Epidemiology, Microbiology, and Risk Assessment of Waterborne Pathogens Including Cryptosporidium

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ABSTRACT

Cryptosporidium parvum has been an emerging cause of illness in food and water for the last 15 years (19, 20, 27, 29, 34). In drinking water, the 1993 outbreak of cryptosporidiosis in Milwaukee (20) raised the level of concern for this organism. Because of this intense interest, the risk assessment for exposure to C. parvum presents an interesting case study in the application of quantitative risk assessment methods. This paper will outline the application and validation of such methods.

The quantitative microbiological risk assessment approach follows the framework proposed for chemical risk assessment (23), and broadly includes the following steps: hazard assessment, exposure assessment, dose–response analysis, risk characterization and risk management. An elaboration of this method, specifically for microbial risk assessment, has been developed by a committee of the International Life Sciences Institute (ILSI) (18). In this paper, the basic National Academy of Sciences steps will be followed.

For microorganisms, hazard assessment (i.e., identification of a pathogen as an agent of potential significance) is generally a straightforward task, except for opportunistic agents. The major tasks of quantitative microbiological risk assessment are focused on exposure assessment, dose–response analysis, and risk characterization. The task of risk management is one of deciding the necessity of any action based on the risk characterization outputs and incorporates significant policy and trans-scientific concerns. However, a risk analyst needs to be aware of the levels of significance that might require an action to be taken.

One outcome of the hazard analysis is a decision as to the principal consequence(s) to be quantified in the formal risk assessment. With microorganisms, consequences may include infection (without apparent illness), morbidity, or mortality; furthermore, these events may occur in the general population, or at higher frequency in susceptible subpopulations. Although mortality from infectious agents, even in the general population, cannot be regarded as de minimus (16), the general tendency (in water microbiology) has been to regard infection in the general population as the particular hazard that should receive protection. This has been justified based on a balancing between the degree of conservatism inherent in using infection as an endpoint and the (current) inability to quantify the risks to more susceptible subpopulations (21).

The purpose of an exposure assessment is to determine the microbial doses typically consumed by the direct user of a water (or food). In the case of water microbiology, this may necessitate the estimation of raw water microorganism levels followed by estimation of the likely changes in microbial concentrations with treatment, storage, and distribution to the end-user (26, 31). In the case of shellfish water quality, additional information on the bioaccumulation of pathogens and possible loss due to depuration and other processes is necessary. In the case of wastewater discharges to receiving water, the effect of dilution and die-off between the point of discharge and the point of exposure must be considered.

A second issue arising in exposure assessment is the amount of ingested material per exposure. As a default number, 2 liters/person-day is used to estimate drinking water exposure (21), although this may be conservative (32). For contact recreational exposure, 100 ml/day has often

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been assumed as an exposure measure (10); however, actual data to validate this number are lacking. For shellfish exposure, market surveys may be used (33), although it is believed that there may be subpopulations with substantially higher than typical consumption levels.

In quantitative microbiological risk assessment, for many microorganisms, human dose–response studies are available that can be used to estimate the effects of low level exposure to microorganisms. In prior work, it has been found that these studies may be adequately described by one of two semimechanistic models of the infection process. In the exponential model that may be derived from the assumption of random occurrence of microorganisms along with a constant probability of initiation of infection by a single organism (r), the probability of infection (P_I) is given as a function of the ingested dose (d) by:

\[ P_I = 1 - \exp(-rd) \]  

(1)

For many microorganisms, the dose–response relationship is shallower than reflected by equation 1, suggesting some degree of heterogeneity in the microorganism–host interaction. This can be successfully described by the beta-Poisson model that can be developed from equation 1 if the infection probability is itself distributed according to a beta distribution (7, 8, 11). This model is described by two parameters, a median infectious dose (N_{50}) and a slope parameter (\alpha):

\[ P_I = 1 - \left[ 1 + \frac{d}{N_{50}}(2^{1/\alpha} - 1) \right]^\alpha \]  

(2)

Figure 1 depicts the effect of the slope parameter on the dose–response relationship; in the limit of \( \alpha \rightarrow \infty \), equation 2 approaches equation 1.

Given a set of dose–response data, i.e., exposure of populations to various doses of microorganisms and measurement of response (such as infection), the best fitting parameters of a dose–response relationship may be computed via standard maximum likelihood techniques. The method has been illustrated for human rotavirus (16, 26) and protozoans (31). Confidence limits to the parameters can then be found and used as a basis for low dose extrapolation.

The process of risk characterization combines the information on exposure and dose–response into an overall estimation of likelihood of an adverse consequence. This may be done in two basic ways. First, a single point estimate of exposure (i.e., number of organisms ingested) can be combined with a single point estimate of the dose–response parameters to compute a point estimate of risk. This may be done using a best estimate, designed to obtain a measure of central tendency, or using an extreme estimate, designed to obtain a measure of consequence in some more adversely affected circumstance. An alternative approach, which is receiving increasing favor, is to characterize the full distribution of exposure and dose–response relationships and to combine these using various tools (for example, Monte Carlo analysis) into a distribution of risk. This approach conveys important information on the relative imprecision of the risk estimate, as well as measures of central tendency and extreme value (2, 6).

One important outcome of the risk characterization process using a Monte Carlo approach is the assessment of the relative contribution of uncertainty and variability to a risk estimate. Variability may be defined as the intrinsic heterogeneity that leads to differential risk among sectors of the exposed group, perhaps resulting from differential sensitivities or differential exposures. Uncertainty may be defined as the factors of imprecision and inaccuracy that limit the ability to quantify risk exactly. Uncertainty may be reduced by additional resources, for example devoted to characterization of the dose–response relationship. Variability represents a lower limit to the overall risk distribution.

The results of a risk characterization are used in risk management. The understanding of appropriate action levels for decision making with respect to microorganisms is still at an early stage. However, in the case of waterborne protozoa, it has been suggested than an annual risk of infection of 0.0001 (i.e., 1 in 10,000) is appropriate for drinking water (21). However, it has been argued that this level may be overly stringent (12).

DOSE–RESPONSE FOR C. PARVUM

Dupont et al. (5) performed controlled human feeding trials on the risk of infection from ingestion of oocysts of C. parvum. Data, along with the best fit and 95% confidence limits to the exponential dose–response model are shown in Figure 2. The exponential dose–response model was fit to these data using the method of maximum likelihood; the beta-Poisson did not provide a statistically significant improvement in fit (13). From this analysis, the best estimate for the parameter \( r \) in equation 1 is 0.0042.

EXPOSURE ISSUES FOR C. PARVUM

Estimation of exposure to waterborne pathogens entails the estimation of two components. First, it is necessary to estimate the amount of tap water that might be ingested directly (or with minimal processing, such as boiling to make hot beverages). The tap water consumption distribution has been evaluated for the U.S. population as a whole and stratified by age and gender by Roseberry and Bur-
Master (32). In ordinary (point estimate) risk assessment in the United States, frequently 2 liters/capita per day is taken as a population average (4).

The second necessary input to exposure estimation is the concentration of infectious organisms in the water to be ingested. In the case of C. parvum, the assessment of this quantity is particularly problematic. Available methods do not differentiate between live, infectious organisms and noninfectious organisms, nor are they strictly specific for human strains of parasites. In addition, the low and variable recovery of oocysts from water samples (occasionally under 10% of spiked oocysts are recovered) makes the interpretation of count data quite difficult (3, 35).

**RISK CHARACTERIZATION AND VALIDATION**

The largest known waterborne outbreak of disease occurred in March to April 1993, resulting from an apparent breach of treatment in one of the Milwaukee, Wisconsin water treatment plants, resulting in a widely disseminated exposure to Cryptosporidium in finished water. This event is believed responsible for over 400,000 cases of illness (20). Using the information presented in the outbreak investigation, in conjunction with the dose–response curve, we examine whether the occurrence of the Milwaukee outbreak was consistent with the infectivity as noted in controlled laboratory investigations.

Based on the investigation of the Milwaukee outbreak, the following information can be gleaned (20):

1. Based on the distribution of onset cases, the most likely duration of contamination ($t$) appears to have been about 21 days, with a possible range of 15 to 30 days. A triangular distribution is used to model this uncertainty.
2. The attack rate ($r$) (based on the entire metropolitan area) determined from an epidemiological survey was 0.21. Based on the sample size employed, the attack rate distribution was described as normal with a standard deviation of 0.01.

To complete the analysis, information is needed on the water ingestion rates of the exposed population. In the absence of site-specific information, the distribution of Roseberry and Burmaster (32) is used, in which the daily water ingestion rate ($q$) (in ml) is log-normally distributed with a mean of 1,948 ml and a standard deviation of 827 ml. One further detail must be assumed to complete the calculation. As with the assumptions used earlier, it is assumed that each day of exposure constituted an individual and identical risk.

From these assumptions, we wish to determine what the average oocyst concentration would be during the exposed period (assuming level exposure) consistent with the attack rate, duration of exposure, and dose–response information.

Given a total risk during the outbreak ($x$) of 0.21, from equation, and assuming that each day’s exposure results in an independent and identical risk, then from equation 3, the average daily risk ($p$) (based on 21 days of exposure) can be determined to be $p = 0.0112$.

$$p = 1 - (1 - x)^{1/21} = 1 - (1 - 0.21)^{1/21}$$

Now substituting this daily risk into the exponential dose–response model, and using the best fit value of the exponential dose–response parameter ($r$) = 0.0042, the daily dose can be estimated to be:

$$d = -\frac{\ln(1 - p)}{r} = -\frac{\ln(1 - 0.0112)}{0.0042} = 2.7$$

From the mean daily water consumption of 1,948 ml, the estimated mean concentration during the outbreak is determined to be 2.7/1,948, or 1.4 oocysts/liter.

During the course of the outbreak, a number of samples were taken for protozoan analysis. In eight samples of finished and distribution system water, four positive samples were obtained with a geometric mean among the positive samples of 0.025/liter; however, these samples were taken during the latter stages of the outbreak (28). Samples of ice manufactured during the outbreak were obtained and analyzed as well. Based on two sets of duplicate samples analyzed by membrane filter concentration, a geometric mean of 0.079/liter was obtained (28)—however, it is believed that as much as a 90% loss could have occurred in oocyst concentration from freezing and thawing (30). Applying this correction, the geometric mean oocyst concentration could have been 0.79/liter. With this correction, then it would appear that the level of Cryptosporidium intrusion into the distribution system necessary to cause the observed outbreak in Milwaukee—assuming the validity of the dose–response relationship—is consistent with the observed levels found by measurement. When the errors involved in estimating the attack rate and duration are taken into account, it can be shown that the apparent risk experienced brackets the expected risk from the dose–response relationship (14).

**CAVEATS**

The application of quantitative risk assessment to the problem of waterborne cryptosporidiosis appears promising based on the above analysis of the Milwaukee outbreak and also (not shown) based on comparison to the outbreak in...
Bradford (UK) (1). However, there are several aspects that must temper the optimism (in addition to the analytical issues already discussed above).

First, there is some recent evidence that an initial exposure to *C. parvum* may result in some decrease in sensitivity to subsequent exposure (24). Further work needs to be done to assess the quantitative extent and duration of this apparent decrease in sensitivity.

Second, although the oocyst levels observed during a number of outbreaks are greater than those noted during apparent nonoutbreak situations (15), it should be noted that outbreaks have occurred in the absence of detectable oocysts in water. Most notable is the Las Vegas outbreak that was associated with mortality in the immunocompromised population, with no apparent effects in the general population, with no apparent effects in the general population despite the occurrence of elevated oocyst levels in outbreaks that have previously been reported (15). In July through September of 1998, there was a prolonged boil water order in Sydney Australia, triggered by the finding of apparent *Cryptosporidium* (and *Giardia*) in the raw and finished water. Although detailed scientific investigations have yet to appear, preliminary reports have suggested that this prolonged contamination incident may have occurred without discernable increases in illness in the general population (22). It must be noted, however, that consideration of the numerous individual and institutional barriers suggests that only a very small fraction of individuals in the general population with cryptosporidiosis will present themselves for medical attention and be adequately diagnosed and counted in the public health registers (25).

Hence, while there appears to be a correspondence between results from feeding studies on large-scale population impacts, in some circumstances—due to imperfections in analytical methodology and possible population variations in sensitivity—both false-negative and false-positive risk estimates may occur.

Finally, in both the Las Vegas situation, and in Milwaukee, substantial excess (or early) mortality occurred in the immunocompromised (and in Milwaukee, the elderly) populations (9, 17, 20). Therefore, protection of the general population does not necessarily safeguard the more sensitive subpopulations. This raises an important question of public health policy, i.e., to what degree should a common utility system such as water supply be expected to be protective of the more sensitive segments of the community?

**CONCLUSIONS**

In conclusion, the methods of quantitative microbiological risk assessment can be used to evaluate the consequences of exposure to waterborne *Cryptosporidium*. However there are a number of issues that are in need of more careful resolution before the issues of false-positive and false-negative findings can be adequately resolved.

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**REFERENCES**


