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Conference Abstract

Pathogen survival in groundwater: Influence of redox and organic matter

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Abstract

Microbial pathogen survival within the environment can be variable and can depend on many criteria, including environmental conditions e.g. oxygen concentration, temperature, pH, sunlight, etc. (e.g., Horswell et al. (2010)). Groundwater has been shown to enable the prolonged survival of pathogenic organisms due to the absence of sunlight and relatively stable temperatures (Cook and Bolster 2007). In other studies, however, survival has been lower in groundwater when compared with a sterile environment (e.g. sterilised groundwater or artificial groundwater) due to the presence of competing organisms and adverse conditions of pH and redox.

To elucidate these discrepancies two experiments were designed: The first, hypothesised that, due to Campylobacters' low tolerance to high oxygen levels, survival in oxic (dissolved oxygen (DO) levels over 5 mg per L) would be less than in anoxic groundwater (DO levels below 2 mg per L). The second hypothesised that the survival of the pathogen *Salmonella typhimurium*, in groundwater, will be enhanced by organic carbon.

METHODS

Campylobacter experiment:

Campylobacter jejuni isolated from the Havelock North drinking water source was used (designated HN16) (Gilpin et al. 2020). To compare the survival of the outbreak strain with type strain *Campylobacter*, NCTC 11351 was used.

Salmonella experiment:

For this experiment, environmental isolates were used rather than laboratory strains. *Salmonella*, isolated from a stream in Wellington, New Zealand, was identified as *Salmonella enterica serovar Typhimurium*. The *Escherichia coli* used was a phylogroup A, isolated from stream sediment in Whangarei Falls, New Zealand.

Mesocosm experiments were established containing groundwater (oxic and anoxic for *Campylobacter* experiment and ultrafiltered, groundwater, groundwater amended with 1% or 10% dissolved organic carbon (DOC). pH, dissolved oxygen (DO), and temperature were monitored over the experimental period. The temperature was maintained at 12-14°C during both experiments.

Samples (5 mL) of the groundwater from each jar were taken aseptically at set time points over the experimental period. Samples were then serially diluted in sterile peptone water to give a dilution series from 10^{-1} to 10^{-4}. Samples were analysed by plating onto selective media.

RESULTS

Campylobacter experiment:

The results presented demonstrated differences in the survival of the two *Campylobacter* strains tested and differences in survival of *Campylobacter* HN16 depending on groundwater type. Fig. 1 shows the average concentration of *Campylobacter* strains in groundwater types over time. The results presented are average of three replicates. Over the whole experimental period survival of *Campylobacter* HN16 was greatest in anoxic groundwater, and only a 1 log reduction was observed (Fig. 1), equating to a 79.6% survival after 16 days. The die-off rate of *Campylobacter* HN16 in anoxic groundwater was calculated to be 0.0873 days and T90 6.85 days.

Salmonella experiment:

Die-of rates for *Salmonella* were similar over the course of the experiment when no or low levels of DOC were present (Fig. 2a). At high levels of DOC, however, *Salmonella* showed similar survival to the control. After 84 days only a 1 Log decrease was observed. In comparison, *E. coli* died off at a faster rate than *Salmonella* in all mesocosms (Fig. 2). It is interesting to note that in the high DOC mesocosms after day 56 counts of *E. coli* remained at 10^3 per mL until the end of the experiment.

Conclusions and significance

Both experiments demonstrated the survival of pathogenic microorganisms in varying groundwater conditions. The variation in the outbreak strain *Campylobacter* compared to the type strain indicated variation within species that may lead to enhanced survival in the environment. The *Salmonella* experiment indicated the presence of additional organic carbon can enhance the survival of pathogens in groundwater. In addition, the variation between the microbial indicator *E. coli* and *Salmonella* provides evidence of differences in the survival of microbes in the environment and indicates caution is needed when considering the survival of pathogens in groundwater if reliance is made on microbial indicator organisms.

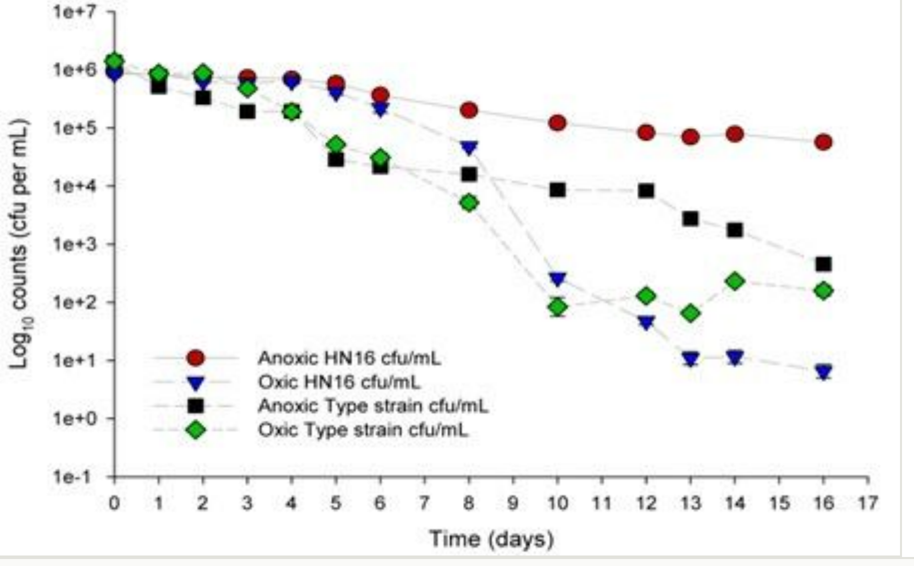

Figure 1. doi

Campylobacter outbreak strain (HN16) and type strain NCTC 11351 survival in anoxic and oxic groundwater. The symbols are average (mean) counts (n = 3), lines are the standard error of the mean.

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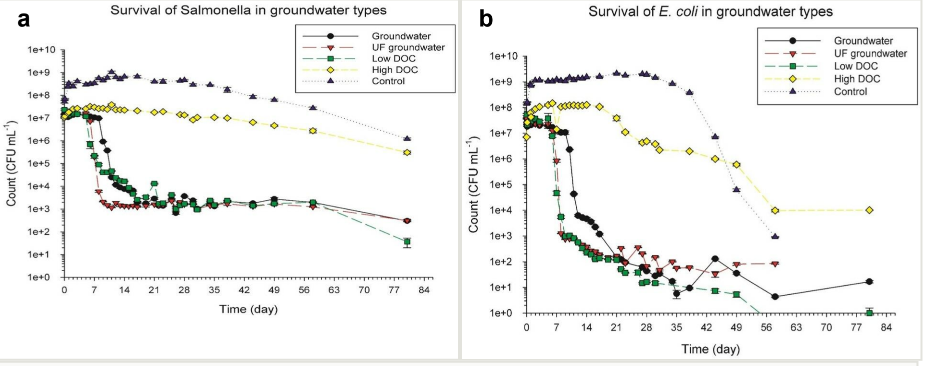

Figure 2.

Salmonella and *E. coli* survival in different groundwaters. UF denotes ultrafiltered groundwater, low DOC is 1% dissolved organic carbon, and high DOC is 10% dissolved organic carbon. Control counts are shown as comparison to treatments

a: *Salmonella typhimurium* survival in groundwater types over time. The symbols are average (mean) counts (n = 3), lines are the standard error of the mean. doi

b: *E. coli* survival in groundwater types over time. The symbols are average (mean) counts (n = 3), lines are the standard error of the mean. doi

Conflicts of interest

The authors have declared that no competing interests exist.

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