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Determining Average Concentrations of *Cryptosporidium* and Other Pathogens in Water

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Analysis of water for propagules of *Cryptosporidium* and other pathogens is now common, especially since promulgation of the U.S. EPA's Information Collection Rule. At low concentrations, available analytic methods frequently result in nondetection (ND samples), thus complicating interpretation. Most existing methods for averaging data that include ND samples produce biased averages, sometimes severely so. Treating ND samples as if one propagule had been found overestimates true concentrations excessively at low concentrations, yet provides no "safety factor" at high concentrations. Averaging only positive results and working with an upper 90th or 95th percentile of the data behave similarly. New procedures are therefore derived to provide improved estimates of mean concentrations and confidence limits for those means. These estimates are calculated for a given set of samples by dividing the total number of propagules counted by the total effective volume of water from which they were isolated. These statistics provide both a safety buffer that does not decline at high concentrations and an easily interpretable meaning. A correction for incomplete recovery is described. Example data from New York City's Catskill Aqueduct at Kensico Reservoir are used to illustrate the methods presented. The statistical methods derived apply to concentration data for any discrete particles.

Introduction

Interest in concentrations of the protozoan pathogens *Cryptosporidium* spp. and *Giardia* spp. in public water supplies has recently been increasing, especially since the outbreak of the former in Milwaukee, WI, in 1993 (*1*, *2*). In the United States, consideration of these organisms is becoming even more widespread under the Information Collection Rule (ICR) recently promulgated by the U.S. Environmental Protection Agency (*3*). Among other things, this rule requires large-scale water suppliers to test their waters for certain pathogens to aid in setting possible nationwide drinking-water standards for those organisms.

Monitoring programs for pathogenic organisms in drinking-water supplies can have multiple scientific and regulatory purposes. These include characterizing how typical concentrations in public water supplies vary regionally and with source type and treatment methods to serve as a basis for future regulation, estimating average concentrations from one site and time period for comparison with averages from other sites and time periods, estimating averages for use in fate and transport studies designed to guide future control strategies, and supplying data for risk analyses. The analytical methods developed in this paper contribute to most of these purposes and apply not only to oocysts of *Cryptosporidium* but also to any organisms transmitted as discrete, countable propagules.

Current methods for measuring *Cryptosporidium* concentrations are time-consuming and expensive, yet far from perfect (*2*). For example, recovery efficiencies can be low (*3*, *4*), and it may be difficult to identify the organisms correctly [e.g., algae may interfere (*5*)]. Thus, improving detection methods is an active research area (*2*). When oocyst concentrations in water are low, the methods frequently lead to nondetection and more generally have low precision. Dealing with sequences of concentration measurements that include many ND samples appears to be especially difficult but is the major focus of this paper.

Suppose that 52 weekly concentration measurements for *Cryptosporidium* oocysts were available for the year 1996 at a particular site in a water-supply system and that analysts had counted no oocysts in 50 of the samples, one oocyst in one, and two in one. Several methods have been proposed for summarizing, reporting, or acting upon such a set of results. They include the following: (A) the "no-zeros" method [averaging all 52 weeks' results, but treating the 50 zero counts as if one oocyst, or sometimes one-half, had been seen instead (*6*, *7*)], resulting values are termed detection limits by Fout et al. (*11*); (B) the "positives-only" method [averaging only the positive results (*6*−*9*), i.e., two in this example, and ignoring the rest]; (C) the "percentile" method [ordering the data from smallest to largest and determining the concentration that falls at the upper 90th or 95th percentile of the data set (*6*)]. Here a further decision is required if 90% (95%) of the samples are nondetects.

We suggest that it is preferable to use an average (possibly a weighted one) of all 52 concentrations, counting zeros as zeros just as every other count is taken at face value. One possible weighting is based on the "effective volumes" of the samples, to be defined below. Related methods for estimating upper confidence limits for the true average concentrations will also be presented. In what follows, we will compare some advantages and disadvantages of summarizing a series of oocyst concentration measurements in these various ways.

Experimental Section

Oocyst Sampling Process. The process usually used to obtain and analyze *Cryptosporidium* samples consists of several stages (*2*, *10*, *11*), including initial filtration, filter washing, centrifugation, flotation, and so on. In the analyses to follow, the true but unknown oocyst concentration in the bulk water, which we will call μ_c (in propagules per hectoliter; 1 hL is 100 L), is assumed to vary in some unknown way from one sampling date to another. For example, it might vary seasonally, or it might follow one distribution after rain events and another in dry weather; thus, it could easily be bimodal (see Appendix I). (Appendices are available as Supporting Information. See end of paper for details.) We will be most concerned with estimating the mean bulk water concentration, μ_c, over some period of time and with obtaining an upper confidence limit for that parameter.

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For any given sample, a volume V of water is first filtered from the bulk water. In the samples used as examples for this paper, V was typically about 11.4 hL (300 gal). We assume that any oocysts present in the bulk water *at any given time* are distributed randomly and independently, so that the number of oocysts N_1 present in the sampled volume V follows a Poisson distribution (*12*, *13*). (Note that we make no assumptions about the distribution of the samples taken over time.) With many ND samples, it is difficult to test this assumption however.

If the oocysts were not distributed independently but instead tended to cluster together, e.g., on particles of debris, then the Poisson distribution would apply to the clusters rather than to the individual cells. Such clustering, which would not bias the estimates of mean concentrations but would increase the variances of sample results and therefore affect confidence limits, will be considered further in the section on confidence limits and is also discussed in Appendix I.

In the laboratory analysis of a given sample, only a fraction of the total filtered material is ultimately inspected under the microscope (*11*). We denote as f the fraction of material deliberately subsampled. In addition, it is possible at several stages of the sample collection and analysis process for some cysts to be lost inadvertently. We define the overall fraction thus lost to be λ, and the fraction retained (recovery rate) to be $\rho = 1 - \lambda$. Overall, the average proportion of oocysts present in the original sample that will be counted is then $\phi = f\rho$. Thus, the number of oocysts counted, K, will have a binomial distribution (*12*), with probability ϕ that each of the N_1 propagules originally filtered will be counted.

We show in Appendix II that a binomially distributed subsample drawn from a Poisson-distributed sample also has a Poisson distribution. Specifically, if a sample of volume V is filtered from bulk water carrying a concentration of μ_c oocysts hL^{-1}, then the number filtered, N_1, will have a Poisson distribution with mean $\mu_N = V\mu_c$, and the number counted, K, will have a Poisson distribution with mean $\mu_K = \phi V\mu_c$. This result will be used below to obtain confidence limits for mean concentrations. We refer to the quantity $\phi V = f\rho V$ as the "effective volume" (or EV) of the sample. On the average, the number of oocysts counted will be equivalent to the number that would be counted if a volume equal to the EV had been filtered in the first place and all oocysts in that volume had been counted.

Example Data. To illustrate the statistical methods to be developed here, we use a data set obtained by the pathogen monitoring program of the Department of Environmental Protection, New York City Bureau of Water Supply, Quality, and Protection (*7*, *14*). The 109 samples considered in this paper were taken from a facility where untreated source water leaves Kensico Reservoir, Westchester County, NY, and enters the Catskill Aqueduct for transport to the city. Samples were taken prior to chlorination of the bulk water and obtained almost weekly for the period from October 1993 through December 1995. They are part of a much larger data set (*14*).

Accounting for Nondetection in Estimating Average Oocyst Concentrations. A critical feature of these Catskill Aqueduct data [and of data from at least some other water supplies (*6*, *14*, *15*)] is that the organisms are sufficiently rare that they go undetected in many samples. (For the Catskill data, detection occurred in fewer than 15% of the samples overall and with declining frequency through time.) Data like these are fundamentally different from chemical concentration measurements, for example, because the oocysts being detected are discrete, countable entities—a given sample of water may contain zero, or one, or two, or ...oocysts. Because of this, it is virtually impossible for the concentration estimated from any single sample to equal the concentration in the bulk water from which the sample was drawn. For example, dividing 1 L of water containing one oocyst into 10 equal samples would yield one sample with a concentration of 10 per liter and 9 with zero concentrations. No single 0.1 L sample could possibly estimate the bulk concentration correctly, although the average would be correct.

In practice, many water samples are likely to contain no oocysts, while others will contain more than the average number (*13*). Compensating for the low precision of single concentration estimates requires many samples and proper averaging.

In the Introduction, we noted several purposes of pathogen monitoring programs. How to serve those multiple purposes in the face of high rates of nondetection is the subject of this and the next section. In particular, we consider ways to estimate mean oocyst concentrations from a series of samples when no oocysts are detected in many of them.

It is clear that the no-zeros method of data analysis will lead on average to overestimating mean concentrations, since a potentially large number of zeros is increased to positive values and no other sample results (some of which are likely to be overestimates) are reduced to compensate. As a result of this bias, the no-zeros method would seem to be protective of public health. However, there is a catch. When the oocyst concentration in the bulk water is very low, sampling will produce many ND samples, and the degree of overestimation will be great. On the other hand, when high concentrations predominate, ND samples become rare, and the tendency to overestimate disappears. Thus, the method overestimates at low concentrations that may be of negligible concern but fails progressively to do so at those higher concentrations that might pose a health risk.

An alternative, and we believe preferable, data analysis method is to take all sample results (including zeros) at face value as estimates of what was in the bulk water, with the goal of obtaining an unbiased estimate of the true mean concentration. This is especially important if the purpose of monitoring is to estimate accurately what concentrations exist in waters of various types or to use the data in fate and transport studies or in best-estimate risk analyses.

Even when a statistic like estimated mean concentration is correct on average, particular sample estimates will usually be either too low or too high. To ensure against underestimation, e.g., to protect public health, we propose using upper confidence limits for the mean concentrations, as considered in the next section. As will be shown, the limits obtained are roughly a fixed amount higher than the corresponding means. The difference between an estimated mean and its upper confidence limits does not decline to zero at high concentrations as occurs with the no-zeros method.

To put these points in quantitative terms, we work with V, f, and K as already defined above, with μ_c (the mean concentration of oocysts, in hL^{-1}) and with the concentration estimate $C(K) = K/(fV)$ that results from a count of K oocysts. (For the time being, we assume that $\phi = f$, i.e., that inadvertent losses are negligible but will account for such losses in a later section.) The issue then becomes what estimate to use for μ_c when $K = 0$, i.e., when no oocysts are counted by the analysts. Let us define μ_s (subscript s for "sample") as the mean (expected value) of concentration estimates obtained by sampling from water with concentration μ_c and by setting $C(0) = 0$. Also, we define μ_{sNZ} as the mean obtained by replacing $C(0)$ with $C(1)$, i.e., with $C(\text{nondetection}) = 1/(fV)$ as is done with the no-zeros method.

We prove in Appendix III that $\mu_s = \mu_c$, i.e., that taking zeros at face value gives the correct, unbiased concentration on average. That appendix also shows that when zeros are inflated to unity, the ratio of the average estimate based on using no-zero values to the true concentration is

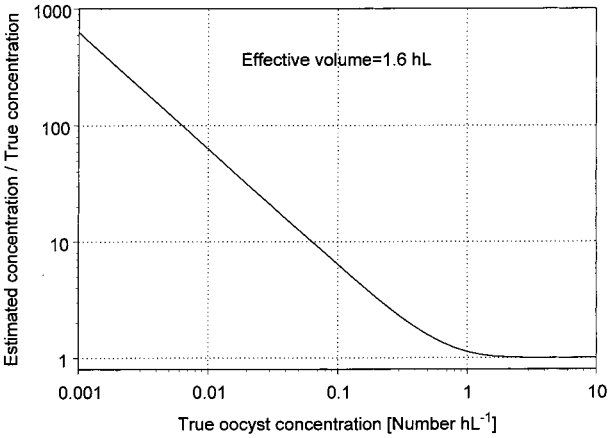

FIGURE 1. Bias introduced by averaging concentration detection limits into a mean concentration (the no-zeros method), relative to the unbiased procedure of treating ND (nondetection) samples as zero when estimating mean concentrations. The comparison, represented by the ratio of the no-zeros mean to the true mean, is made for an effective sample volume like that in the Catskill Aqueduct samples. Note that the bias can be exceptionally high at low concentrations where protection that might be afforded by the bias is unimportant, but it disappears at high concentrations where a safety factor might be more useful. The ratio approaches unity for all concentrations higher than those shown.

$$\frac{\mu_{sNZ}}{\mu_c} = 1 + \frac{1}{fV\mu_c \exp(fV\mu_c)} \qquad (1)$$

This ratio, which tends toward unity as μ_c becomes large, is plotted against m_c in Figure 1. This expression describes the bias introduced by replacing zeros with ones so that they are not available to balance out overestimates that occur in other samples in which some detection occurs.

If one wishes to have an unbiased estimate of the concentrations actually present, zeros must clearly be treated as zeros, not as ones. If one wished for some purposes to build in a safety factor, it seems undesirable to apply a factor that disappears at high, potentially dangerous concentrations, as does the inflation caused by the no-zeros method (Figure 1). The next section provides an alternative approach based on confidence limits for ensuring against underestimation at all concentrations, including high ones.

We now use the Catskill Aqueduct monitoring data to compare the estimates of quarterly mean concentration calculated using (a) estimates equal to the total number of propagules observed divided by the total effective volume of water investigated (Method 1, our preference), (b) simple averages of the sample concentration estimates (Method 2), (c) the no-zeros approach, (d) averages of positive results only, and (e) the upper 95th percentile of the data. The data considered are for the total of "presumed but unconfirmed" plus "confirmed" oocysts (*10, 11*), although in the 109 samples in the data set, only a single oocyst was confirmed.

The data available from pathogen monitoring programs are complicated to analyze because the effective volume of water examined varies from sample to sample. In the present case, this occurred because adjustments were made as the program became established, because filters may clog or the original amounts of water filtered varied for other reasons, and because laboratory analysts counted propagules from varying proportions of the filtered material. From a statistical point of view, it would be preferable for all samples to be of the same effective size, but they seldom are in practice, so we must account for the variation in sample size.

We consider here two ways to estimate a mean concentration from a series of samples with varying effective

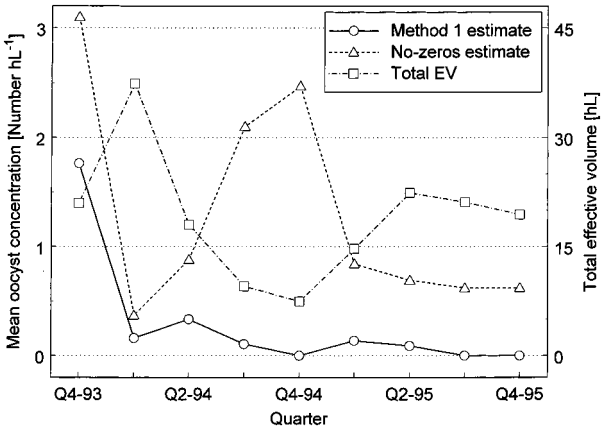

FIGURE 2. Quarterly mean oocyst concentrations in water entering the Catskill Aqueduct from Kensico Reservoir prior to chlorination. These means are calculated by weighting the result of each sample by its effective volume. The left vertical axis applies to both the unbiased means estimated by treating ND samples as zeros (circles) and the biased means estimated by treating each nondetection as if one oocyst had been seen (triangles). The right-hand axis represents the total effective sample volume for each quarter (squares), which affects both the reliability of each result and the bias introduced by the no-zeros method.

volumes, i.e., with method 1—(sum of propagules counted)/(sum of effective volumes)—and method 2—mean of (propagules counted/effective volume), that is, the average of the individual sample concentrations. These choices have the following advantages and disadvantages:

Method 1. When multiple estimates of varying reliability are averaged, it is common in statistics to calculate a weighted mean, using the inverse of the estimated variance of each observation to give more reliable values greater weight. As described in Appendix IV, that procedure leads to method 1, in which the estimated mean concentration is:

$$\hat{\mu}_{cw} = \frac{\sum EV_i(K_i/EV_i)}{\sum EV_i} = \frac{\sum K_i}{\sum EV_i} \qquad (2)$$

This is simply the total number of oocysts counted divided by the sum of all the effective volumes.

Method 2. The second method weights each concentration estimate equally, regardless of the effective amount of water contributing to the estimate. It has the disadvantage that less reliable samples, those based on smaller quantities of water, are counted equally with larger, more reliable samples. However, it is conceivable (though not supported by analyses of Kensico Reservoir data) that smaller amounts of water might be filtered (or that smaller fractions of filtered material might be examined in the lab) when water contains more solids and, concomitantly, that concentrations of pathogen propagules might tend to be higher in waters carrying more solids. If that were true, then method 1, which weights larger samples more, would tend to weight cleaner samples more as well and thus yield a mean concentration estimate that was biased low.

In the remainder of this paper, we will emphasize method 1 but have also used method 2 and will discuss the differences briefly. The resulting means for the Catskill Aqueduct data are presented in Figure 2, which shows both the method 1 means and the highly biased estimates resulting from the no-zeros method. As predicted, the no-zeros estimates tend to overestimate more at low concentrations than at high ones, but the relationship is not as simple as with the theoretical curve of Figure 1. The reason is that the effective volume (EV = fV), which governs the size and frequency of

TABLE 1. Comparison of Several Methods for Summarizing Results of a *Cryptosporidium* Monitoring Program[a]

			EV-weighted		simple averaging					
quarter	*N*	% ND samples	mean	upper 95% CL	mean	upper 95% CL	no zeros, mean	positives-only, mean	95th percentile	95th percentile (no zeros)
4 1993	12	50	1.76	2.32	2.44	3.09	3.10	4.89	12.62	12.62
1 1994	11	73	0.16	0.32	0.14	0.29	0.37	0.51	0.57	0.57
2 1994	12	67	0.33	0.66	0.32	0.64	0.88	0.95	1.39	1.91
3 1994	12	92	0.11	0.50	0.31	0.81	2.10	3.77	1.70	4.01
4 1994	12	100	0.00	0.40	0.00	0.40	2.47	NA[b]	0.00	5.00
1 1995	11	91	0.14	0.43	0.12	0.42	0.84	1.38	0.69	1.19
2 1995	14	93	0.09	0.28	0.10	0.30	0.69	1.36	0.48	0.98
3 1995	13	100	0.00	0.14	0.00	0.14	0.62	NA	0.00	0.69
4 1995	12	100	0.00	0.15	0.00	0.15	0.62	NA	0.00	0.68

[a] Approximately weekly data for water from New York City's Catskill Aqueduct (prior to chlorination) are summarized on a quarterly basis. *Cryptosporidium* concentration estimates are in oocysts hL^{-1}. Samples with no oocysts detected have been treated as zeros for the values shown in column 10, while column 11 shows the results of combining the no-zeros and 95th percentile calculations. [b] NA, not applicable.

the ND values, is not a constant here as it was for Figure 1. In fact, the EV ranged from 0.18 to 4.61 hL for individual samples in this data set. To indicate this variation, the quarterly totals of effective volume are plotted as squares in Figure 2. Note that the bias caused by inflating zeros to ones rises as EV declines.

Table 1 compares the results of summarizing the data using (a) EV-weighted averaging (method 1), (b) 95% upper confidence limits for the EV-weighted means, (c) simple averaging (method 2), (d) 95% upper confidence limits for the simple means, (e) the no-zeros method, (f) the positives-only method, and (g) upper 95th percentiles. The positives-only values were calculated as straight averages of the concentrations reported by the lab, without volume weighting. The percentile values in the table were computed using the quantile function of the S-Plus statistical software package; this function estimates percentiles not actually present in the data set by linear interpolation between percentiles that are present (*16*). The percentiles were calculated in two ways—treating ND samples as zeros or treating them as the reported detection-limit values.

For reference, the ratio of the method 2 to method 1 averages ranged from about 0.86−2.8, and the corresponding ratios for the confidence limits were 0.91−1.62. The largest difference occurred in the third quarter of 1994 when the one oocyst detected in 12 samples was found in a sample of small volume. Confidence limits for the method 2 means were obtained by (a) multiplying the estimated mean concentration by the average effective volume to get an average count, (b) treating this as an approximate Poisson variable, (c) obtaining the confidence limit for that count by linear interpolation between the next lower and next higher integer, and (d) converting that back to a confidence limit for the concentration.

When many samples result in nondetection, both the positives-only and percentile methods neglect a high proportion of the data and rely almost entirely on extreme values. ND samples carry important information about average concentrations, and that information is totally discarded with these two methods, not just inflated as in the no-zeros method. As Table 1 shows, these two methods produce summary statistics that are nearly as excessive as, or even more excessive than, the no-zeros estimates in comparison with the method 1, EV-weighted estimates.

Another major feature of Figure 2 is the much higher mean oocyst concentration in the fourth quarter of 1993 as compared to that in the eight succeeding quarters. Possibly *Cryptosporidium* concentrations at this site were tracking a reported decline throughout North America over the last several years, perhaps caused by cyclic variations in environmental levels of this organism (*6*). Alternatively, as noted by one reviewer, many analysts probably counted other fluorescent particles as oocysts when this procedure was first used (*5*), and that may have happened here. Since that first quarter, the unbiased estimated means for this untreated source water have remained below 0.34 oocysts hL^{-1}, more than 3 orders of magnitude below the 10^3/hL (10/L) that would trigger monitoring of treated water under the ICR (*3*). These values have not been adjusted for imperfect recovery efficiencies, but even a recovery efficiency as low as 5% would lead to an upward adjustment of just over an order of magnitude.

Confidence Limits for Mean Oocyst Concentrations. Whenever means of concentrations or other quantities are estimated, it is desirable also to estimate the reliability of those estimates. One common way of gauging reliability is with confidence limits, and this section provides methods for calculating those limits for the kind of data analyzed here. The confidence limits derived can also be used to provide a safety buffer, in that we could choose to work with the upper 90% (or other percentage) confidence limit for a mean instead of or in addition to the actual estimated mean.

For pathogen data with many ND samples, the estimated concentrations would have a distribution that is strongly skewed to the right, even if all samples came from water with a constant background concentration. Because the example data analyzed here were taken at roughly weekly intervals and because the bulk water concentration no doubt varied from week to week, the distribution of weekly bulk water means is also being sampled from, and we have no information about the shape of that distribution.

We therefore need a method for calculating confidence limits for means of oocyst concentrations that takes the special characteristics of these data into account. If we continue to assume that oocysts are distributed randomly and independently in the bulk waters that are sampled, this is not difficult. Consider the case of the quarterly mean concentrations described in the previous section, each of which is based on $n \approx 12$ samples. In Appendix V, we note that the total number of oocysts K_{tot} counted in n samples has a Poisson distribution with a mean equal to the sum of the means of the individual samples (*17*). We then describe how to obtain upper confidence limits for that total count and finally how to convert those numbers into confidence limits for the EV-weighted mean concentrations. Figure 3 shows upper confidence limits for true but unknown mean counts, based on selected percentage limits and *observed* counts, and Table V-1 in Appendix V provides numerical values. When divided by the total effective volume, these yield the confidence limit for $\mu_{c,mean}$. Confidence limits for other probabilities and counts are given in refs *12* and *17*.

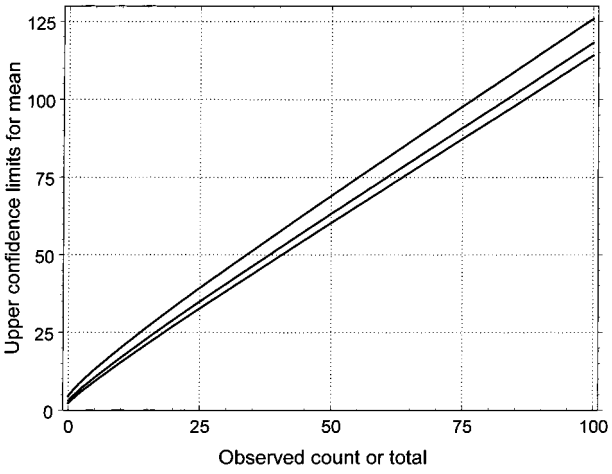

FIGURE 3. Upper confidence limits for counts, for both single samples and for sums of samples when all are Poisson distributed. Curves (top to bottom) represent 99%, 95%, and 90% limits for the mean count. Corresponding confidence limits for concentrations can be obtained by dividing the count limits by the associated effective volumes.

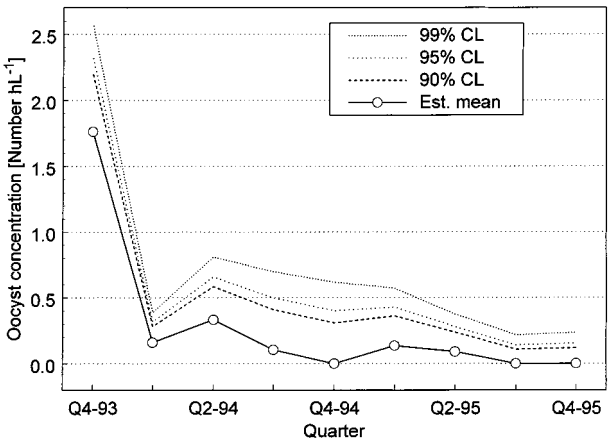

FIGURE 4. Estimated effective volume-weighted mean concentrations of total *Cryptosporidium* oocysts at the Catskill Aqueduct for each quarter between October 1993 and December 1995. The solid line shows the unbiased means, based on treating ND samples as zeros. The successively higher lines represent one-sided upper confidence limits for the true quarterly means (90%, 95%, and 99% respectively). The confidence limits depart most from the central estimates in April−December 1994 because the effective sample volumes were lowest then (Figure 2). For example, compare the confidence limits for the fourth quarter in 1994 with those in 1995.

The limits obtained in this way are interesting in several ways. First, confidence limits can be obtained for the concentration estimated from a single sample as well as for mean concentrations based on multiple samples. Confidence limits can be calculated for samples or groups of samples even if no oocysts whatever are detected. This surprising result is possible because the upper 90% confidence limit for a Poisson-distributed variable is that value of the Poisson mean for which the probability of observing a count equal to or less than that actually seen (e.g., zero here) is 10% (*18*, *19*). Suppose two different sets of samples lead to the same total count of three oocysts. The estimated mean concentrations and the associated confidence limits will differ between the two data sets if their total effective volumes differ.

Figure 4 shows the upper, one-sided confidence limits for the quarterly mean oocyst concentrations at the Catskill Aqueduct. Note that the confidence limits depart most from the estimated means in quarters when the total effective sample volumes were relatively small (e.g., the second through fourth quarters of 1994; see Figure 4). Figure VI-1 (Appendix VI) shows an analysis of the same data when they are aggregated as running averages over 1-year periods. Because each mean is there based on more weekly samples (≈50 instead of ≈12), the estimates should be more reliable. That is shown by the confidence limits being closer to the estimated means than they were with the data aggregated by quarter.

With these confidence limits, we now have a way to ensure to a known extent against underestimating mean concentrations. Specifically, we believe that if one wishes to "err on the high side" in estimating concentrations to protect public health, then it makes more sense to do this by using upper confidence limits than by arbitrarily treating all ND samples as if one oocyst had been seen. As shown in Figure 1, the overestimation caused by this no-zeros procedure varies dramatically between low and high concentrations, with overestimation by several orders of magnitude occurring at low concentrations. As shown in Figures 4 and VI-1, that tendency is much reduced when confidence limits are used in place of no-zeros estimates. That is, the confidence limits are higher than the estimated averages by more or less fixed amounts (although there is some variation with estimated volumes). A further advantage of confidence limits is that one can choose a desired level of confidence for a given application; for example, lower level confidence limits might be appropriate for untreated source waters, but a higher level for finished water in a distribution system. In contrast, the no-zeros method provides an arbitrary and variable amount of overestimation that is hard to interpret.

Potential Effects of Oocyst Clustering. The confidence limits shown in Figures 4 and VI-1 are based on the assumption that oocysts move independently in the bulk water. As noted earlier, if the propagules instead moved on independent clusters, then method 1 would continue to estimate correct means on average but upper confidence limits would be too low. Appendix VII describes a simulation in which oocysts were assumed to move in clumps of 1, 2, 5, and 10.

Although clustering should not and did not affect the means obtained, it did lead to upper confidence limits that were too low (Table VII-1). For example, when all oocysts were assumed to move in clumps of 10 at a bulk water concentration of one oocyst/hL, the true mean concentration exceeded the estimated upper 95% confidence limit in about 15% of the simulated years rather than in 5% of the years as should have occurred. This scenario is probably extreme, but it indicates a possible limitation to our proposed method for estimating confidence limits for waters in which clustering were common. Appendix VII closes with a recommendation for research into the degree to which pathogen propagules may travel in clumps.

Corrections for Incomplete Oocyst Recovery. As noted in the Introduction, the analyses so far have proceeded on the assumption that all oocysts in the water filtered would be retained on the filter and that none would be lost inadvertently during laboratory analysis. However, the methods used to obtain data like those discussed here are imperfect, and publications "have usually reported recovery efficiencies of less than 50% for both cysts and oocysts" (*4*). In some instances, recovery efficiency may be as low as 5% (*3*). One aim of current research is to improve this efficiency (*2*). If estimated oocyst concentrations are not to be biased low, we need a way to correct for incomplete recovery. If an estimate ρ of the overall recovery efficiency is available, then our method can accomplish this correction with the use of $\phi = f\rho$ in place of f in the foregoing analyses. Unfortunately

ρ cannot be known from routine sampling, but it can be and has been estimated in research studies (*3*, *4*). Clearly, good estimates of recovery efficiencies under various conditions are worth having, if we wish to have unbiased estimates of oocyst concentrations present in different waters. Nahrstedt and Gimbel (*13*) have analyzed recovery losses in more detail and have allowed for a distribution of recoveries in place of a single estimate.

Discussion

Careful consideration of the statistical sampling properties of the methods usually used to measure cyst and oocyst concentrations in water leads to several practical recommendations. In the Catskill Aqueduct samples considered here, oocysts were seldom detected. However, the statistical methods derived in this paper (especially dividing total counts of oocysts actually observed by total effective water volume sampled) can be used to obtain both relatively unbiased estimates of mean concentrations and confidence limits for those means. These estimates are more logical than averages calculated by acting as if one oocyst had been seen in all samples in which none were actually detected (the no-zeros method) or by two other methods considered. The upward bias caused by these other procedures might seem protective of public health, but that bias, and hence the protection it might afford, disappears at high concentrations where having a safety factor seems most desirable. The confidence limits associated with the method 1 estimated means maintain their protective buffer even at high concentrations.

Through most of the paper, we assumed that no oocyst losses occurred in the filtering, sample preparation, or counting stages, except for the oocysts in the known fraction of the filtered solids that is not inspected microscopically. Incomplete recovery appears to be common, however. As long as the net recovery rate can be estimated, then estimated mean concentrations and confidence limits for those means can be adjusted in an unbiased way if the estimates are divided by that net recovery rate.

Although we have illustrated our methods with data from a water supply with low oocyst concentrations, nothing limits their use to low concentrations. It would be easier to test for clustering in waters with higher concentrations, and we recommend that that be done. Finally, we note that the analytical results described here apply equally well to concentrations of any discrete particulate matter. Anyone performing mass-balance studies with such particles may wish to use the estimation techniques and interpretative suggestions provided in this paper.

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Supporting Information Available

Poisson and negative binomial distributions, proof that a binomially distributed subsample take from a Poisson-distributed sample also has a Poisson distribution, weighting by effective volumes as an approximation to inverse-variance weighting, proof that nondetects must be treated as zeros to yield unbiased means, calculating confidence limits for effective volume-weighted means oocyst concentrations, Catskill aqueduct data plotted as running annual means, potential effects of oocyst clustering, and references (12 pp). Ordering information is given on any current masthead page.

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