pumped. Viruses are enumerated by plaque assay and epifluorescence microscopy. To quantify the virus inflow at each horizon, a purpose-built packer flow meter was used to determine the up-hole discharge of water in the borehole at the sampling depths. A preliminary test without the injection of viruses was conducted to test the experimental equipment and to assess the degree of contamination of the site from previous experiments. The fluorescent dye used previously was found only at concentrations marginally above background, but PRD1 bacteriophages were found at concentrations in the order of 10^3 ml^{-1}, indicating that these viruses survive longer (more than two years) under field conditions than predicted by previous laboratory experiments. Following the results of the preliminary trial, modifications are being made to the instrumentation in preparation for the forthcoming full scale tracer test.

Keywords: virus, bacteriophage, groundwater, sandstone, epifluorescence,

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1 Introduction

The spread of viral infection through groundwater continues to be a subject of concern. Maunula et al. (2005) report that groundwater was the method of transmission in 15 out of 18 identified outbreaks of norovirus in Finland between 1998 and 2003. Many cases of waterborne enteric viral disease occur through faecal contamination, and so the reuse of treated sewage in artificial recharge schemes in cities requires that a risk assessment of the virus hazard be undertaken. Although many outbreaks occur through transmission through shallow aquifers, human enteric viruses have been found at depth in the Permo-Triassic Sandstone aquifer underlying the city of Nottingham in the UK (Powell et al, 2003). This finding was unexpected since the transit time from the source of contamination (assumed to be near surface) to depth is believed to be much greater than the expected survival time of the viruses. Consequently, a set of field and laboratory tests was conducted to characterise the transport of viruses in the sandstone. The Permo-Triassic Sandstone aquifers in the region are extensively used for water supply and underlie several other major cities, including Birmingham, Liverpool, and Manchester, and are described by Barker and Tellam, (2006). The sequence investigated comprises weakly to well-cemented fluvial and aeolian sandstones, with occasional thin, usually decimetre scale, mudstones and palaeosols (Fig. 1). The median pore sizes are typically 10-50 μm, much larger than the diameter of virus particles (often less than 100 nm). The sandstones are fractured, but modelling suggests that the regional scale permeability is close to that of the matrix (Hitchmough et al., 2007).

Figure 1: An outcrop of the sandstone showing both sedimentary structures and fractures

Field and laboratory column studies were carried out between March 2004 and August 2005 using bacteriophages as human enteric virus surrogates (Joyce et al., 2007). These studies were followed by further detailed laboratory investigations on the survival of enteric viruses in groundwater and the effect of natural colloids on the migration of viruses in sandstone (Pedley et al., 2007; Joyce et al., in press). The field studies were carried out at the University of Birmingham campus between two boreholes in the sandstone and consisted of a set of inter-borehole tracer tests using the bacteriophages PRD1, φX174, H40/1 and MS2 with a fluorescent dye as tracers. Although each virus was transported from one borehole to the other, attempts to identify the principal transmission pathways, by targeting fractures and high permeability horizons for detailed testing, proved unsuccessful. The aim of the present study is to identify these pathways by a more systematic study of the boreholes and to attempt to relate them to characteristic features in the geological sequence. To achieve this, a set of tracer tests has been designed with novel instrumentation allowing six horizons to be investigated simultaneously. This paper describes the experimental design and results from preliminary testing.

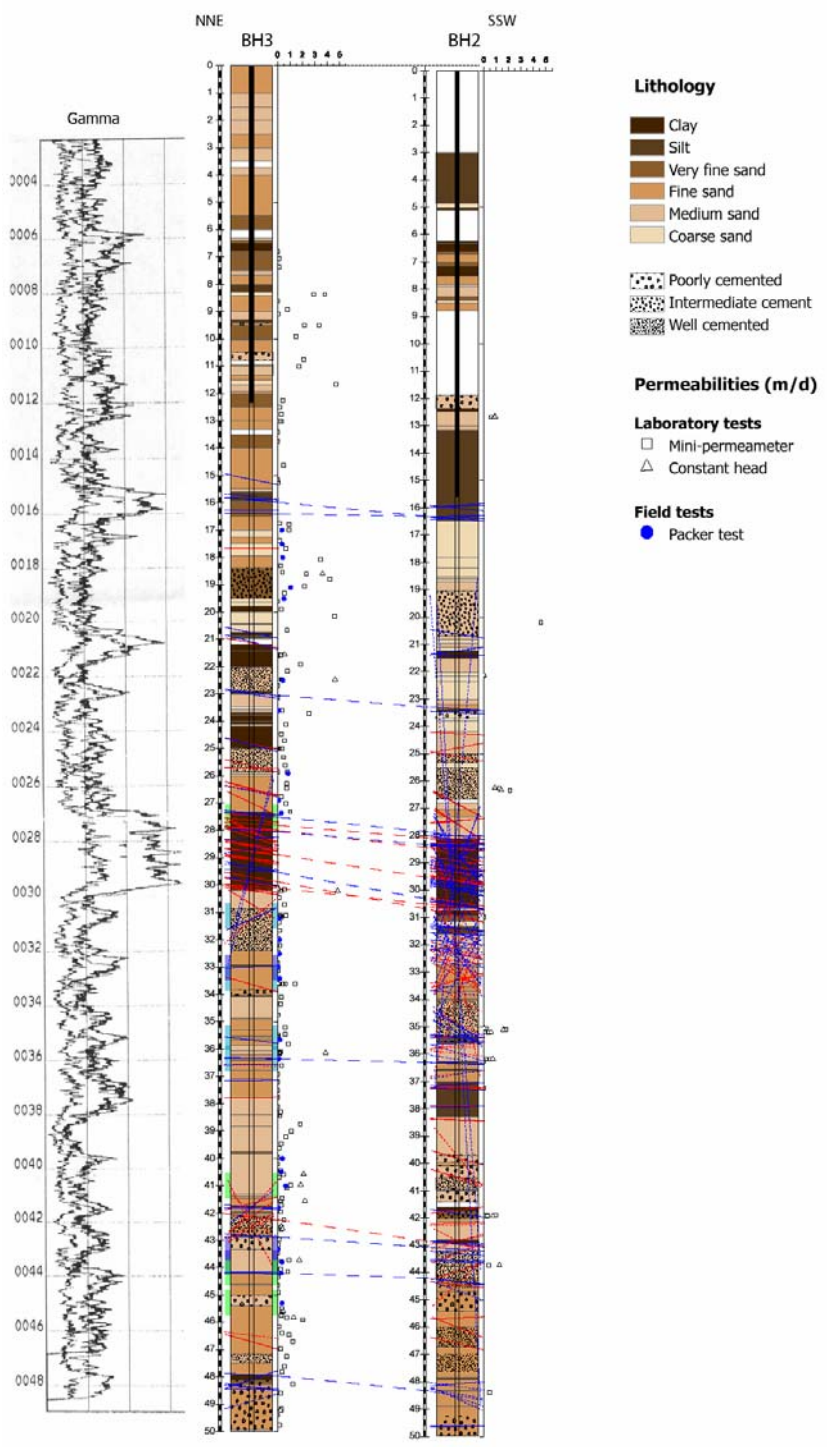

Figure 2: Synthesis of the geophysical and lithological logs and hydraulic test results on the test boreholes

2 The test site

The test boreholes (BH2 and BH3) are located on the University of Birmingham campus. Both are cored to 50 mbgl. BH2 is cased to 15.6 mbgl and BH3 to 12.2 mbgl. Figure 2 shows a summary of the lithological description of the cores from both boreholes and the planar features observed in optical televiewer logs. The dashed line between the boreholes shows a possible correlation between planar features observed in each hole, based upon geometry alone. Open squares show the hydraulic conductivity based upon mini-permeameter tests on core, and the open triangles the results from falling head tests on core. The close circles show the hydraulic conductivity determined from short-interval packer tests in each hole. Two superimposed natural gamma logs are presented for BH3. The gamma log for BH2 is not shown, but correlates well with that for BH3. The solid line in the centre of each hole represents that part of the hole that is cased. The field tests in 2004 and 2005 were conducted by injecting in BH2 and abstracting from BH3. However, cross-hole packer testing (Ferguson, 2006) indicated that the section of BH3 above 15 mbgl is effectively isolated hydraulically from BH2, and hence that pumping BH3 draws in water from levels unconnected to BH2. Thus for the new series of tracer tests, BH2 has been selected as the abstraction borehole. The cross-hole packer tests also showed that there is little hydraulic connection between the zones above 27 mbgl in each borehole and the zones below 37 mbgl in either hole.

3 New tracer test design

As in the previous tracer tests, bacteriophages and a fluorescent dye (fluorescein) will be injected into one borehole and abstracted from the second, continuously pumped, borehole. In addition to the main pump (Grundfos MP3), the abstraction borehole (BH2) will be instrumented with a sampling system that routes water from five predefined levels in the borehole to monitoring devices at the surface. Following unsatisfactory trials with multiple sampling pumps, the present system employs a single Grundfos MP1 submersible pump to sample from each of five levels sequentially. This is achieved using a system of computer controlled solenoid valves that route the water samples through the pump via inlet and outlet manifolds. Viruses are collected at the surface in Argonide NanoCeram® virus filter traps. Each trap collects samples from a particular sampling level and is changed daily and taken to the laboratory for analysis. Thus, the virus sample represents an integrated viral throughput. In addition to sampling from the borehole at five levels, a sixth virus trap is connected to a sampling line from the main pump discharge. Effluent water from each of the virus traps is monitored for fluorescein using a logged Schnegg fluorimeter.

Since the sampled borehole is being continually pumped, the water sampled from each level represents a mixture of the water from lower levels. The relationship between the number of viruses flowing into one section of the borehole is related to the number of viruses recovered from the traps by Equation (1).

$$M_n(t) = \frac{Q_n}{\alpha Q_n^s} m_n(t) - \left(\frac{Q_{n-1}}{\alpha Q_{n-1}^s} - 1 \right) m_{(n-1)}(t) \qquad (1)$$

where

$M_n(t)$ is the cumulative number of viruses at time t that has entered the nth section of the borehole from the bottom.

$m_n(t)$ is the cumulative number of viruses at time t that is recovered from the nth virus trap.

Q_n is the constant discharge due to pumping at the top of the *n*th section of the borehole.
Q_n^s is the constant sampling pump discharge in the *n*th virus trap.
α is the efficiency of each virus trap.

The use of Equation (1) depends on knowing the up-hole discharge at each sampling level, which is not simple to achieve during the tracer test. However, since the discharge during the tracer tests is at a constant rate, the up-hole discharges are measured before and after the test at the same discharge rate. A preliminary survey has been conducted in each borehole to help identify the sampling locations for the full tracer test. This was achieved using a spinner flow meter installed in a purpose built packer system, which ensures that the entire upflow at the measurement depth contributes to the measurement, whilst maintaining very small head losses across the device.

Viruses are recovered from the traps by elution as described in US EPA (2001a) using 1.5% beef extract and 0.05M of glycine, adjusted to pH 9.5 with sodium hydroxide (NaOH). The solution is passed through the filter trap slowly using a peristaltic pump, and the eluate adjusted to between pH 7.0 and 7.5 and filtered through a 0.45µm sterilizing filter. The number of viruses is estimated by plaque assay using the Double Agar Layer (DAL) procedure (Adams 1959), according to the protocol set out in US EPA (2001b,c,d). Initial tests indicate the efficiency of the virus trap and elution process in recovering influent viruses (α) to be high at approximately 91%.

Hourly spot measurements of total virus concentrations in the water discharged from the borehole will be made using an automatic sampler, with laboratory analysis by epifluorescence microscopy. This technique estimates the phage abundance using a DNA cyanine based nucleic acid stain (YO-PRO-1). The stain is prepared as described in Hennes and Suttle (1995). YO-PRO-1 is diluted to 50 µM in an aqueous solution of 2mM NaCN (sodium cyanide), and 80 µl of stain are placed in the bottom of a Petri dish. 100 µl water samples are diluted with 700 µl of deionised water. Diluted samples are filtered using a 0.02 µm pore size Al_2O_3 Anodisc 25 membrane filter (Whatman) with a premoistened backing filter (pore size 0.45µm). The Anodisc membrane samples are placed side up on the YO-PRO-1 drops. The Petri dishes are placed in the dark for 2 days at room temperature. The filter is then washed twice with 800µl of sterile deionised water. The membranes are transferred to a glass slide, immediately covered with a drop of spectrophotometry-grade glycerol and a cover slip. A Zeiss Axioplan 2 epifluorescence microscope with blue filter at magnification of 1000 time magnification is used to view the viruses.

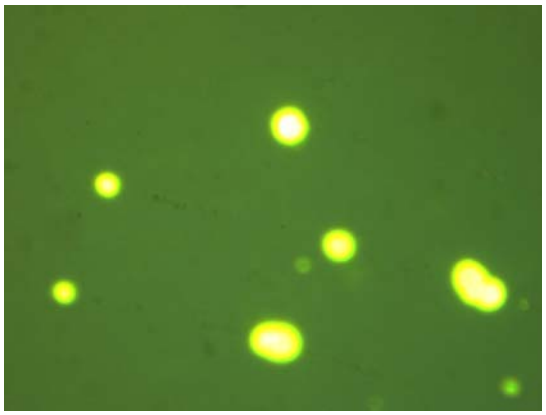

Figure 4: Epifluorescence image of PRD1 bacteriophages

4 Results to date

The results of the borehole discharge survey are shown in Figure 5. Apart from determining the up-hole discharges (Figure 5a), this test gives the inflow profile up each borehole (Figure 5b). From the results of the geophysical logging and the hydraulic testing, including the up-hole discharge tests, the subsurface sampling levels in the first full tracer test will be at 18, 21.5, 29.5, 37.5 and 45.75 mbgl.

During 2004 and 2005, microcosm studies in which bacteriophages were suspended in dialysis bags in a borehole approximately 90 m from the test site, indicated a PRD1 inactivation rate in groundwater of approximately 0.024 day^{-1} (Joyce et al., 2007). This rate would suggest a PRD1 concentration in September 2007 of at most 1.2×10^{-8} times that injected in August 2005. However, during the 2007 tests, concentrations of approximately 1.7×10^{3} pfu/ml were recovered from the pumped borehole, representing 2.6×10^{-6} times the injected concentration. This high concentration occurs despite the existence of natural horizontal and vertical head gradients in the aquifer tending to remove the viruses from the vicinity of the borehole, and the fact that all three holes at the site had been pumped during hydraulic testing (without virus assays). It appears, therefore, that although PRD1 does not migrate readily through a large proportion of the aquifer thickness, the presence of the rock (and the possible attachment to its surface) protects the virus against inactivation. Thus, although transport pathways through the sandstone appear to be rare, this in itself does not provide the effective protection previously expected.

Further tests with injections of fluorescent dye are planned in the remainder of 2007, with a full tracer test with injection of PRD1 scheduled for early 2008.

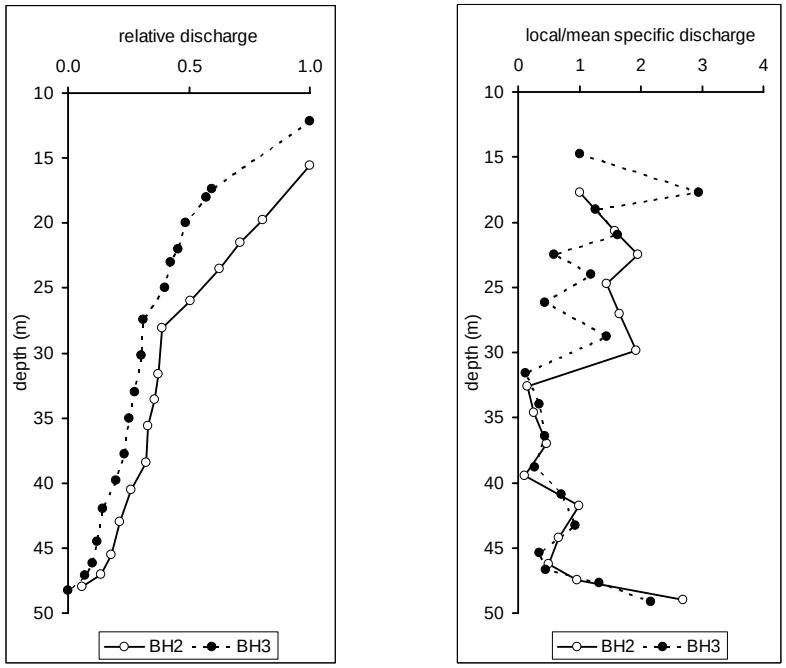

Figure 5: (a) Discharge as a function of depth in BH2 and BH3 expressed as a fraction of the total discharge (b) Specific discharge in the aquifer as a function of depth in BH2 and BH3 expressed as a fraction of the mean specific discharge

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