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Use of Geostatistics to Predict Virus Decay Rates for Determination of Septic Tank Setback Distances

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Water samples were collected from 71 public drinking-water supply wells in the Tucson, Ariz., basin. Virus decay rates in the water samples were determined with MS-2 coliphage as a model virus. The correlations between the virus decay rates and the sample locations were shown by fitting a spherical model to the experimental semivariogram. Kriging, a geostatistical technique, was used to calculate virus decay rates at unsampled locations by using the known values at nearby wells. Based on the regional characteristics of groundwater flow and the kriged estimates of virus decay rates, a contour map of the area was constructed. The map shows the variation in separation distances that would have to be maintained between wells and sources of contamination to afford similar degrees of protection from viral contamination of the drinking water in wells throughout the basin.

The significance of viruses in drinking water is just beginning to be recognized (16). Over half of the waterborne outbreaks of disease in the United States are classified as acute gastroenteritis of unknown etiology (14). However, results of recent retrospective serological studies suggest that many of these gastroenteritis outbreaks are caused by Norwalk virus and Norwalk-like viruses, as well as rotaviruses (12). It has been estimated that Norwalk virus alone is responsible for 23% of the reported waterborne outbreaks in this country (13). As the efficiency of methods for detecting viruses in environmental samples and cultivating them in the laboratory is increased, it is likely that viruses will be identified as the cause of even more waterborne outbreaks of disease in this country (9).

Septic tanks discharge more than 800 billion gal (3.028×10^{12} liters) of wastewater to the subsurface every year and are the most frequently reported sources of groundwater contamination (21). Enteric viruses can survive in septic tanks (10; S. L. Stramer, Ph.D. dissertation, University of Wisconsin, Madison, 1984), and their progress from the septic tank to the soil absorption field and into the underlying groundwater has been monitored (10, 23, 24; Stramer, Ph.D. dissertation). Viruses can survive for prolonged periods in groundwater (27) and have remained infective in the environment long enough to cause several waterborne outbreaks of disease.

Septic tank effluent was the source of a Norwalk-like virus that caused over 400 people to develop gastroenteritis at a resort camp in Colorado (7). McCabe and Craun (15) described an outbreak of 98 cases of infectious hepatitis which was traced to the use of commercial ice. A dye tracer was used to show that the septic tank serving a home occupied by persons who had recently had infectious hepatitis was the source of contamination of the well water used to make the ice. Another outbreak of infectious hepatitis involving 17 persons occurred when three drinking-water wells were contaminated by effluent from a septic tank located 1.8 to 3 m away (25).

In the past, septic tank systems were installed to ensure that the effluent would percolate efficiently through the soil and not rise to the surface. Little consideration was given to the potential for groundwater contamination by the effluent. However, in many areas, the density of the systems has increased to the point at which the capacity of the soil to effectively treat the effluent has been overcome (5). As groundwater is increasingly relied upon as a source of potable water (16), public attention is focusing on limiting groundwater contamination.

Prohibiting the use of septic tanks, especially in areas of high population density, would likely result in a decrease in the amount of groundwater contamination and thus a decrease in the number of waterborne disease outbreaks in this country. The costs of installing municipal sewage service, a common alternative to septic tanks, are generally prohibitively high, especially in rural areas (18). Therefore, it is unlikely that septic tanks will be eliminated as a means of disposal of domestic wastewater in the near future. Indeed, in 1980 it was estimated that approximately 25% of all new homes being constructed in the United States have septic tank systems for disposing of domestic wastes (22).

A more realistic approach to decreasing the potential for contamination of groundwater and waterborne disease by septic tank effluent would be to regulate septic tank placement to minimize the negative impacts on the quality of nearby drinking water. Most states have attempted to do this by imposing minimum separation distances of 10.7 to 91.4 m between drinking-water wells and septic tanks, with an average distance of about 15.2 m (17). However, waterborne disease outbreaks have occurred when the septic tank was located further from the well than the prescribed distance; several of these outbreaks have been reviewed recently (26). One of the contributory factors may have been the imposition of the regulations over a statewide area, with little consideration for local hydrogeologic conditions. The purpose of the present study was to determine the variation in separation distances that would be required to afford the

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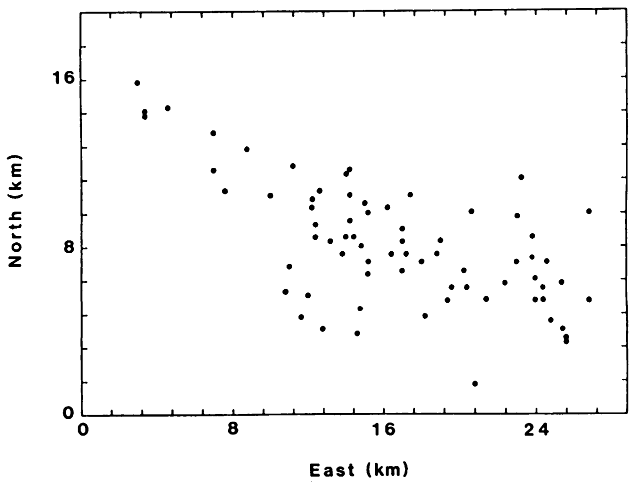

FIG. 1. Location of groundwater sample collection sites. Origin corresponds to Arizona quadrant D, township 14, range 13, section 31, C, C, C.

same amount of protection from viral contamination to different drinking-water sources over a citywide area.

MATERIALS AND METHODS

Sample collection. Water samples were collected from 71 public drinking-water supply wells (Fig. 1). The samples were collected aseptically in sterile polypropylene containers after the lines had been flushed for 2 to 3 min. Water temperature was measured at the time of collection. It was assumed that the measured temperature was approximately the same as that of the groundwater. All samples were placed on ice for transport to the laboratory.

Determination of virus decay rates. Water samples (50 ml) were placed in sterile polypropylene containers and inoculated with *Escherichia coli* B coliphage MS-2 at a final concentration of approximately 10^6 PFU ml^{-1}. Polypropylene containers were chosen because virus adsorption to this material is minimal (2). The containers were incubated in water baths at the measured in situ groundwater temperature ±1°C (20 to 30.5°C). On days 0, 1, 2, 3, 5, 7, 10, 15, and 20, 1-ml subsamples were withdrawn after the container had been vortexed for approximately 30 s; the subsamples were assayed to determine the number of infective virus particles remaining. Virus assays were performed in triplicate by the agar-overlay plaque technique of Adams (1), with *E. coli* ATCC 15597 as the host bacterium.

Virus decay rates were calculated by using linear regression, with time as the abscissa and the number of infective virus particles remaining as the ordinate. All decay rates are reported as positive numbers: the larger the number, the more rapidly the viruses were inactivated.

Theoretical considerations of geostatistics. Unlike classical statistics, which generally assumes independence among samples, geostatistical analyses are based on the observation that a sample value is often affected by its position in space relative to other samples. Kriging allows one to estimate the value of a characteristic at a desired location by using known values of that characteristic at nearby locations. The unknown value is estimated by weighting the known values on the basis of their spatial orientation and the underlying spatial correlation structure relative to the desired location (3, 4, 6, 11, 19).

To decide whether it is appropriate to analyze a data set with geostatistical techniques, it must first be determined that the data are spatially correlated. If the data are spatially correlated, then the samples close to one another will be more similar (i.e., will have a lower variance) than those located farther apart (i.e., with a higher variance). At a certain separation distance, called the range of influence, the variance reaches the level that would be expected if the samples were not spatially correlated (i.e., independent).

The pattern of spatial correlation is described by calculating a semivariogram. The variance of all pairs of sample measurements a given separation distance (h) apart is calculated with the following equation (11):

$$\gamma(h) = [1/2N(h)] \sum_{i=1}^{N(h)} [Z(x_i) - Z(x_i + h)]^2$$

where $\gamma(h)$ is the semivariogram, $N(h)$ is the number of pairs of samples separated by a distance h ($h = x_i - x_j$), $Z(x)$ is a sample value at point x (x is a vector) and $Z(x + h)$ is the sample value at distance h from point x. Likewise, the variances of all pairs of sample measurements located $2h$, $3h$, $4h$, . . ., nh distances apart are calculated. A graph is then drawn with the separation distance between samples on the abscissa and the semivariogram on the ordinate (Fig. 2). This experimental semivariogram is then modeled by using one of several equations. One of the most commonly used equations is the spherical model $\gamma(h) = C_0 + C_1[3h/2a - 0.5(h/a)^3]$ for $0 < h \leq a$, $\gamma(h) = C_0 + C_1$ for $h \geq a$, and $\gamma(h) = 0$ for $h = 0$, where a is the distance at which the samples become independent of one another (the range of influence) and $C_0 + C_1$ is the value of γ at which the graph levels off (the sill). The sill is the variance that would be expected if the sample values were not spatially correlated. The term C_0 is the nugget effect and represents either the measurement error or the variability at a smaller scale than the smallest sampling interval.

The value of a characteristic at the desired location can be estimated by using the values at surrounding points within a given radius. For this purpose, 5 to 10 neighboring points were used in the estimation process. Generally, the contribution of these points to the estimation of the value at the desired location is weighted on the basis of the distance that point is from the point where the desired value is unknown.

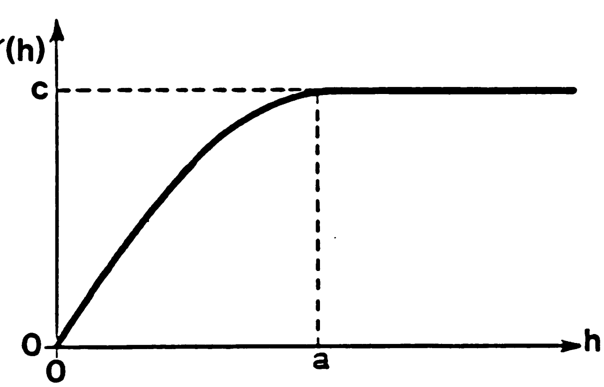

FIG. 2. Spherical model semivariogram. h is the separation distance between samples, a is the range of influence, c is the sill, and γ(h) is the semivariogram.

In other words, the closer a neighboring point is to the desired point, the more heavily its value is weighted when the unknown values are estimated. The following equation is used in the estimation process:

$$Z_k^*(x_0) = \sum_{i=1}^{n} \lambda_i Z(x_i) \quad (1)$$

where $Z^*_k(x_0)$ is the estimated value at the point x_0, λ_i is the weight associated with a sample location x_i, and n is the number of samples used in the estimation process. To obtain an unbiased estimator, the weights in equation 1 are subject to the constraint

$$\sum_{i=1}^{n} \lambda_i = 1$$

Because values for $Z(x_i)$ are known (i.e., the data) and the experimental semivariogram has been modeled, the only values needed to calculate a $Z^*_k(X_0)$ are the weight factors. These weights are found by using the kriging equation

$$\sum_{i=1}^{n} \lambda_i \gamma(x_i - x_j) + \xi = \gamma(x_0 - x_j); \quad j = 1, 2, \ldots, n \quad (2)$$

where a Lagrange multiplier (ξ) is introduced to minimize the variance of errors (subject to the constraint on the weight factors [3, 11]). Equation 2 can be written as the following matrix:

$$\begin{bmatrix} \gamma_{11} & \gamma_{21} & \cdots & \gamma_{n1} & -1 \\ \gamma_{12} & \gamma_{22} & \cdots & \gamma_{n2} & -1 \\ \cdot & \cdot & & \cdot & \cdot \\ \cdot & \cdot & & \cdot & \cdot \\ \cdot & \cdot & & \cdot & \cdot \\ \gamma_{1n} & \gamma_{2n} & \cdots & \gamma_{nn} & -1 \\ 1 & 1 & \cdots & 1 & 0 \end{bmatrix} \begin{bmatrix} \lambda_1 \\ \lambda_2 \\ \cdot \\ \cdot \\ \cdot \\ \lambda_n \\ \xi \end{bmatrix} = \begin{bmatrix} \gamma_{01} \\ \gamma_{02} \\ \cdot \\ \cdot \\ \cdot \\ \gamma_{0n} \\ 1 \end{bmatrix}$$

The weights and Lagrange multiplier found by solving this system of equations are used in equation 1 to estimate the value of a property at each desired location.

Geostatistical analyses. Experimental semivariograms were calculated for virus decay rates by using the program

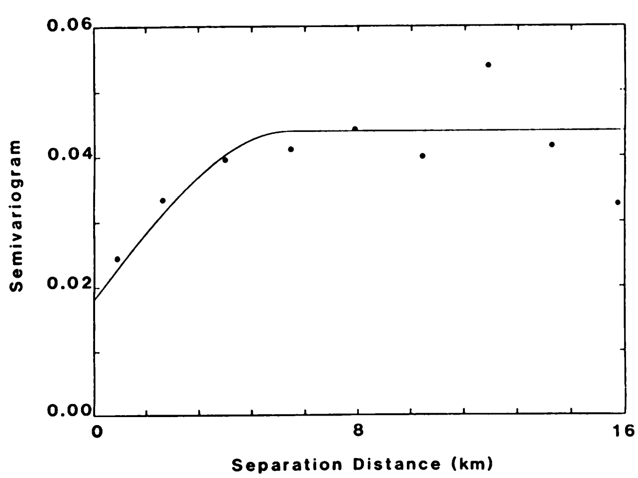

FIG. 3. Experimental virus decay rate semivariogram (●) modeled by using the spherical equation (——) with a sill of 0.044, a nugget of 0.018, and a range of influence of 6 km.

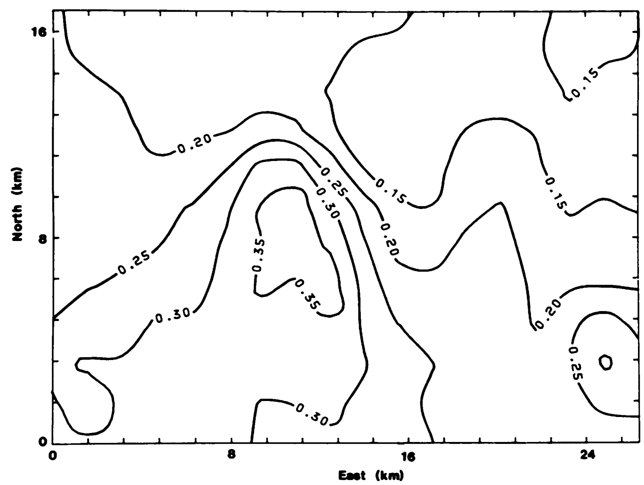

FIG. 4. Contour map of virus decay rates ($-\log$ PFU day^{-1}) in Tucson groundwater. Origin is the same as in Fig. 1.

GAMA3 (11). Model semivariograms were calculated by using VARI.FTN and validated by using a jackknifing option in the program KRIGCJ.FTN. Virus decay rates were kriged by using the computer program KRIGCJ.FTN. The documentations for VARI.FTN and KRIGCJ.FTN are contained in *GESTAT: Geostatistical Programs for Analyzing Stationary Random Variables* (S. R. Yates, manuscript in preparation). All contour maps were generated with the program SURFACE-II (20).

Estimation of separation distances. Separation distances between wells and potential sources of contamination were calculated as the distance required to achieve a 10^7-fold decrease in virus number as the water moves from the point of introduction of the viruses to a source of drinking water. Distances were calculated by using Darcy's law (8)

$$D = (tKi)/n_e \quad (3)$$

where D is the separation distance in meters, t is the travel time (days), K is the hydraulic conductivity (meters day^{-1}), i is the hydraulic gradient (meters meter^{-1}), and n_e is the effective porosity of the aquifer.

Hydraulic gradients were calculated from a water table elevation map obtained from the City of Tucson. Hydraulic conductivity data were calculated on the basis of transmissivity values provided by the state of Arizona Department of Water Resources. Travel times were calculated by using the kriged estimates of virus decay rates as the number of days required for a 10^7-fold decrease of virus inactivation.

RESULTS

The decay rates of MS-2 coliphage in the groundwater samples ranged from 0.068 to 0.71 log PFU day^{-1}. The decay rates were highly correlated with the incubation temperature ($r = 0.93$, $P = 0.01$), with higher inactivation rates at higher temperatures.

Virus decay rates were found to be spatially correlated, as shown by the semivariogram (Fig. 3). The experimental semivariogram was modeled by using a spherical equation, with a sill of 0.044, a nugget of 0.018, and a range of influence of 6.0 km.

A contour map of the decay rates of viruses in Tucson groundwater generated by using kriged estimates is shown in

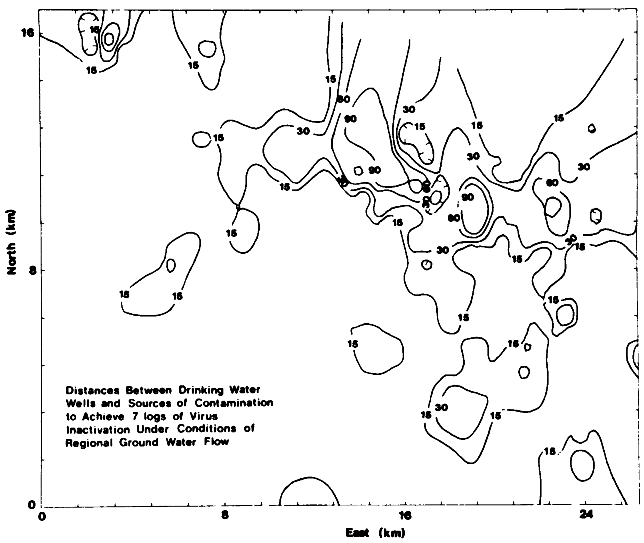

FIG. 5. Separation distances in meters. Origin is the same as in Fig. 1.

Fig. 4. The separation distances (meters) that would have to be maintained between wells and septic tanks to achieve 7 logs of virus inactivation and that were predicted by using the kriging equation and equation 3 are shown in Fig. 5.

DISCUSSION

A comparison of Fig. 4 and 5 shows that in the areas with the lowest virus decay rates (0.15 log PFU day^{-1}), some of the largest separation distances were calculated. This area corresponds to a zone of recharge along the banks of the Rillito River, which flows only as a result of high rainfall or snowmelt runoff. The groundwater temperature near the river was the lowest measured in the city, averaging approximately 20°C. Previous experiments have shown that, of all measured water characteristics, including pH, nitrate, sulfate, iron, hardness, temperature, ammonia, and total dissolved solids, only temperature is significantly correlated with the inactivation rate of viruses in groundwater (27). In these studies of more than 100 groundwater samples, temperature was the single most important predictor of virus decay; thus, it was felt that the low temperature measured near the river was responsible for the relatively slow virus inactivation rate.

The aquifer in this area has transmissivities up to 0.03 m^2 s^{-1} (compared with 0.0014 m^2 s^{-1} elsewhere in the basin) and hydraulic gradients up to an order of magnitude higher than elsewhere in the basin, resulting in high rates of groundwater flow near the river. The combination of slow virus decay rates and high groundwater flow rates resulted in large separation distances in the area near the river. Contours for separation distances of up to 90 m are shown in Fig. 5. Separation distances as large as 120 and 150 m were calculated in this area; these contours are not shown, in the interests of clarity.

As with all attempts to mathematically model environmental processes, the results must be interpreted with complete knowledge of the assumptions that were used in constructing the model. Several simplifications were incorporated into the model for this first attempt to predict separation distances. First, coliphage MS-2 was used as the model virus in the experiments to determine decay rates. It has been shown previously that there is no significant difference in the decay rates of MS-2, poliovirus -1, or echovirus -1 in groundwater samples when incubated under the same conditions (27). However, recent work has shown that hepatitis A virus may persist for much longer periods than the other enteroviruses studied (M. D. Sobsey, personal communication), so that the use of MS-2 may lead to an underestimation of the survival times of hepatitis A virus in groundwater.

Another simplification in this modeling effort was to omit the effects of well pumping on the groundwater flow pattern and to consider only the regional groundwater flow characteristics. If pumping were included in the model, the required separation distances would be increased in the areas surrounding the wells, because pumping would increase the rate of groundwater flow to the wells.

The thickness of the unsaturated zone will also affect the potential for viruses to reach groundwater, since viruses are removed by adsorption to soil particles as the water percolates through the soil matrix. The effects of the unsaturated zone were not directly included in this simple model but have been taken into consideration indirectly, as discussed below.

Although the use of MS-2 coliphage as a model virus and the omission of the effects of pumping wells on the groundwater flow may have resulted in an underestimation of separation distances, this has been compensated for by calculation of the distance as a function of the travel time required for 7 logs of virus inactivation. If it is assumed that 1 virus particle in 1,000 to 10,000 liters of water can be detected, then 10^3 to 10^4 virus particles per liter could be introduced into the groundwater and be inactivated (i.e., for a total of 7 logs of inactivation) as the water moved from the point of introduction of the viruses to the point of water abstraction. Under most circumstances, there is only a small probability that 10^3 to 10^4 viruses per liter would penetrate into the groundwater after being retained in the septic tank for several days and traveling through the soil absorption field and the unsaturated zone, especially if the septic tank soil absorption system is properly designed, installed, and maintained.

This study has shown that there is a high degree of variation in the decay rates of MS-2 coliphage in the groundwater collected from wells over a relatively small area. This, coupled with the variation in the hydraulic gradient and hydraulic conductivity throughout the basin, resulted in predicted separation distances between wells and sources of potential contamination that range from 15 to larger than 150 m.

Because of the simplicity of the model and the assumptions we have made, these separation distances may not correlate exactly with conditions in the field. However, our distances do illustrate the wide variation in separation distances that would be needed to afford different wells similar degrees of protection from viral contamination over a small (citywide) area. In addition, this model provides a way to make site-specific predictions of virus decay rates.

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