Comparative survival of enteric viruses and bacteria in Atlantic Ocean seawater

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Abstract The survival of Escherichia coli, Salmonella typhi, Shigella sonnei, poliovirus type 1 and a parvovirus (Minute Virus of Mice) was determined in seawater. Seeded seawater was incubated in the laboratory at 6, 12, 20 and 28ºC for up to 40 d. In-situ survival studies were done seasonally (winter, spring, summer and fall) using seeded microbial dialysis equipment placed in the Atlantic Ocean off coastal North Carolina at water depths of 3–10 m. In laboratory studies all test microbes survived longer at lower temperatures with typical times for 90% inactivation (T90) of 1–3 d at the highest temperature and >10 d at the lowest temperature. Of the microbes tested, E. coli survived least well while S. typhi and Sh. sonnei survived similar to or greater than enteric viruses. Parvovirus survival was similar to that of poliovirus. Under in-situ conditions, E. coli also survived least well of all microbes tested with T90 values of 0.9–3.9 d depending upon season. All other test microbes had generally similar survivals. Overall, microbial survival in seawater was greater under laboratory conditions than under in-situ conditions. There was no clear association between microbial survival and water temperature. The lower survival of E. coli compared to the bacterial and viral pathogens under laboratory conditions raises concerns because it is a key microbial indicator of faecal contamination.

Keywords Seawater; survival; bacteria; viruses; bacterial injury; inactivation

Introduction The survival of enteric microbes in seawater is a public health concern for marine bathing, other primary contact recreation and bivalve molluscan shellfishing. The survival of enteric viruses and bacteria in seawater has rarely been compared. Furthermore, when bacterial survival was studied, the methods and media employed for bacterial enumeration were often inadequate for recovering injured bacteria. Studies have repeatedly shown that indicator and pathogenic bacteria are injured in environmental waters and that conventional assay methods for them greatly underestimate their actual concentrations. The purpose of this study was to determine the survival of selected enteric bacteria (E. coli, Shigella sonnei, and Salmonella typhi) and viruses (poliovirus and parvovirus) in seawater under both laboratory and in situ conditions using bacterial culture enumeration methods capable of recovering injured cells.

Materials and methods

Bacteria growth and assay
The enteric bacteria E. coli B (ATCC 11303), S. typhi (ATCC 19430) and Sh. sonnei (ATCC 29031) were grown in tryptic soy broth overnight at 37°C. Cells were harvested by centrifugation, washed with 0.5% buffered peptone water (pH 7.2) and resuspended in filter-sterilised seawater for addition to test waters. Test bacteria were enumerated for viable counts by an injury-repair procedure consisting of initial spot plating on tryptic soy agar (TSA), incubating for 1 h at room temperature followed by overlaying with a thin layer of molten violet red bile agar and incubating at 37°C overnight (Speck et al., 1975). Concentrations were expressed as colony forming units (CFU)/mL.
Virus growth and assay
Poliovirus type 1 LSC, (PV1) was grown in and assayed by the plaque technique on confluent layers of BGMK cells (Sobsey et al., 1978). Stock virus, as infected BGMK cell lysate, was purified and dispersed by fluorocarbon extraction. The parvovirus Minute Virus of Mice (MVM) was grown in and assayed by the plaque technique on L cells (Sobsey et al., 1980). Stock virus was harvested as infected cell lysate from roller bottles and purified and dispersed by fluorocarbon extraction.

Laboratory experiments
Seawater (≥32 mg/L salinity), collected seasonally as subsurface grab samples from a site 1.2 km offshore from Beaufort Inlet in Onslow Bay, was dispensed in 50 mL amounts in 125 mL fluted, borosilicate Erlenmeyer flasks, seeded with test bacteria and viruses to achieve initial concentrations of about 10^4–10^7/mL and replicate flasks were incubated in the dark at 6, 12, 20 and 28°C with rotary shaking. Flasks were sampled initially and at intervals of 1–3 d to determine surviving test microbe concentrations over periods of 8–16 weeks.

In-situ (field) experiments
On a seasonal basis, McFeters-type diffusion chambers (McFeters et al., 1974) fitted with 15nm pore size polycarbonate (Nuclepore) membranes and covered externally with 106-mesh polypropylene netting were filled with seawater and seeded with test microbes. Chambers were attached to the anchor chains of buoys located in 10–15 m of water about 2 km offshore from Atlantic Beach, North Carolina. Dialysis chambers were sampled initially and then at intervals of 1–2 weeks for up to 16 weeks to determine surviving test microbe concentrations.

Statistical analysis of microbial inactivation data
Mean concentrations of each test microbe were used to calculate the proportions of each initial microbe remaining at time = t (N_t) relative to their initial concentrations at time = 0 (N_0) as log_{10} proportions, N_t/N_0 and these data were used to estimate the times for 90% (1 log_{10}) reductions. Analysis of variance was used to test for statistically significant differences (at the 5% level) among experimental treatments or conditions and Duncan’s multiple range test was used to compare any two of a series of treatments or conditions for statistically significant differences (at the 5% level).

Results and discussion
Table 1 shows that there was increased inactivation of all test microbes at higher temperatures at all seasons and for the mean values of experiments for seasonal waters. The inactivation rates for each microbe and test temperature were not significantly different among the four seasonal water samples. However, negative relationships between water temperatures and microbial inactivation rates were significantly different at the 5% level. In general, inactivation rates at 6°C differed significantly from those at the other temperatures studied but differences between inactivation rates at the three higher temperatures (12, 20 and 28°C) were not always significant. *E. coli* B was inactivated more rapidly than any other test microbe under a given set of test conditions. Inactivation rates among other test microbes were not significantly different.

Reductions of test microbes at different seasons under field conditions in dialysis chambers placed 3 m and 10 m below the surface are summarised in Table 2. In general, test microbes were reduced more rapidly under field conditions than at similar temperatures in the laboratory experiments (Table 1). Survival times were greatest for the two viruses, PV1
and MVM, but only during winter conditions when temperatures were lowest. Seasonal differences in microbe survival were noted and were significant for *E. coli*, *S. typhi* and PV1 (P < 0.01). However, there were no consistent relationships between inactivation rates and water temperatures for all test microbes. Some test microbes survived longest at the lowest temperatures (PV1 and MVM) but others survived longest at more moderate temperatures (*E. coli* and *S. typhi*). Test microbe survival in dialysis chambers placed 3m and 10m below the seawater surface did not differ regardless of season.

**Discussion and conclusions**

The survival of enteric bacteria and viruses in seawater, expressed as time in days for 90% or 1 log₁₀ inactivation, varied from less than 1 d to >28 d depending on the test microbe, temperature, season and other experimental conditions. Studies investigating the fate of enteric viruses and bacteria in seawater have implicated many factors influencing their survival and persistence. Consistent with previous studies, water temperature influenced the survival of test bacteria and viruses in the laboratory experiments of this present study with 2–11 fold lower times for 90% inactivation at 28°C than at 6°C. However, this relationship between microbial inactivation rate and temperature was not consistently observed in the *in-situ* field experiments perhaps because of other factors influencing microbial survival.
The ability of the indicator bacterium, *E. coli*, to predict the inactivation of the pathogenic microbes of this study differed between laboratory and field studies and between the test pathogens. In laboratory studies *E. coli* was inactivated significantly faster than *S. typhi* and *Sh. sonnei* but it was not inactivated significantly faster than PV1 and MVM. However, during *in-situ* field studies, the inactivation of *E. coli* was not significantly different from the inactivation of the bacterial and viral pathogens studied. The reasons for differences in the inactivation rates of test bacteria between laboratory and *in-situ* field experiments are not known but may be related to differences in environmental conditions. Laboratory studies were done in sealed flasks allowing for the accumulation of materials from the bacteria and other microbes present, including dissolved and particulate organic and inorganic materials. In contrast, *in-situ* field experiments were done in dialysis chambers that made it possible for dissolved constituents to diffuse in and out of the chamber. It is possible that materials accumulating in the laboratory flasks produced effects on the bacteria that contributed to either their survival or inactivation; such effects did not occur for bacteria in dialysis chambers used *in-situ* because such materials did not accumulate and because other materials could diffuse in.

It is noteworthy that enteric viruses did not survive appreciably longer than enteric bacteria under field conditions. This observation, which is contrary to what has been previously reported in the literature, may be due to the use of resuscitation and repair plating procedures for the enumeration of bacteria. It is likely that the data of previous studies were underestimates of the actual concentrations of viable and culturable bacteria because of the use of sub-optimal plating procedures. For this reason, the use of resuscitation and repair plating procedures for indicator and pathogenic bacteria in water is to be encouraged in order to improve estimates of the actual concentrations of the enteric bacteria present. If this is done, perhaps enteric bacteria will also be more reliable indicators of enteric viruses in seawater. Future efforts are recommended to develop and validate better microbial indicators of enteric bacterial and viral pathogens in water and to incorporate injury repair techniques into enumeration methods for enteric bacterial and viral indicators and pathogens in seawater.

**References**


