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Coliphages and indicator bacteria in birds around Boston Harbor

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Droppings from feral populations of pigeons, geese and herring gulls from the urban/suburban environment around Boston Harbor, MA, USA contained up to 10^6 somatic coliphages, 10^8 enterococci, 10^9 thermotolerant coliforms and 10^2 F-specific coliphages per gram of feces. Somatic coliphages, enterococci and thermotolerant coliforms were common in the feces of all three kinds of birds but F-specific coliphages were found in droppings from only three of 32 gulls. Thus these sources of bacterial and viral indicators should be considered when dealing with the ecology of fecal pollution indicators. Moreover, microbial indicators of fecal or sewage pollution originating from bird droppings may be mistaken for indicators that come from humans. This may cause an overestimate of the hazard from human pathogens in water and confound attempts to locate sources of fecal or sewage pollution.

Keywords: indicator bacteria; coliphages; Boston Harbor; birds; pigeon; herring gull; Canada geese

Introduction

The threat of viral and bacterial transmission through water has prompted many studies concerning the use of microbial indicators such as somatic coliphages, enterococci, coliforms and F-specific coliphages to evaluate water safety [6,8]. Studies that examine the presence of microbial indicators in the feces of farm and zoo animals have been conducted because of concern that indicators of human pollution may not be specific to humans [5,9,13]. For instance, F-specific coliphages have been serotyped in order to find human-specific variants [13]. Phages of *Bacteroides fragilis* have attracted attention as indicators because of their specificity to human waste [7,14,16]. Wyer *et al* [18] stated that 'non-outfall sources of faecal indicators can and do affect the compliance of bathing waters even after expensive engineering projects have been successfully implemented'.

Four common indicators of fecal pollution: somatic coliphages, F-specific coliphages, thermotolerant coliforms and enterococci were measured in the droppings of three species of birds. We measured indicators in the droppings of pigeons (*Columba livia*), Canada geese (*Branta canadensis*) and herring gulls (*Larus argentatus*). These birds are highly mobile, urban (suburban), feral and regarded as vermin besides living in and around Boston Harbor and its watershed. Ricca and Cooney [15] found somatic coliphages, F-specific coliphages, thermotolerant coliforms and enterococci in Boston Harbor, an urban harbor where human fecal pollution is a concern. Surface run-off is a source of fecal pollution for rivers and streams [11]. Our goal was to see if we could detect these microbial indicators from non-human sources using methods commonly used by public health agencies. We found substantial numbers of somatic coliphages, thermotolerant coliforms and enterococci from all three species of birds. However, F-specific coliphages were present in low numbers or absent.

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Materials and methods

Microorganisms

The F-pilus-harboring *Salmonella typhimurium* WG49 [10] was used for the initial isolation and enumeration of F-specific coliphages. The F^+ strains *Escherichia coli* C3000 (ATCC 15597) and *E. coli* HS(pFamp)R [3] and the F^- strains *E. coli* C (ATCC 13706) and *S. typhimurium* WG45 [10] were used as phage hosts when spot-testing plaques from *S. typhimurium* WG49. Each of the five bacterial host strains was given to us by Mark D Sobsey, University of North Carolina, as was coliphage MS2. Coliphage MS2 was used as a positive control for F-specific coliphages, and phage ϕX174, provided by Mark Doolittle, University of Massachusetts, Boston, was used as positive control for somatic coliphages. *E. coli* C was used both as host for somatic coliphages and as a positive control for thermotolerant (fecal) coliforms. *Enterococcus fecalis* (Carolina Biological Supply Co, Burlington, NC, USA) was used as a positive control for enterococci.

Field testing

Thirty-two samples of fresh bird droppings were collected for each of the three species of birds in the summer of 1997. All bird droppings were placed in cold tryptone-glucose-yeast-extract (TGYE) broth in plastic centrifuge tubes using sterile toothpicks and kept on ice until tested for the presence of indicators. One-gram samples of pigeon droppings were collected from nesting sites under a highway bridge in Weymouth, MA. Each sample was placed in 5 ml of cold TGYE broth. One-gram samples of herring gull droppings were collected from an active landfill in Milton, MA and placed in 5 ml of cold TGYE broth. Twenty-gram samples of goose feces were collected from a golf course in Braintree, MA and placed in 20 ml of cold TGYE broth. The fecal samples were shaken and allowed to settle when

testing for bacteria, or centrifuged (6000 × *g* for 10 min at 4°C) when testing for phage. Dilutions of the supernatant phases (in phosphate-buffered saline, PBS) were tested for each of the four indicators.

Bacterial and coliphage counts

Membrane filtration (0.45-μm pore-size nitrocellulose filters) was used to enumerate the bacteria [1,17]. Membrane filtration of thermotolerant coliforms and enterococci was performed on dilutions of fecal samples in PBS. For thermotolerant coliforms, membranes were incubated overnight at 44.5°C on mFC agar with rosolic acid (Difco Laboratories, Detroit, MI, USA). Blue colonies were counted. For enterococci, filters were incubated for 48 h at 41°C on mE agar [17]. The membrane filters were then transferred to Esculin-Iron Agar (EIA, Difco) and incubated for 15 min at 41°C. Pink-to-maroon colonies that blackened EIA were counted.

Standard double-agar-overlay plaque assays [2] were used to determine phage numbers. Plaque counts were made by plating a mixture of logarithmic-phase host bacteria and sample dilutions on TGYE agar. Somatic coliphages were counted after overnight incubation at 37°C using *E. coli* C as host while F-specific phages were detected after overnight incubation at 37°C with *S. typhimurium* WG49. Phages isolated from *S. typhimurium* WG49 were spot-tested [12] on *E. coli* C3000 and *E. coli* HS(pFamp)R to verify that they were F-specific coliphages. Phages were also spot-tested on *S. typhimurium* WG45 to see if any were somatic *Salmonella* phages and on *E. coli* C to see if any were somatic coliphages.

Results and discussion

Indicator count trends are shown in Figure 1. Somatic coliphages were found in over three quarters of the fecal samples from pigeons and herring gulls and in about half of the samples from geese. Somatic coliphage plaque counts from goose droppings averaged about ten times less than the numbers (10^2–10^4 pfu g^{-1}) in herring gull or pigeon droppings (Figure 1a). Enterococci were detected in over 80% of the fecal samples from all three kinds of birds. Enterococci numbers ranged from less than one CFU g^{-1} to 10^8 CFU g^{-1} with counts highest in herring gulls (Figure 1b). Thermotolerant coliforms were present in all samples from all three birds (Figure 1c). The range of thermotolerant coliforms in goose droppings (10^1–10^5 pfu g^{-1}) was lower than the ranges for pigeons (10^5–10^9) and herring gulls (10^3–10^8). No F-specific coliphages were found in pigeon and goose feces (Figure 1d). Only three of the 32 samples of herring gull droppings had F-specific coliphages and plaque counts on these plates (10^0–10^2) were low.

The high numbers of somatic coliphages, thermotolerant coliforms and enterococci from bird droppings in this study were in the same range as numbers found previously in humans and animals [5,7,9,13]. However, F-specific coliphages were encountered less often and in lower numbers. This is in agreement with results obtained by Dhillon *et al* [5] and Osawa *et al* [13] but differs from the high numbers detected by Havelaar *et al* [9] and Grabow *et al* [7].

Grabow *et al* [7] found F-specific coliphages in South African seabirds more frequently than we found them in herring gulls and they detected F-specific coliphages in

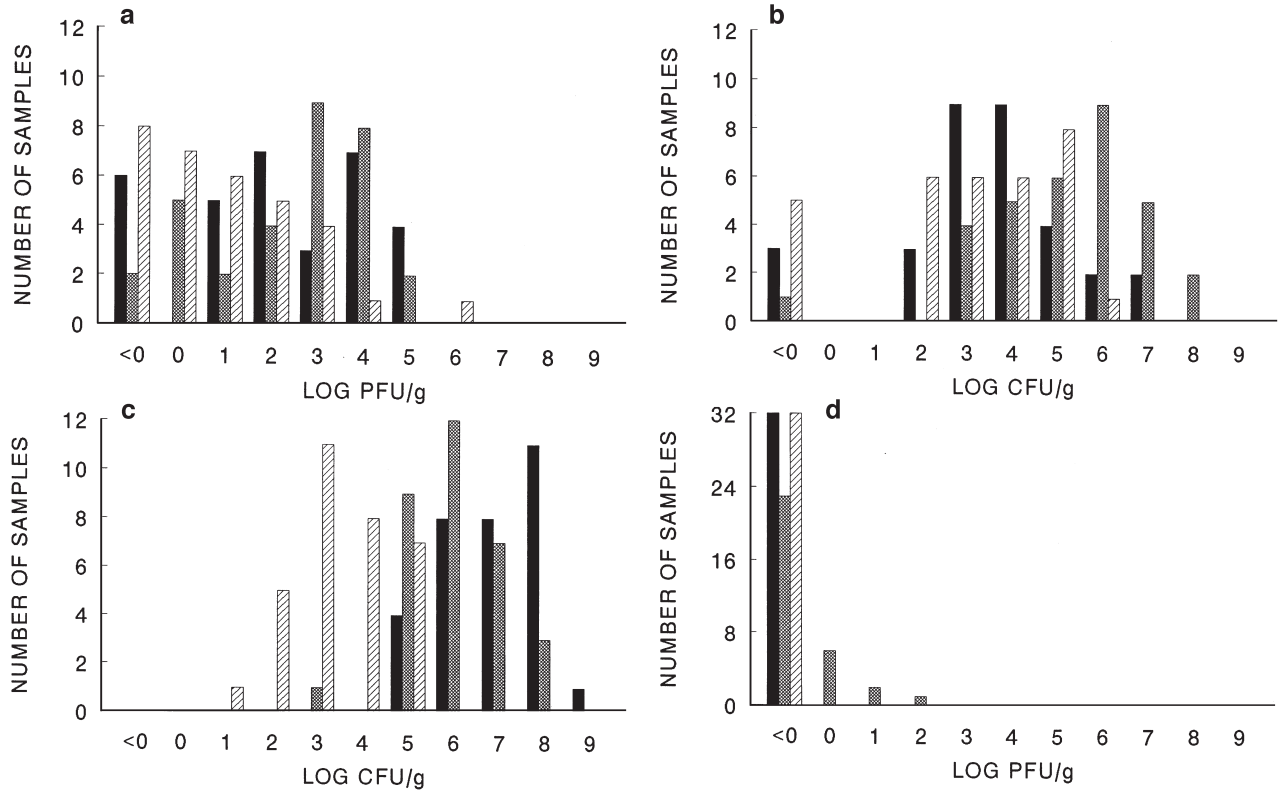

Figure 1 Frequency of somatic coliphages (a), enterococci (b), thermotolerant coliforms (c) and F-specific coliphages (d) in pigeon ■, gull ▨ and goose ▨ droppings.

geese whereas we found none. Their method for detecting phages involved an enrichment step and the resulting increase in sensitivity may be why they found more F-specific coliphages than we did. We used the double-agar overlay plaque assay common for microbiological water quality studies in order to determine if customary analyses can be influenced by indicator microorganisms from birds.

Herring gull droppings contained phages that formed plaques on *S. typhimurium* WG49. Spot tests of plaques from *S. typhimurium* WG49 on different hosts showed that some of these phages can infect *S. typhimurium* WG45 and can also infect *E. coli* C. In other words, they are somatic *Salmonella* phages as well as somatic coliphages. Phages that can infect both *E. coli* and *S. typhimurium* have been isolated from sewage [4]. Since *S. typhimurium* WG49 was designed to differentiate somatic coliphages from F-specific coliphages, the existence of somatic coliphages in the environment that can infect *Salmonella* 'somatically' means that somatic *Salmonella* phages can be confused for F-specific phages. Therefore, although bird droppings are probably not a major source of somatic *Salmonella* phages in the environment, care must be taken when sampling at places where some kinds of birds congregate.

These results indicate that pigeons, herring gulls, geese and probably other birds as well can contribute somatic coliphages, thermotolerant coliforms and enterococci to Boston Harbor. These indicators may be added directly to harbor water or they may enter via storm sewers, rivers or runoff from the land. Feral populations of birds should be considered as a source of indicator organisms in some water quality studies.

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