Salmonella spp. numbers much greater than indicator bacteria in environmental waters

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Introduction

Since the acceptance of the Germ Theory of Disease, society has been interested in water quality and its influence on public health. Awareness of the dangers of contaminated drinking water led to questions about the best means of indicator bacteria. As early as 1880, Von Frisch suggested the use of Klebsiella pneumoniae and E. Alhydrogenes as suitable indicators due to their presence in human feces (Galtshik, 1988). Five years later, Theodor Escherich discovered Bifidus coli (now Escherichia coli) whose presence was also observed in high numbers in the feces of warm-blooded animals (Crook, 1885). Several workers at that time argued for the use of total coliform as the gauge of fecal contamination in water (Hustman and Raby, 1977). However, in 1905, Alfred MacConkey proclaimed that many coliforms in water were not fecal origin (MacConkey, 1988). This led scientists to focus their attention more on E. coli while using total coliforms as a very loose guideline, which, even over a century later, is still the focus of current water quality monitoring.

Water quality is of as much interest now as it was back then when Pasteur and Koch were helping publicize the Germ Theory of Disease. Since then, there have been many discoveries of pathogens which can be transmitted via the fecal–oral route. Some of these pathogens include bacterial species such as E. coli (O157:H7), Campylobacter spp. and Salmonella spp. (Centers for Disease Control and Prevention, 2017), enteric viruses, and certain protozoans, to name a few—all of which cause some form of gastroenteritis which, in some cases, can be fatal. This understanding has increased both the interest and the need to ensure safe water quality for human contact.

The question posed by this study is: How effective are E. coli counts in predicting the counts of potential pathogens, in this case, specifically Salmonella species, in local streams in south-central Virginia? Recent reports from the literature show mixed results. Some studies have shown little to no correlation between Salmonella spp. and indicator bacteria (Pinto et al., 1998) while others have shown a significant correlation between Salmonella spp. and indicator bacteria (Fitzharris et al., 2000).

Materials and Methods

Salmonella isolation and enumeration

Water samples were collected from three locations: Appomattox River (APR), Sayler’s Creek (SY), and Green Creek (GC). The samples were collected by lowering a sterile container mid-stream and collecting the water for up to 2 minutes. Samples were then placed in ice and transported back to the laboratory for processing. Two field duplicates were taken during each collection event. Samples were usually processed within 1-2 hours of collection.

In the laboratory, the samples were passed through membrane filtration. One mL of sample was diluted with sterile, buffered water and filtered through 0.45 µm pore size filter membranes (Millipore, Bedford, MA) and transferred to 50 mL petri plates containing 1.5 mL of Tetrathionate enrichment broth (Eiken) and incubated at 44°C for 48 hours. The samples were then transferred to 50 mL petri plates containing 1.5 mL of sterile brain heart infusion broth and incubated an additional 24 hour at 44°C.

The plates were enumerated by counting all colony forming units (CFU) that were thought to be Salmonella spp. based upon colonial morphology (i.e. color and morphology). This was usually all of them except for those appearing as yellow. Any CFUs with a hint of pink, orange, or red was counted as positive. Many of the representative colonies were transferred to Triple Sugar Iron (TSI) agar plates for confirmatory testing.

E. coli and Salmonella enumeration

The same test water samples were used for the assessment of total coliform and E. coli via Defined Substrate Test using the Colilert (Melsa, Westbrook, ME) Quant tray 2000 system. Twenty-five ml of water sample was diluted with 75 ml of buffered lactose broth and then processed based on the manufacturer’s instructions and incubated at the same conditions as the filter membranes.

After incubation, the numbers of wells altering chromogenicly were counted for total coliform enumeration. The tray was then subjected to 240 g (160 BA) so as to acidify the triphenyl tetrazolium chloride for E. coli enumeration. The results were compared to a most probable number system with a quantitative range of 1 to 2512 CFU per 100 mL when using a 25 mL sample dilution. According to Bokema et al. (1998), confirmatory testing of coliform bacteria and E. coli using Colilert media is not necessary.

Statistical Analyses

For each Salmonella enumeration date, the average of two 1 mL field duplicate samples was taken and multiplied by 100 to represent the number of suspect bacteria per 100 mL standard volume. All enumerations of total coliforms and E. coli were also determined with respect to 100 mL volumes per consumption.

One-way analysis of variance (ANOVA) was used to determine differences between pooled counts of E. coli and Salmonella spp. (dependent variable) from all sample sites and dates. Bacterial counts from individual stream sites were then compared using student t-tests and were analyzed to compare the standard deviations in counts of Salmonella spp. and E. coli for each site. The F-test allowed determination of the correct student t-test equation to be used in comparing bacterial counts from the individual sites.

The least-squares method of regression analysis was conducted using log transformed values to determine the degree of correlation between counts of Salmonella spp. and E. coli across the term of the study.

Results

Table 1 presents the raw data of paired bacterial counts obtained to date for 100 mL volumes. Figures 1 and 2 illustrate differences in bacterial counts per sample date at the two sampling sites for which we have the most data—Sayler’s Creek (SY) and Green Creek (GC) (Fig. 1 and 2, m; n). The ANOVA results showed that the variances differ significantly (<0.05) between the pooled raw data and individual sites alone except for APP2. All of the F-tests were completed on both the pooled raw data and on the individual sites alone except for APP2. All of the results from the F-tests showed that the standard deviations were all significantly different (see Table 6).

Discussion

Our results suggest that there is no correlation between raw numbers of the bacterial indicator E. coli and Salmonella spp. found within samples of our stream water (Table 1). Closer examination of Table 1 reveals an order of magnitude difference between the indicator species and the potentially pathogenic species count from the same water sample. This difference may be explained by the reported difference in hardness of the two genera of bacteria, with Salmonella spp. being quite hardy (e.g., resistance to environmental change) as compared with E. coli. These results support other published data on the correlation between indicator bacteria and potentially pathogenic bacteria. Hillman et al. (2004) found no correlation between E. coli and select enteropathogenic species such as Campylobacter spp. Ahmet et al. (2008) and Schieweck et al. (2010) also showed mixed results, including E. coli, with potential pathogens, including Salmonella spp. concluding that there was poor correlation between the two groups. Schwartz et al. (2002) examined the relationship between indicator bacteria and enteric viruses and found low correlation.

Using E. coli as indicator bacteria originated from a concern that sewage contamination of drinking water sources posed a significant health risk. This logic is sound in that sewage contamination contains high numbers of E. coli and high numbers of E. coli are present; contamination is likely to be human related. However, mounting evidence suggests that numbers of E. coli and other indicator bacteria show mixed results when correlated with pathogens genera of bacteria, viruses, and protozoa.

Several suggestions have been offered to explain the lack of correlation between numbers of E. coli and pathogen counts. Lura et al. (2010) investigated the ability of E. coli to survive in marine sediments and concluded that E. coli can outperform pathogen species. Soto-Delartre et al. (2004) found that E. coli could effectively out-compete competitors in dry soil. Whitman et al. (2006) added that E. coli can persist in forest soils as well as soil that is prone to being a major water source for long periods of time. These studies suggest that the ability of E. coli and other indicator bacteria to exist as continuous non-point sources could potentially influence their effectiveness as indicator bacteria.

The purpose of indicator bacteria is to assess the quality of water by attempting to generally predict the degree of pathogenic presence. This purpose would benefit from future studies aimed at identifying better indicator bacteria that more closely correlate with specific pathogens. Relative to Salmonella, this may be achieved by examining a harder indicator bacteria that may correlate better with E. coli. However, future data collections continue to support the correlation as Figure 3, it may be possible to predict Salmonella spp. number of E. coli numbers based upon a mathematical model effectively arguing for the continued use of E. coli as an indicator bacteria.

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<th>E. coli (CFU/mL)</th>
<th>Salmonella spp. (CFU/mL)</th>
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Table 5 shows the results of the student t-tests that were completed on the individual sites alone except for APP2. All of the student t-test results showed that the means were all significantly different.