EVALUATION OF COLIPHAGES AND FECAL COLIFORMS DENSITIES ON THE PRINCIPAL CANAL OF THE IRRIGATION SYSTEM IN THE SOUTHWESTERN OF PUERTO RICO

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ABSTRACT

Coliphages viruses were monitored in conjunction with fecal coliform bacteria in the main canal of the irrigation system of southwestern Puerto Rico during the November 9, 2001 to March 2, 2002 time period. Coliphages were detected by the direct plate assay and using Escherichia coli C3000 as host bacteria. The membrane filter method was used for the fecal coliform detection on mFC culture media. Fecal coliforms were present on 100% of samples against 65% on coliphages and a ratio of 62:1 fecal coliform to coliphage was calculated on 100 mL samples (n = 72). Statistical analyses of data indicate a significant (t 70, 0.05 = 1.993, p < 0.001) positive correlation between coliphage virus and fecal coliforms densities. Not significant differences between mean densities of the same indicator were detected through different canal points for both coliphage and fecal coliforms. As a result, the canal probably does not have any stationary source of fecal contamination and the virus and bacteria indicators detected may be coming from the fecal contamination of the Loco Reservoir water.

Key Words: Coliphage, Fecal Coliform

INTRODUCITON

One of man’s more important resources is water. Water from rivers, lakes, reservoirs, and groundwater aquifers are widely used for consumption and recreation purposes. This water is subject to varying degrees of fecal pollution, and consequently fresh waters are a vector transmission of many pathogenic bacteria, viruses, and protozoa. Fecal pollution can reach water resources by human activities, such as sewage treatment plants and communities without sewage control. Many diseases are related to fecal polluted water, but enteropathogenic microorganisms cause most of them. The presence of enteric pathogens in waters is of great concern. For this reason the microbiological safety of water is very important to protect the public health.

For decades the fecal coliform group of bacteria has been used as an indicator of water quality with respect to the presence of human pathogens. An indicator organism is one that indicates the possible presence of pathogenic organisms. However, commonly used fecal coliform bacteria as indicators of fecal contamination on waters are
believed to have limited predictive value on tropical waters. Fecal coliform bacteria that have been found in tropical waters do not always imply fecal contamination and its presence can be attributed to be part of the natural flora in the tropics.

Bacteriophages, virus that attack bacteria, have been proposed as indicators of fecal pollution on tropical waters. Specifically, coliphages, a group of bacteriophages that infect *Escherichia coli* bacteria. These are present on the natural flora of the intestine of warm-blooded animals and they have been identified only in tropical waters that have been characterized as fecally polluted. Its presence is therefore associated with fecal contamination since no other source of coliphages has been found on the environment. Another advantage of coliphages is that the coliphage assay is easy, cheap, and results are obtained in a short period of time (12 hours). Coliphages have been also proposed as an indicator of enteroviruses presence, because the related behavior of coliphages to those viral particles. Viral pollution is an issue that is emerging as a public health concern.

As a result of the danger to public health due to the presence of pathogens, it is extremely important to determine an indicator that can ensure the microbiological safety of water. Coliphages assay could be used to indicate both the bacteriological and virological safety of water and its characteristics as the indicator could be used to decrease enteropathogenic diseases on people. This study was conducted to evaluate coliphages as an indicator of fecal pollution, elucidate the occurrence of fecal coliforms and coliphages in the irrigation system of southwestern Puerto Rico, and identify possible fecal contamination sources on the area.

**METHODOLOGY AND RESULTS**

Water samples from six points at the main canal of the irrigation system of Lajas, Puerto Rico (Figure 1) were tested for fecal coliforms and coliphages during the November 9, 2001 to March 2, 2002 time period. As a quality control procedure the temperature, pH and DO were measured on different sampling points to evaluate their change parameters through the canal and verify if those changes have affected the fecal coliforms and coliphage densities measured on this project. The fecal coliforms and coliphage densities are evaluated and statistically compared to determine possible relationships between indicators and to determine possible sources of contamination through the canal.

The densities of fecal coliforms and coliphages were expressed as 100 ml sample volumes. The fecal coliforms were present in 72 (100 %) of the samples against the coliphages that were present only in 47 (65 %) of the samples. A ratio of 62 fecal coliforms per coliphage (62:1) was calculated. The average of all densities of fecal coliforms and coliphages were statistically compared with a student’s t test, with \( n-1 \) degrees of freedom and two tails alpha value of 0.05 (\( \alpha = 0.05 \)). Fecal coliforms resulted in a significant higher average value than coliphage densities.

Correlation analysis was performed between fecal coliforms and coliphage density. The correlation coefficient was used to measure the strength of the relationship between the two indicators. Figure 2 shows the scatter diagram, the least squares regression line, the regression equation, and the \( r^2 \) value. The correlation coefficient is positive and has the following regression equation: \( y = 31.007x + 249.1 \). As the slope of the regression equation is positive the correlation between the variables is also positive. Statistical analysis, \( n = 72 \), shows values of \( r = 0.4732 \), which represents a medium linear relationship between samples. To see if the sample value of \( r = 0.4732 \) is of sufficient magnitude to indicate that fecal coliforms and coliphages are correlated a hypothesis test was conducted. The null hypothesis established assumes that the two variables were not correlated (\( H_0: \rho = 0 \)). The sample distribution was assumed to be distributed as Student’s t distribution with \( n-2 \) degrees of freedom (\( df = 70 \)). The critical values of \( t \) for two tails with alpha equal to 0.05 (\( \alpha = 5 \)) are \( \pm 1.993 \). The computed value of \( t \) was 4.494 (\( t = 4.494 \)). Since the computed value of \( t \) exceeded the critical value of \( t \), the null hypothesis was rejected. Rejected the null hypothesis implies that fecal coliforms and coliphage densities are significantly positive correlated with a \( p \) value less than 0.001 (\( p < 0.001 \)).

Statistical analysis with Student’s t test was used to determine possible pollution sources, probably located through the main canal of the irrigation system of Lajas, Puerto Rico. The average of fecal coliforms and coliphage densities, shown on Figures 3 and 4, were statistically analyzed for each sample point. If the mean value of a point was statistically different it will indicate that upstream from this point a fecal coliform or coliphage source may be located. Densities between sample points were compared against each for coliphages and fecal coliforms. Data Analysis using the Excel program of Microsoft Corporation, t-test: Paired Two Sample for Means, was used to
compute \( t \) values, assuming the null hypothesis that the two means are not different \( (H_0: \mu = 0) \). The test was performed \( n - 1 \) degrees of freedom \( (df = 11) \) and critical values of \( t \) for two tails with alpha equal to 0.05 \( (\alpha = 0.05) \), of \( \pm 2.20 \). Since none of the computed values of \( t \) for both fecal coliforms and coliphage densities did not exceed the critical value of \( t \) the null hypothesis was accepted, concluding then that fecal coliforms and coliphage averages on different sampling points are not significatively different.

CONCLUSION

The above study was conducted to evaluate coliphages as indicators of fecal pollution, elucidate the occurrence of fecal coliforms and coliphages, and identify possible fecal contamination sources in the irrigation system of Lajas, Puerto Rico. The occurrence of coliphage and fecal coliforms in this water was unprecedented since no previous research had been performed. The concentration of fecal coliforms was significantly higher \( (t_{70, 0.05} = 1.993, p < 0.001) \) than that of coliphages. The fecal coliforms were present in 100% of the samples against the coliphages that were present only on 65% of the samples and a ratio of 62 fecal coliforms per coliphage (62:1) was calculated.

Measures of pH, DO, and temperature were correlated with the indicators, coliphages and fecal coliforms. None of the water quality parameters showed statistical \( (t_{70, 0.05} = 1.993) \) correlation with the fecal coliforms or coliphage densities. Only the DO parameter showed big changes on the different sampling points. The lowest DO values were detected on point 1. This reduction in oxygen may be caused by bacteriological oxygen demand occurring on the pipe that carries water from Loco Reservoir to the beginning of the canal, where water does not have any contact with air. Another reason for the low oxygen levels could be that the water comes with low levels of oxygen from the reservoir itself. This means that water quality parameters do not apparently affect densities of the two variables.

Furthermore, correlation analysis was performed on coliphage and fecal coliforms concentrations. The correlation coefficient was used to measure the strength of the relationship between each indicator of water quality. The two fecal related indicators measured were significantly \( (t_{70, 0.05} = 1.993, p < 0.001) \) positively correlated with each other, that is, when one increased in number, the other also increased in number. Changes in coliphage concentrations are apparently related to changes of fecal coliforms concentrations.

Mean fecal coliform densities on each point were statistically compared against each other for coliphage and fecal coliform densities. The analysis concludes that fecal coliforms and coliphage average on different points are not significatively different \( (t_{11, 0.05} = 2.20) \). As a result, the canal probably does not have any stationary sources of fecal contamination, and maybe those indicators, virus and bacteria, that were detected are coming from the fecal contamination of the Loco Reservoir water. Although statistical analysis did not determine possible areas of fecal pollution, the irrigation system may be polluted by animals such as pigs, horses, and goats. The presence of those warm-blooded animals in the areas surrounding the canal of the irrigation system was very evident during sampling periods. However, scientifically, the true source of those indicators is still unknown.

Coliphage densities correlate with the densities of the fecal indicator bacteria. This relationship between fecal coliforms and coliphage deserves future discussion because of the current status of the fecal coliform bacteria as an indicator. The results of this analysis suggest that a relationship or correlation exists. Results observed showed a significant relationship of coliphages and fecal coliforms densities, but this study failed to prove that coliphages were good indicators of fecal contamination because of a lack of comparative values in other waters.

These results lead to the conclusion that the coliphage indicator could be an effective tool to evaluate fecally contaminated water in tropical areas, since water could have fecal coliforms as natural environmental flora but the coliphages should come from a fecal source. The results of this study suggest that a valid assessment of the risk associated with contaminated recreational fresh water cannot be made using only coliphages as an indicator. The use of coliphage analysis can underestimate the number of fecal coliforms and the possible presence of environmental pathogens even in the absence of fecal pollution. Fecal coliform bacteria could also be underestimating the potential public health significance of pathogenic enteroviruses in water and fail to determine true fecally polluted water. It seems imperative to include coliphage assay as a water quality monitoring method to ensure that no microbiological population can affect human health. Perhaps multiple methodologies can be used in
combination to ensure the safety of water, virological and bacteriological. This strategy could help to avoid searching for an indicator that meets all criteria.

Figure 1. AREA OF STUDY ON PUERTO RICO MAP SECTION
Figure 2. **CORRELATION BETWEEN COLIPHAGE AND FECAL COLIFORMS DENSITIES**

\[ y = 31.007x + 249.1 \]

\[ R^2 = 0.2239 \]

CFU = Colony forming unit, PFU = Plaque forming unit.

Figure 3. **ARITHMETIC MEAN FOR COLIFORM DENSITIES PER SAMPLING POINTS**

CFU = Colony forming unit.
Figure 4. ARITHMETIC MEAN FOR COLIPHAGE DENSITIES PER SAMPLING POINTS

$n = 12$

PFU = Plaque forming unit.