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PII: S0043-1354(01)00119-1

*Wat. Res.* Vol. 35, No. 16, pp. 3934–3940, 2001

Printed in Great Britain
0043-1354/01/$ - see front matter

# RISK ASSESSMENT FOR *CRYPTOSPORIDIUM*: A HIERARCHICAL BAYESIAN ANALYSIS OF HUMAN DOSE RESPONSE DATA☆

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*(First received 1 May 2000; accepted in revised form 1 November 2000)*

**Abstract**—Three dose–response studies were conducted with healthy volunteers using different *Cryptosporidium parvum* isolates (IOWA, TAMU, and UCP). The study data were previously analyzed for median infectious dose ($ID_{50}$) using a simple cumulative percent endpoint method (Reed and Muench, 1938). $ID_{50}$s were derived using two definitions of infection: one as subjects having oocysts detected in stool by direct fluorescence assay, and the other by a clinical finding of diarrhea with or without detected oocysts (Chappell *et al.*, 1998; Okhuysen *et al.*, 1999). In the present study, the data were analyzed using the broader definition of infection (i.e., presence of oocysts in stool and/or diarrheal illness characteristic of cryptosporidiosis). Maximum likelihood dose–response parameter estimates for UCP, IOWA, and TAMU were 2980, 190, and 17.5, respectively. Based on these estimates, the $ID_{50}$s of the three respective isolates were 2066, 132, and 12.1. The three oocyst isolates were considered representative of a larger population of human-infecting strains and analyzed as combined data using a hierarchical Bayesian model. Hyperparameters defined the distribution of dose–response parameters for the population of strains. Output from Markov Chain Monte Carlo analysis described posterior distributions for the hyperparameters and for the parameters of the IOWA, TAMU, and UCP strains. Point estimates of dose–response parameters produced by this analysis were similar to the maximum likelihood estimates. Finally, the utility of these results for probabilistic risk assessment was evaluated. The risk of infection from single oocyst doses was derived for a mixture of the three isolates (where IOWA, TAMU, or UCP are equally likely), and for an oocyst selected at random from the larger population of strains. These estimated risks of infection were 0.018 and 0.028, respectively. 

*Key words*—*Cryptosporidium parvum*, dose–response, volunteer studies, Bayesian statistics, meta-analysis

## NOMENCLATURE

| | |
|---|---|
| $i$ | group of human subjects |
| $N_i$ | number of subjects in the $i$th group |
| $D_i$ | dose for the $i$th group (number of oocysts ingested by each subject in the group) |
| $X_i$ | number of infected subjects in the $i$th group |
| $k$ | dose response parameter (exponential model) |
| P( ) | probability of infection |
| $L$( ) | likelihood function |

*Greek letters*

| | |
|---|---|
| $\mu$ | mean (a parameter of the distribution of $\ln(k)$) |
| $\sigma$ | standard deviation (dispersion parameter for the distribution of $\ln(k)$) |

## INTRODUCTION

*Cryptosporidium parvum* (*C. parvum*) is a waterborne microbial pathogen that is known to infect immunocompetent and immunocompromised humans. Diarrhea and abdominal cramping characterize cryptosporidiosis, the illness caused by this protozoan. Nausea, low-grade fever, malaise and occasional vomiting may also occur. Most *Cryptosporidium* oocysts are removed from water by conventional filtration, but because they are resistant to conventional drinking water disinfectants, those that are not removed are of major concern to regulatory agencies, the water industry, and consumers.

Currently, the best information on the infectivity of *C. parvum* comes from three dose–response studies conducted at the University of Texas-Houston Health Science Center (DuPont *et al.*, 1995; Chappell *et al.*, 1999; Okhuysen *et al.*, 1999). In each study, healthy volunteers ingested a single known dose of viable *C. parvum* oocysts. Each study involved a

☆The views expressed in this paper do not necessarily represent the views of the US Environmental Protection Agency or the United States.

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different isolate of *C. parvum*: the IOWA, TAMU, and UCP isolates. The three *C. parvum* isolates used in the volunteer studies were examined for their genetic polymorphism. A multilocus analysis revealed that all three were genotype 2 organisms, which can infect both human and other mammalian species. Two of the isolates (Iowa and UCP) were originally collected from naturally infected calves during a diarrheal episode. Both of these isolates have been passaged in the laboratory setting for a number of years. The TAMU isolate was collected from a veterinary student who became infected while participating in a necropsy on an infected foal. This isolate had been passaged twice prior to the volunteer studies. During the experiment, 5–7 passages of the oocysts were carried out in calves to provide fresh oocysts for volunteer challenges. Three to 6 volunteers were studied per challenge until full dose response curves were constructed. Thus, dose response studies for an individual isolate required 11–14 months to complete. To monitor for any changes in the isolates with calf passage, full dose response curves were done in neonatal mice concurrently with each volunteer challenge. Although the human dose response studies used oocysts from several calf passages, no significant differences (or trends) in mouse infectivity was noted with any of the isolates.

The distribution of subjects by study (*C. parvum* strain) and dose is shown in Table 1, together with the numbers presumed infected.

Previous studies (Chappell *et al*., 1999; Okhuysen *et al*., 1999) utilized a simple cumulative percent endpoint estimation method (Reed and Muench, 1938). In the present and previous studies (Chappell *et al*., 1999; Okhuysen *et al*., 1999) two definitions of infection were considered, namely "confirmed infection" and "presumed infection". Fecal oocysts were detected by direct fluorescence assay (DFA), a technique with a detection limit of about 10,000 oocysts per mL (Weber *et al*., 1991). Persons who were positive for oocysts by DFA were considered to have confirmed infections regardless of their clinical outcome (i.e. the development of symptoms). Further, not all persons shedding detectable oocysts developed a diarrheal illness.

DFA positivity confirmed the replication of the organism if oocysts were found in the feces at any time after 36 h post inoculation. Oocysts found earlier were thought to represent inoculum "flow through" and may not have been indicative of infection. In contrast, some oocyst-negative volunteers were noted to have unformed stools and gastrointestinal symptoms that were indistinguishable in onset, duration and character from ill volunteers who were concurrently shedding oocysts. Due to the DFA detection limit, light infections or a disruption in the parasite life cycle would not have produced enough oocysts to be recognized. To explore this possibility, a more sensitive, experimental technique (flow cytometry) was used to test selected samples from these persons. A high percentage of these samples yielded low numbers of oocysts. Thus, it appeared that these individuals were also infected, but were shedding oocysts in numbers that were below the DFA detection limit. The individuals who had significant clinical manifestations, but who were negative for oocysts by DFA were said to have "presumed" infections. These observations suggest that if DFA positivity alone is accepted as the standard of infection, the number of cases is likely to be underestimated. Thus, these two definitions have been used to capture the full extent of infection in challenged volunteers.

Table 1. *Cryptosporidium parvum* infectivity in healthy, adult volunteers. Each column lists the number of volunteers belonging to each category

| Dose | Subjects | Infected, but no oocysts detected | Presumed infected |
|---|---|---|---|
| IOWA isolate: | | | |
| 30 | 5 | 1 | 2 |
| 100 | 8 | 1 | 4 |
| 300 | 3 | 0 | 2 |
| 500 | 6 | 0 | 5 |
| 1000 | 2 | 0 | 2 |
| 10,000 | 3 | 0 | 3 |
| 100,000 | 1 | 0 | 1 |
| 1000,000 | 1 | 0 | 1 |
| TAMU isolate: | | | |
| 10 | 3 | 1 | 2 |
| 30 | 3 | 1 | 2 |
| 100 | 3 | 2 | 3 |
| 500 | 5 | 2 | 5 |
| UCP isolate: | | | |
| 500 | 5 | 2 | 3 |
| 1000 | 3 | 1 | 2 |
| 5000 | 5 | 1 | 2 |
| 10,000 | 4 | 0 | 4 |

## MAXIMUM LIKELIHOOD ESTIMATES FOR INDIVIDUAL EXPONENTIAL DOSE–RESPONSE EQUATIONS

Studies were analyzed individually, assuming exponential dose–response relationships. Under the exponential model, there is no minimum infectious dose, as a nonzero risk is predicted with any nonzero dose. Studies with animal and tissue models have shown single oocysts do cause infections, but there is, at present, no collaborating evidence in human studies. As a one-parameter model, the exponential is the simplest that does well at fitting the study data. More complex models, such as the Beta-Poisson, exhibit different behavior at low doses, some predicting much greater risk than that predicted by the exponential. We invoke Occam's razor and proceed with the exponential. The probability of infection depends on the dose, $D$, and the unknown dose–response parameter, $k$ (Haas *et al*., 1999):

$$\text{Probability of infection} = P(D,k) = 1 - \mathrm{e}^{-D/k}.$$

The likelihood of observing $X$ infections out of $N$ subjects exposed to dose $D$ is a binomial probability function that depends on the dose–response parameter:

$$L(X|N,D,k) = N!P(D,k)^X(1-P(D,k))^{N-X} /(X!(N-X)!).$$

For a single study involving $n$ different dose levels, the likelihood function can be evaluated for the entire data set. Expressed in terms of the unknown dose–response parameter, the likelihood function $L(k)$ is:

$$L(k|X,N,D) = \Pi L(X_i,|N_i,D_i,k).$$

For example, the likelihood function from the UCP study was the following product:

$$L(3|5,500,k) \times L(2|3,1000,k) \times L(2|5,5000,k) \times L(4|4,10000,k).$$

Figure 1 shows the likelihood function for the UCP study. This function attained its maximum value when $k$ was 2980, the maximum likelihood parameter estimate (MLE) for the UCP study.

Maximum likelihood estimates for the IOWA and TAMU studies were 190 and 17.5, respectively. If these values are accepted as accurate estimates for the three studies, then the curves in Fig. 2 would communicate how the probability of infection is related to the number of oocysts ingested. Clearly, these maximum likelihood functions predicted vastly different probabilities of infection among the isolates in the displayed range.

Closely related to the dose–response parameter was the median infectious dose, or $ID_{50}$. The $ID_{50}$ is a dose that is expected to cause infection in half of the susceptible persons who would ingest that number of oocysts. Based on the MLEs, the $ID_{50}$s for UCP, IOWA, and TAMU strains were 2066, 132, and 12.1, respectively.

## BAYESIAN ESTIMATION OF INDIVIDUAL EXPONENTIAL DOSE–RESPONSE PARAMETERS

The three studies were conducted over different ranges of doses. The ratio of maximum to minimum dose was 4.5 logs ($10^6/30 = 33{,}333 = 10^{4.5}$) for the IOWA study, 1.7 logs for the TAMU study, and 1.3 logs for the UCP study. The IOWA study was designed with a wide range of doses to ensure that the median infectious dose was included. Although the other two studies were planned under the same degree of prior uncertainty, their adaptive design allowed for narrower search ranges. The adaptive design was carried out in the following manner: one group of subjects was dosed at a moderate level, and the next group's dose level depended on the outcome for the previous group. If the first group produced no infections, then the second group would be dosed at a higher level. If the first group produced all-infections, then the second group would be dosed at a lower level. Although the three studies began with the same relative uncertainty regarding dose–response parameter, the adaptive designs succeeded in bracketing the median infectious doses with smaller numbers of subjects exposed over smaller dose ranges.

The present analysis utilized the same noninformative prior density for each study. The simplest prior is defined for the natural log of $k$ as a uniform density; $\pi(\ln(k)) = \text{constant}$. The posterior density function for $\ln(k)$ is simply proportional to the likelihood.

Figure 3 shows the posterior density functions for the three strains' dose–response parameters. While

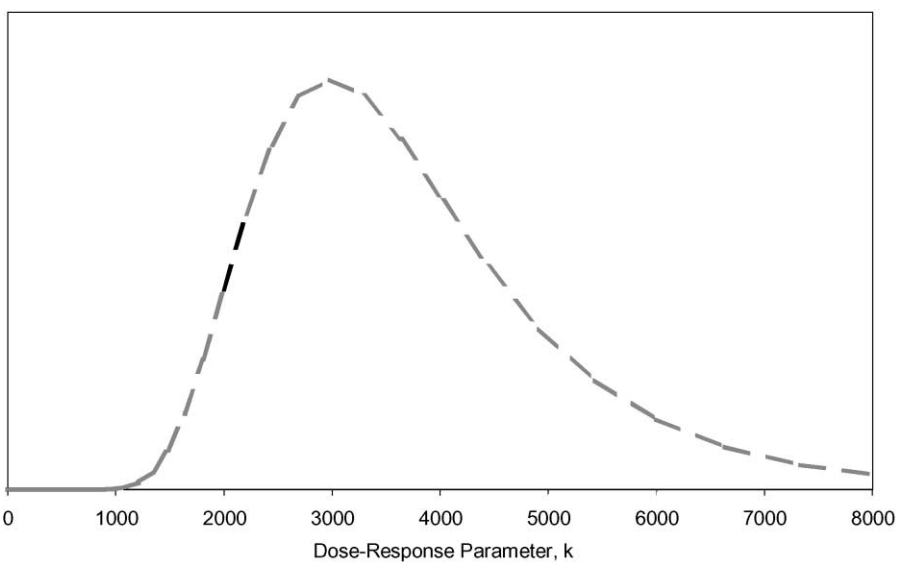


Fig. 1. Likelihood function for the UCP study.

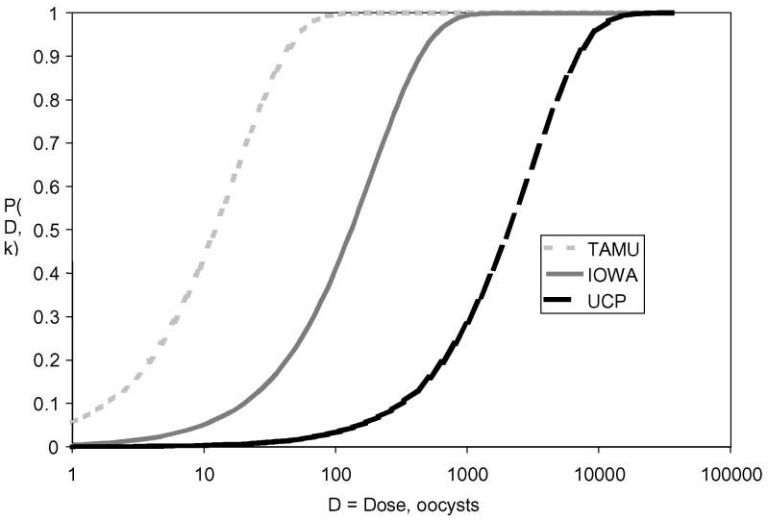


Fig. 2. Maximum likelihood dose–response curves.

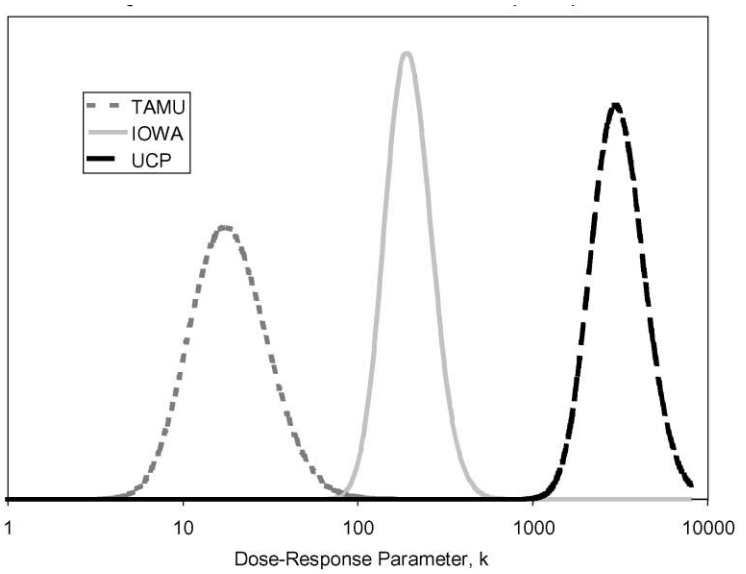


Fig. 3. Posterior densities for three dose–response parameters.

Fig. 2 shows the dose–response functions corresponding to maximum likelihood values of $k$, Fig. 3 shows that those estimates were uncertain. Notice that the bell-shaped curve for IOWA is the sharpest, having the narrowest base, while that for TAMU has the widest base. This is principally due to the different numbers of study volunteers. With the largest number of subjects, the IOWA study produced the most precise dose–response estimate.

Integrating over these posteriors for dose–response parameters, the expected $ID_{50}$s for UCP, IOWA, and TAMU are 2290 [80% credible interval (1400,3400)], 141 [80% credible interval (92,200)], and 14.8 [80% credible interval (6.8,25)], respectively.

In contrast, the confirmed infections (counting only those cases where oocysts were detected in the stool) lead to the posteriors of Fig. 4. The greatest shift compared to Fig. 3 is that of TAMU, due to stool-negative results for only half of the subjects exhibiting symptoms of cryptosporidiosis. The smallest shift is that of IOWA, due to only two stool-negative subjects who developed symptoms. Believing these to be false negatives, we proceed to assess the results using the definition of presumed infection.

## META-ANALYSIS OF THE THREE STUDIES

Meta-analysis utilizes the results from independent studies (such as the IOWA, TAMU, and UCP) in combination to produce improved estimates of the parameter or set of parameters being studied. Here,

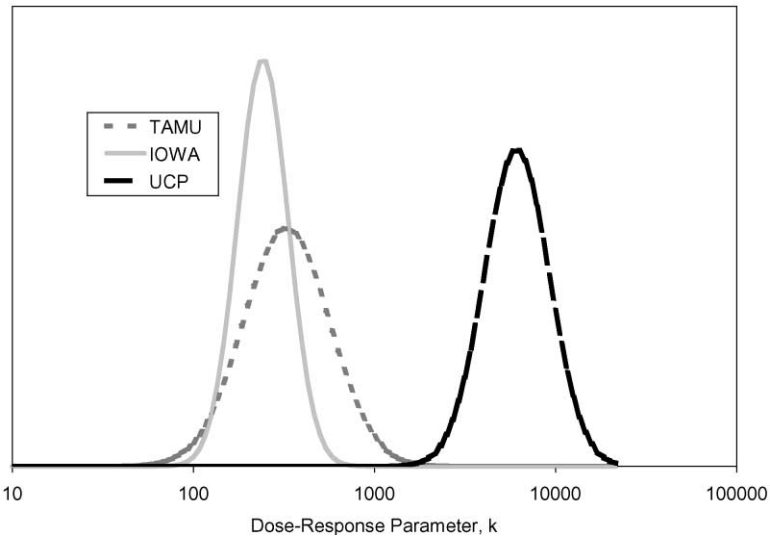


Fig. 4. Posteriors under old definition of infection (oocysts detected in stool).

we employ meta-analysis to characterize the population of *Cryptosporidium* strains from which the three isolates were selected. We have already learned that the three strains have significantly different dose–response parameters. However, these were only three isolates belonging to an unknown, larger population of *C. parvum* strains that cause infection in humans. There is no information to determine the extent to which these three strains represent the population of strains. For the purpose of this meta-analysis, we assumed that:

- the three are a random sample from the larger population of strains,
- the exponential dose–response relationship holds for all strains (but with different parameters), and
- the distribution of all of these different dose–response parameters is lognormal, so that their natural logs are normally distributed with some unknown mean ($\mu$) and standard deviation ($\sigma$).

Called "hyperparameters," $\mu$ and $\sigma$ describe the distribution of dose–response parameters ($k$'s) for the population of infectious *C. parvum* strains. The first parameter, $\mu$, could be interpreted as the natural log of the median dose–response parameter. Fully half the infectious *C. parvum* strains would have dose–response parameters less than $e^{\mu}$ and half would have parameters greater than $e^{\mu}$. It seems reasonable that $e^{\mu}$ is somewhere in the range of parameters estimated for the three individual studies, say [20, 2000]. $\text{Ln}(200) = 5.3$ would therefore appear to be a very reasonable value for this hyperparameter.

The second hyperparameter, $\sigma$, describes the strain-to-strain variability of infectivity. Because TAMU and UCP's maximum likelihood estimates differed from that of IOWA by roughly "give or take a factor of ten", it seems reasonable that the geometric standard deviation could be about ten, and the variance parameter $\sigma$, therefore, is in the neighborhood of $\ln(10) = 2.3$.

Our "ballpark" estimates of the hyperparameters are 5.3 and 2.3. For more scientific estimates, we used the likelihood functions defined above in a Markov Chain Monte Carlo algorithm (MCMC) to generate a large number of "samples" from the joint posterior distribution for the hyperparameters and three dose–response parameters (Spiegelhalter *et al.*, 1999; Gilks *et al.*, 1996). The routine also generates samples from the posterior distributions for the TAMU, UCP, and IOWA strains' dose–response parameters.

The MCMC routine was run using the WinBUGS program, version 1.2 (Spiegelhalter *et al.*, 1999). Posterior information was gathered and summarized for the hyperparameters ($\mu$ and $\sigma$) and for the three dose–response parameters (TAMU, UCP, and IOWA). Summary statistics are given in Table 2.

Note that the tabled means for $\mu$ and $\sigma$ compare very well with our "ballpark" estimates. Posterior densities for IOWA, TAMU, and UCP were very nearly identical to those of Fig. 3, above. This shows that each isolate's estimate was only weakly influenced by including the other isolates' data in the hierarchical model. A greater number of isolate studies would have produced more precise estimates of the hyperparameters and those, in turn, could have had stronger influence on the individual parameter estimates. Figure 5 displays the corresponding

Table 2. Summary statistics for MCMC outputs

| Parameter | Mean | SD | 10th Percentile | Median | 90th Percentile |
|---|---|---|---|---|---|
| $\mu$ | 5.5 | 1.1 | 4.1 | 5.5 | 6.8 |
| $\sigma$ | 2.1 | 1.0 | 1.2 | 1.8 | 3.3 |
| IOWA | 205 | 66 | 133 | 194 | 292 |
| TAMU | 27 | 16 | 12 | 23 | 48 |
| UCP | 2950 | 1070 | 1800 | 2730 | 4320 |

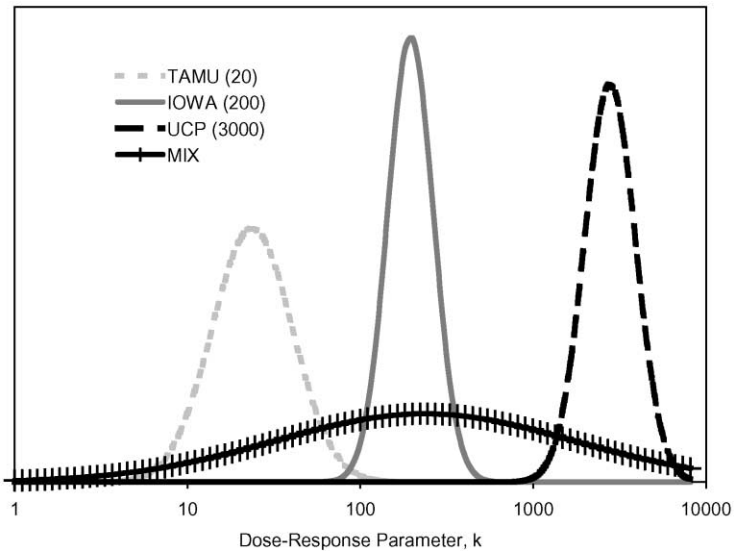


Fig. 5. Posterior densities from the meta-analysis.

probability density functions plus the marginal density function for the dose–response parameter, $k$. This function (labeled MIX) was derived from the joint posterior density for the hyperparameters $\mu$ and $\sigma$.

## USE IN PROBABILISTIC RISK ASSESSMENT

These data suggest a number of approaches to conduct probabilistic risk assessment. Given information on occurrence of viable oocysts, but no information on strain, any of the following assumptions could be made about the oocysts:

- the oocysts are all of the IOWA strain,
- the oocysts are all of the TAMU strain,
- the oocysts are all of the UCP strain,
- the oocysts are a mixture of IOWA, TAMU, and UCP strains, or
- the oocysts are a mixture of all strains whose dose–response parameters are distributed as the marginal posterior for the dose-response parameter.

At present, there is no way to tell which of the above assumptions will give rise to the best risk estimate. Nothing is known about the population of infectious strains in the environment. The three isolates that were studied are all classified as Genotype 2 organisms, but Genotype 1 (human) organisms may pose a different level of risk to humans. A prudent approach is to predict risks under each of the five assumptions.

Of course, assumptions must also be made about secondary spread, immunity, and the dose–response relationships for persons having an immune response. The risk from ingestion of one oocyst is important because most exposures will be at low daily levels. Because none of the study subjects was dosed at the one-oocyst level, we must extrapolate using the exponential dose–response equation.

Based on the ''individual study analysis,'' the expected risk of infection for a dose of one oocyst, selected at random from the three isolates, was 0.018 (Table 3). In comparison, based on the meta-analysis, the risk of infection for a dose of one oocyst (from the population of all strains, not limited to the three that were studied) was 0.028.

Table 3. Risk of infection, given one oocyst per volume ingested

| Assumption | Risk (Infection\|Dose = One Oocyst) Mean [80% Credible Interval] |
|---|---|
| IOWA Only (single analysis) | 0.0053 [0.0035, 0.0076] |
| IOWA Only (meta-analysis) | 0.0053 [0.0034, 0.0074] |
| TAMU Only (single-analysis) | 0.059 [0.028, 0.098] |
| TAMU Only (meta-analysis) | 0.048 [0.022, 0.081] |
| UCP Only (single analysis) | 0.00034 [0.00020, 0.00050] |
| UCP Only (meta-analysis) | 0.00038 [0.00023, 0.00055] |
| Mix of IOWA, TAMU, and UCP (single analysis) | 0.022 [0.011, 0.035] |
| Mix of IOWA, TAMU, and UCP (meta-analysis) | 0.018 [0.009, 0.029] |
| Unknown Strain from Population (meta-analysis) | 0.028 [0.005, 0.066] |

## CONCLUSIONS

The broader definition of presumed infection (to include subjects who exhibited symptoms of cryptosporidiosis, but did not have oocysts detected in stool) increased the number of subjects infected, and reduced the maximum likelihood dose response parameters ($k$'s) and $ID_{50}$s. Combining the data in a meta-analysis produced estimates that only slightly differed from the results from individual analyses.

The main power of the meta-analysis is its ability to describe the population of strains from which the three isolates were selected. Admittedly, three isolates represent a very small number on which to estimate population parameters; however, it is a reasonable start, and the only data available until more studies are completed. Another feature of the analysis is that its output may be used in probabilistic risk assessments that properly reflect the uncertainty due to our limited knowledge of such a small number of strains.

*Acknowledgements*—The volunteer studies were supported in part by the US environmental Protection Agency (CR-819814 and CR-824759) and the National Institutes of Health General Clinical Research Center Grant M01-RR-02558.

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