Risk Analysis, Vol. 22, No. 3, 2002

Cryptosporidium Dose-Response Studies: Variation Between Hosts

Peter F. M. Teunis,[1]* Cynthia L. Chappell,[2] and Pablo C. Okhuysen[3]

The issue of variation is highly important in dose-response analysis: variation among genetically related pathogens infecting the same host, but also variation among hosts, in susceptibility to infection by the same pathogen. This latter issue is addressed here for the protozoan parasite *Cryptosporidium parvum*, the causative agent for many outbreaks of water-borne gastrointestinal illness. In human feeding studies, infectivity has been shown to be low in subjects with high preexisting anti-*Cryptosporidium* IgG-levels. Here we adapt the hit theory model of microbial infection to incorporate covariables, characterizing the immune status of the susceptible host. The probability of any single oocyst in the inoculum to cause infection appears to depend on preexisting IgG-levels. This does not necessarily imply direct protection by the humoral immune system; high IgG-levels may reflect a recent episode of infection/illness, and be an epi-phenomenon associated with other protective responses. The IgG-dependence of the dose-response relation can be easily applied in quantitative risk analysis. The distribution of anti-*Cryptosporidium* IgG levels in the general population is accessible by analyzing serum banks, which are maintained in many Western countries. Using such an approach provides first insights into the variation of susceptibility to infection in the general population.

1. INTRODUCTION

The susceptibility of a human host to infection and/or illness depends on a number of recognized and unrecognized factors that predispose (or protect) an exposed individual from parasite replication. Two well-documented factors are age and immune status. These factors again may depend on multiple other parameters, which are probably more difficult to characterize. Further, infection and illness should be regarded as related, but separate, outcomes since oocyst excretion can occur in humans without any attendant symptoms, and because *Cryptosporidium* isolates given in equal doses vary in their ability to cause diarrhea.

Cryptosporidium challenge studies of healthy volunteers have provided the opportunity to monitor infection and illness under controlled conditions where the prechallenge health, immune status, and oocyst dose were known. These individuals were further followed for six weeks after challenge and studied for oocyst excretion patterns, clinical manifestations, and immunological responses.

For one of the isolates (the ''Iowa'' isolate), infectivity was studied in subjects without preexisting anti-*Cryptosporidium* IgG (ELISA) and in subjects with high preexisting anti-*Cryptosporidium* IgG (DuPont *et al.*, 1995; Chappell *et al.*, 1999).

[1] National Institute for Public Health and the Environment, PO Box 1, 3720 BA Bilthoven, The Netherlands.
[2] Center for Infectious Diseases, University of Texas School of Public Health, Houston, TX 77030, USA.
[3] Division of Infectious Diseases, University of Texas Medical School and Center for Infectious Diseases, Houston, TX 77030, USA.
* Address correspondence to Dr. P. F. M. Teunis, National Institute of Public Health and the Environment, PO Box 1, NL-3720 BA Bilthoven, The Netherlands; tel.: 31 30 274 2937; fax: 31 30 274 4456; Peter.Teunis@rivm.nl.

0272-4332/02/0600-0475$22.00/1

Analysis of this data as determined by comparing ID_{50}s, defined as cumulative percent end point by the method of Reed and Muench (1938), indicated a sharp decrease in susceptibility to infection in the subjects with high preexisting anti-*Cryptosporidium* IgG levels, compared to those with low IgG levels (Chappell *et al.*, 1999).

This finding can be interpreted as an indication for the existence of some protective effect resulting from prior infection with this organism. A high serum IgG level is not necessarily protective by itself, if only because *Cryptosporidium* only infects the intestinal tract and does not cause systemic infections and the principal host defense mechanisms to *Cryptosporidium* are mediated by cellular immunity. Thus, the physiological function of an IgG response to this organism is unclear, but elevated serum IgG levels are probably correlated with recent infection (and/or clinical cryptosporidiosis). Since serum IgG titres are known to decrease slowly after infection by other pathogens (Auranen *et al.*, 1999; de Melker *et al.*, 2000; van Herck *et al.*, 2000), it also seems reasonable to assume that the higher a subject's IgG titre, the more recently this person was infected.

It must be noted that in a separate study, some of the subjects (with low preexisting anti-*Cryptosporidium* IgG) who had been infected in the first experiment (DuPont *et al.*, 1995), were re-exposed one year later. In these subjects, susceptibility to infection (as well as probability of cryptosporidiosis) appeared not to have changed (Okhuysen *et al.*, 1998). Therefore, if there is a protective response, it may be short lived, with a duration less than one year. Consequently, repeated exposures to *Cryptosporidium parvum* are needed to develop protective immunity.

We investigated the relationship between the relative anti-*Cryptosporidium* IgG values and protection from infection. The microbial dose-response model was modified to allow the use of covariables. Since IgG levels are measured in individual subjects, we also need to slightly modify the statistical analysis, to separately incorporate the contribution of each individual subject to the likelihood function.

2. EXPERIMENTAL DATA

Data used here are from two studies: the first established the dose-response relation for oocysts of *Cryptosporidium parvum* (the ''Iowa'' isolate) in seronegative subjects, selected to have low IgG levels (DuPont *et al.*, 1995). These subjects were assumed to not have a recent history of infection with *Cryptosporidium*.

Subsequently, a separate group of volunteers were selected for the presence of anti-*Cryptosporidium* serum IgG. These volunteers were given different doses of the Iowa isolate to compare infectivity in a naïve versus preexposed population of healthy individuals. Results are summarized in Table I.

Registered responses include both fecal detection of *Cryptosporidium* oocysts by means of an

Table I. Dose-Response Data for the Iowa Isolate of *Cryptosporidium parvum* in Seronegative Human Subjects (DuPont *et al.*, 1995) and in Subjects with High Preexisting Anti-*Cryptosporidium* IgG (Chappell *et al.*, 1999)

		Oocyst +		Diarrhea		Clin Def	
Dose	Tot	Neg	Pos	Neg	Pos	Neg	Pos
Iowa							
30	5	4	1	4	1	3	2
100	8	5	3	4	4	4	4
300	3	1	2	2	1	1	2
500	6	1	5	3	3	1	5
1000	2	0	2	2	0	0	2
10000	3	0	3	1	2	0	3
100000	1	0	1	0	1	0	1
1000000	1	0	1	0	1	0	1
	29	11	18	15	13	9	20
Iowa, high IgG							
500	3	3	0	2	1	2	1
5000	6	4	2	4	2	3	3
10000	5	2	3	1	4	0	5
50000	3	1	2	0	3	0	3
	17	10	7	7	10	5	12

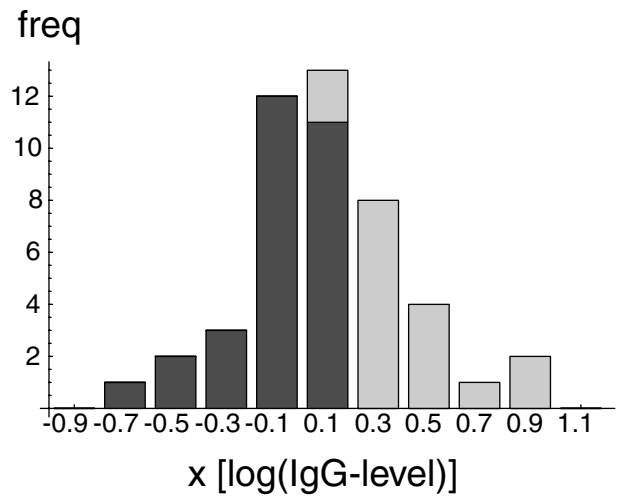


Fig. 1. [10] log IgG levels (absorption in immunosorbent assay) in the low (left) and high IgG groups of subjects.

immune assay (DFA) and clinical symptoms of cryptosporidiosis. As argued elsewhere (Chappell *et al.*, 1999; Okhuysen *et al.*, 1999), either response indicates infection. By the clinical definition used here, having DFA and/or clinical symptoms means a positive response.

Antibody levels, measured by absorption in an enzyme-linked immunosorbent assay (ELISA) are not treated as categorical variables. Instead, each individual subject is used with her or his preexisting IgG value, after log-transformation (since these values are typically determined by serial dilution). The actual (measured) IgG levels of the two subject categories (high and low preexisting IgG levels) do not appear to be in clearly distinct categories, as can be seen in Fig. 1.

3. DOSE-RESPONSE ANALYSIS: INFECTION

By definition for dose-response modeling, the pathogenic organism multiplies within the host (or its intestinal tract). For this to occur, three conditions must be fulfilled: the organism must have been ingested (entry), the organism must have survived until a site suitable for growth is reached (survival), and then the organism needs to be vital enough to multiply (growth). Note that detection of fecal excretion is a *sufficient* but not *per se* necessary condition for infection.

The single hit model is based on the following assumptions: the inoculum is known but for Poisson uncertainty (randomness), organisms act independently, individual probabilities of success do not depend on their numbers (independence), and any single organism can start infection (single hit).

In case of m successive barriers, the probability that any single organism successfully passes all these barriers is p_m. The probability that at least one of the ingested organisms survives and infects the host is

$$\mathrm{P_{inf}}(\mathrm{D}; p_m) = 1 - \mathrm{e}^{-\mathrm{D}p_m}, \tag{1}$$

the exponential dose-response relation according to Haas (1983).

If variation in p_m (between individual pathogens and/or between hosts) is described by a beta distribution with density function $f(p_m; \alpha, \beta)$, the marginal probability of infection can be calculated as

$$P_{\mathrm{inf}}(D; \alpha, \beta) = 1 - {}_1F_1(\alpha, \alpha + \beta, -D) \tag{2}$$

in which ${}_1F_1()$ is the Kummer confluent hypergeometric function (Teunis and Havelaar, 2000). We refer to this function as the ''hypergeometric'' dose-response relation.

When $\beta \gg 1$, and $\alpha \ll \beta$, the simple relation

$$P_{\mathrm{inf}}(D; \alpha, \beta) = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha} \tag{3}$$

given by Haas (1983) holds. Note that this relation is scalable, i.e., the parameter β is a scale parameter. Changes in β cause the beta Poisson dose-reponse relation to be shifted along the dose axis, without changing shape. The hypergeometric relation cannot be scaled. This is due to the nonscalability of the beta distribution for p_m.

3.1. Immune Response as a Covariable

The hypergeometric dose-response function is not the only valid single hit model. Mixture density functions $f(p_m; \boldsymbol{\theta})$ other than the beta distribution may be used, leading to different dose-response relations. Alternative parametrizations may be more convenient than the beta distribution, for instance with regard to covariables, as the mean and variance of the beta distributed p_m are not simple linear functions of the parameters (McCullagh and Nelder, 1989).

For the analysis of data sets with covariate information, the dose-response parameter p_m may be expressed as a function of the covariable(s). One

solution could be to use a parameter transformation. For instance, a covariable x can be factored into the dose-response model using a logit function:

$$\log\left(\frac{p_m}{1-p_m}\right) = a + bx \tag{4}$$

so that a and b may have any real value $(-\infty < a, b < \infty)$. Extension to multivariate models is straightforward. While this gives much more freedom of choice for the distributions of the coefficients (a and b in this example), the resulting marginal dose-response relation cannot be obtained in closed form.

For our analysis, we want p_m to vary with IgG level x, not between 0 and 1, but between arbitrary minimum and maximum levels for highly susceptible subjects with low IgG levels and highly protected subjects with high IgG levels, respectively. To do this, we introduce two additional parameters $c\,(>0)$ and $d\,(>0)$. The dose-response parameter p_m, dependent on covariable x (Equation (4)) then can be written as:

$$p_m(x;a,b,c,d) = \mathrm{e}^{-d} + \frac{\mathrm{e}^{a+bx-c}}{1+\mathrm{e}^{a+bx}} \tag{5}$$

thereby limiting p_m to the interval $\{\mathrm{e}^{-d}, \mathrm{e}^{-d} + \mathrm{e}^{-c}\}$.

The probability of infection now depends on both dose and IgG-level x.

$$P_{\mathrm{inf}}(D,x;a,b,c,d) = 1 - \mathrm{e}^{-p_m(x;a,b,c,d)\cdot D} \tag{6}$$

The dose-response Equation (6) above "wraps" the logistic regression function into the single hit relation. The resulting model still is a single hit model, but also retains the property of having an exponentially shaped maximum possible risk curve (Teunis and Havelaar, 2000).

Say we have dose-response data from subjects with different values of some relevant immune parameter. There are m dose groups $(i = 1, \ldots, m)$. A dose group (dose D_i) consists of n_i subjects, with immune status x_{ij} $(j = 1, \ldots, n_i)$. The binary variable q_{ij} describes the response of each subject (1 for infection, 0 for not infected).

The contribution of a single subject to the likelihood is

$$P_{\mathrm{inf}}(D_i,x_{ij};a,b,c,d)^{q_{ij}} \cdot \left[1 - P_{\mathrm{inf}}(D_i,x_{ij};a,b,c,d)\right]^{1-q_{ij}} \tag{7}$$

for all subjects in every dose group

$$\begin{aligned}\ell(a,b,c,d) &= \prod_{i=1}^{m}\prod_{j=1}^{n_i} P_{\mathrm{inf}}(D_i,x_{ij};a,b,c,d)^{q_{ij}} \\ &\quad\cdot\left[1 - P_{\mathrm{inf}}(D_i,x_{ij};a,b,c,d)\right]^{1-q_{ij}} \\ &= \prod_{i=1}^{m}\prod_{j=1}^{n_i}\left(1-\mathrm{e}^{-p_m(x;a,b,c,d)\cdot D}\right)^{q_{ij}} \\ &\quad\cdot\left(\mathrm{e}^{-p_m(x;a,b,c,d)\cdot D}\right)^{1-q_{ij}}\end{aligned} \tag{8}$$

3.2. Infection Dose Response: Results

When the dose IgG model Equation (5) is fit to the separate data sets, weak evidence for IgG effects is found (increasing p_m with increasing (log) IgG levels in the low IgG data, and decreasing p_m with increasing (log) IgG levels in the high IgG data). Note the values of $\hat{b}$ in Table II. When tested in a likelihood ratio test against the same models with b set to zero (i.e., absence of IgG effect), unfortunately, these interesting effects do not appear to be statistically significant.

The simple model in Equation (4) can also be fit to the separate data sets (high and low IgG). However, when fit to pooled data (high and low IgG), a strong IgG effect is found, but the goodness of fit decreases to an unacceptable level. This is caused by the discrepancy between the dose-response relations of the two groups and the corresponding IgG levels. There appeared to be a 23-fold increase in ID_{50} in the subjects with high preexisting anti-crypto-IgG (Chappell *et al.*, 1999), while the IgG levels of the two groups were overlapping (see Fig. 1).

Since the preexisting IgG levels were the only criterium for selection of volunteers for these two experiments, there are no obvious other variables that could explain this discrepancy. We therefore

Table II. Dose-Response Model in Equation (6) Fitted to Data from Either the Study with Subjects Selected for Low IgG Levels, or the Study with High IgG Subjects, or Pooled Data from Both Studies

	$-2\log\hat{\ell}$	$\hat{a}$	$\hat{b}$	$\hat{c}$	$\hat{d}$
low IgG	25.54	85.7	237.8	1.62	1.90
high IgG	14.34	151.6	−200.7	2.16	2.21
low + high IgG	44.22	29.11	−251.9	1.66	2.12

Note: $\hat{\ell}$ = maximum likelihood. A likelihood ratio test for pooling the two data sets shows that the four parameter model fits the merged data adequately ($44.22 - 25.54 - 14.34 = 4.34$; less than $\chi^2_{0.95}(4+4-4)$.

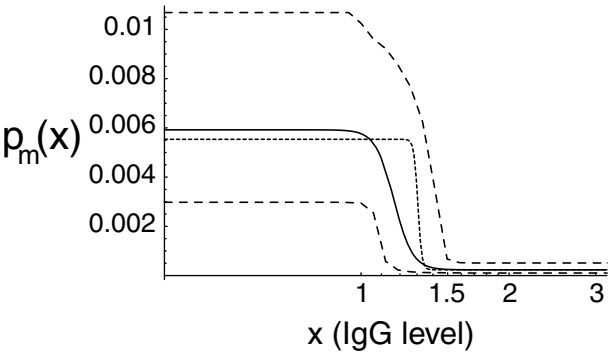


Fig. 2. Dose-response parameter p_m, showing the relation to the IgG level, for the combined High + Low IgG data. Black: maximum likelihood curve, hatched: posterior mode curve. Also shown (hatched) are MCMC-based 95% limits.

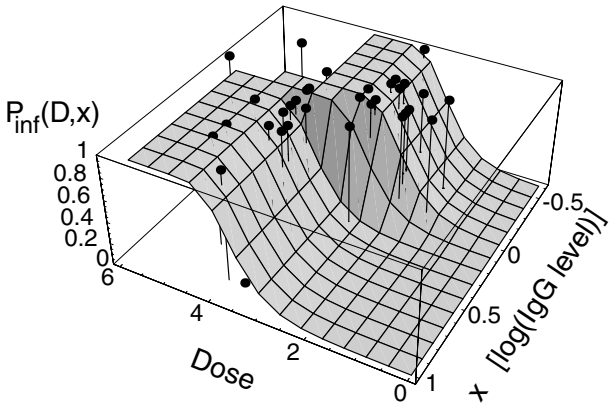


Fig. 3. Posterior mode dose-response relation for the model in Equation (6) fitted to the combined High + Low IgG data. Dots show data (0 = negative response, 1 = infection).

have to accept that there is a steep change in the relation between IgG levels and infectivity. This is illustrated by the results for the four-parameter model. The dose-response relation is a function of two variables, dose and IgG level. A three-dimensional graph of the maximum likelihood estimate of this function is shown in Fig. 3, for parameter estimates obtained from both the high and low IgG subsets at once. It can be seen that when IgG levels exceed about 1.4 units ($^{10}\log 1.4 = 0.15$), the probability of infection decreases sharply.

Most informative for this model is the way the probability of infection for a single organism, the parameter p_m, depends on the covariate. Fig. 2 shows p_m as a function of preexisting log-IgG levels. Also shown here is the uncertainty in the shape of this response function, as determined by Markov chain Monte Carlo methods, as described earlier (Teunis and Havelaar, 2000).

Table III. Posterior Mode Parameters for the Dose-Response Model in Equation (6) Fitted to Data from Either the Study with Subjects Selected for Low IgG Levels, or the Study with High IgG Subjects, or Pooled Data from Both Studies

	$-2\log\hat{\pi}$	$\hat{a}$	$\hat{b}$	$\hat{c}$	$\hat{d}$
low IgG	57.11	6.93	14.9	1.59	2.15
high IgG	45.77	5.15	−6.90	2.11	2.33
low + high IgG	75.95	3.56	−53.3	1.64	2.12

Note: $\hat{\pi}$ = mode of posterior probability.

Parameter values are listed in Table III. Fig. 4 shows the maximum likelihood dose-response relation, with a 95% credible interval, based on the same Markov chain Monte Carlo methods.

4. DOSE-RESPONSE ANALYSIS: ACUTE ILLNESS

For the present, the only illness response we consider is acute gastroenteritis. Although this still implies a spectrum of symptoms, listed in DuPont *et al.* (1995), we assume that, at least in an experimental setting, cryptosporidiosis can be diagnosed with sufficient accuracy. The occurrence of illness is considered conditional on infection. Only infected subjects can become ill. As with infection, we start by defining the basic concepts for the dose-response model.

The components of the model are (Teunis *et al.*, 1999): an *infected host*, many living pathogens are present in (parts of) the gut; a *hazard of illness*, during infection, there is a certain nonzero hazard of becoming ill; and the *duration of infection*, the length of the period that colonization persists.

The length of the infection period is the key variable in these models: a host with strong defenses against the pathogen is assumed to clear infection rapidly. Conversely, a highly virulent pathogen is assumed to be able to sustain intra-intestinal growth for a long period (Teunis *et al.*, 1999).

The probability that illness has occurred by time t after onset of infection is

$$P_{\text{ill,inf}}(t,\gamma) = 1 - \mathrm{e}^{-\int_{v=0}^{t} h(v)\mathrm{d}v} \tag{9}$$

with hazard function $h(t)$, and duration of infection τ. The hazard function can have arbitrary shape, as long as it can be written as a function of t/τ. This

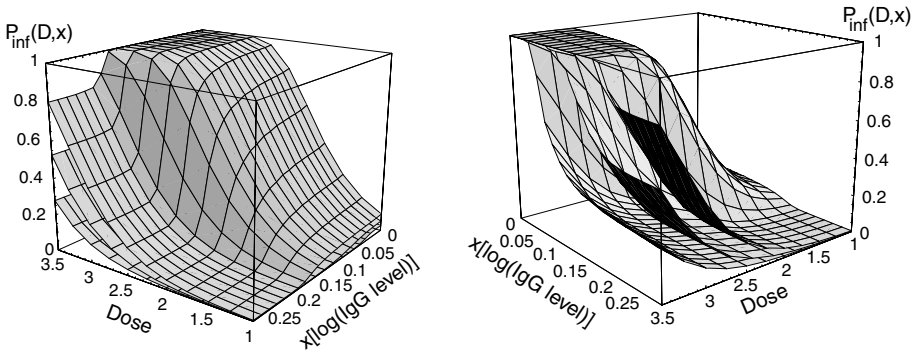


Fig. 4. Dose-response relation for the model in Equation (6) fitted to the combined High + Low IgG data. Posterior mode relation and (MCMC-based) 95% credible bounds.

means that when the duration of infection is twice as long, the integral of the hazard over this period becomes exactly twice as large. The probability of illness can then be written as:

$$P_{\text{ill,inf}}(\tau, \gamma) = 1 - \mathrm{e}^{-\gamma\tau} \tag{10}$$

When τ has a Gamma pdf $g(\tau; r, \lambda)$, the probability of illness

$$\begin{aligned} P_{\text{ill,inf}}(\gamma, r, \lambda) &= \int_{u=0}^{\infty} (1 - e^{-\gamma u}) g(u; r, \lambda) du \\ &= 1 - (1 + \gamma\lambda)^{-r} \end{aligned} \tag{11}$$

Without sacrificing generality, we can set $\gamma = 1$.

$$P_{\text{ill,inf}}(r, \lambda) = 1 - (1 + \lambda)^{-r} \tag{12}$$

Assume that the scale factor λ is a function of applied dose (D) and preexisting log-IgG level (x) as:

$$\lambda(D, x; A, B, C) = AD - Bx + C \tag{13}$$

so that

$$P_{\text{ill,inf}}(D, x; r, A, B, C) = 1 - (1 + AD - Bx + C)^{-r} \tag{14}$$

4.1. Illness Dose Response: Results

The model in Equation (14) can be used for analysis of the illness data for the two experiments with the Iowa isolate (Table I).

In Table IV, the likelihoods for three different variants of the model in Equation (14) are given for the low IgG group, the high IgG group, and for the pooled data from both groups. Comparison of each of these models fitted to the two separate data sets, with the same model fitted to pooled data from both studies, shows a nonsignificant decrease in log-likelihood. We therefore consider the two studies as a single data set. Starting from the model with constant probability of illness ($A = B = 0$), addition of dose dependence ($A \neq 0$) decreases the deviance (difference in $-2 \log \hat{\ell}$) by a significant amount, subsequent addition of (log) IgG dependence only slightly improves the goodness of fit, as can be seen in Table IV. Table V gives posterior mode values for the same models.

Fig. 5a shows the effect of dose on the probability of illness in infected subjects at IgG level 1.0 ($\log x = 0$). Fig. 5b indicates a weak tendency to decreasing risk of illness with increasing preexisting IgG levels. Fig. 6 shows the bivariate dose-response model for illnes, with corresponding (MCMC-based) 95% range.

5. DISCUSSION

The analysis given here corroborates and extends earlier reports, stating that the ID_{50} in volunteers with preexisting antibody was approximately 20-fold higher than in serologically negative volunteers (Chappell *et al.*, 1999).

Support is also given for the observation that the volunteers who developed diarrhea were among those receiving the highest oocyst dosages (10,000 oocysts or more). Again, those receiving low-dose challenges were less likely to develop illness. Interestingly, the volunteers who did develop diarrhea had the same median incubation period (5 days, range 3–12 days) as the seronegative hosts, but experienced a longer illness (6.5 days) with a greater number of unformed stools (10, range 3–35). This is an aspect of

Table IV. Illness Dose-Response Model in Equation (14) Fitted to Data from Either the Study with Subjects Selected for Low IgG Levels, or the Study with High IgG Subjects, or Pooled Data from Both Studies

	$-2\log\hat{\ell}$	$\hat{A}$	$\hat{B}$	$\hat{C}$	$\hat{r}$	df
low IgG	25.89	0	0	0.62	1.00	2
	24.08	1.95×10^{-5}	0	−3.32	25.58	3
	22.21	5.18×10^{-5}	3.34	−4.86	82.36	4
high IgG	10.81	0	0	1.41	1.10	2
	9.66	3.12×10^{-5}	0	−5.22	222.23	3
	9.64	3.65×10^{-5}	−0.56	−1.86	6.76	4
low + high IgG	38.02	0	0	0.77	1.10	2
	34.13	3.78×10^{-5}	0	−4.10	58.06	3
	34.12	4.24×10^{-5}	0.18	−2.83	16.67	4

Note: $\hat{\ell}$ = maximum likelihood, df = degrees of freedom. Three variants are given for each of the three data sets: constant ($A = B = 0$), dose dependent ($B = 0$), and both dose and IgG dependent probability of illness.

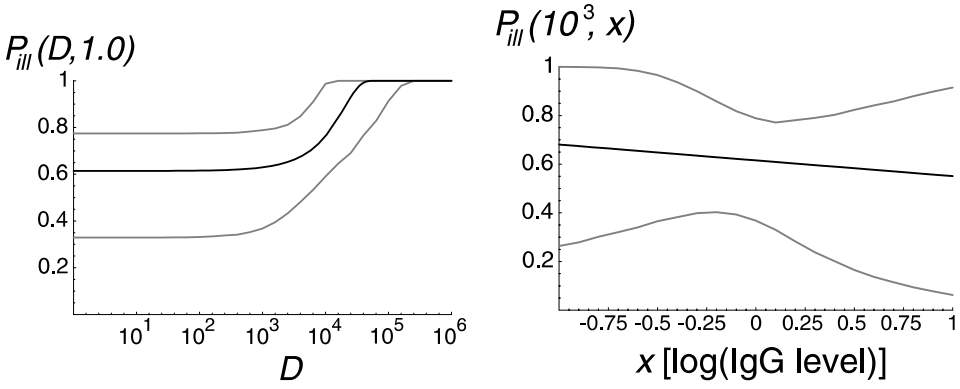
Table V. Posterior Mode Estimates for the Illness Dose-Response Model

	$\hat{A}$	$\hat{B}$	$\hat{C}$	$\hat{r}$
low IgG	0	0	0.62	1.00
	2.05×10^{-5}	0	−2.53	11.88
	5.11×10^{-5}	3.40	−3.95	33.26
high IgG	0	0	1.41	1.10
	3.44×10^{-5}	0	−2.55	15.85
	3.12×10^{-5}	−0.58	−3.09	22.20
low + high IgG	0	0	0.77	1.10
	4.02×10^{-5}	0	−2.57	12.96
	4.21×10^{-5}	0.16	−2.58	13.12

Note: Prior distributions used: A: Uniform(−10, 10); B: Uniform(−10, 10); C: Uniform(−20, 20); r: Uniform(0, 200).

illness that deserves more attention, especially since the hazard model is well suited for studying the temporal aspects of illness (Teunis *et al.*, 1999).

A number of volunteers who developed a diarrheal illness had no detectable oocysts in their stools by IFA. This observation was especially prominent in volunteers who had been previously exposed to the parasite. The diarrhea in these individuals did not differ in the incubation time, duration, or any of the characteristic symptoms as compared to those who had confirmed infections. The cause of this phenomenon is not known, but several explanations exist. First, these diarrheal episodes may be the result of a hypersensitivity to the organism. In this scenario, the exposure alone would provide a sufficient stimulus for the development of diarrhea without replication of the organism. However, with other antigens, the hypersensitivity response typically requires two to three days or less to develop, a shorter time to onset than was observed with these volunteers. Second, an infection may have



(a) Dose dependence at $x = 1.0$ (b) IgG dependence at $D = 10^3$

Fig. 5. Bivariate illness dose response for the Iowa strain of *Cryptosporidium*. Left: dose response at an IgG level of 1.0 units ($\log x = 0$). Right: IgG dependence of the response at a dose of 1,000 oocysts. Best fitting (maximum likelihood) curve and (in grey) MCMC-based 95% limits.

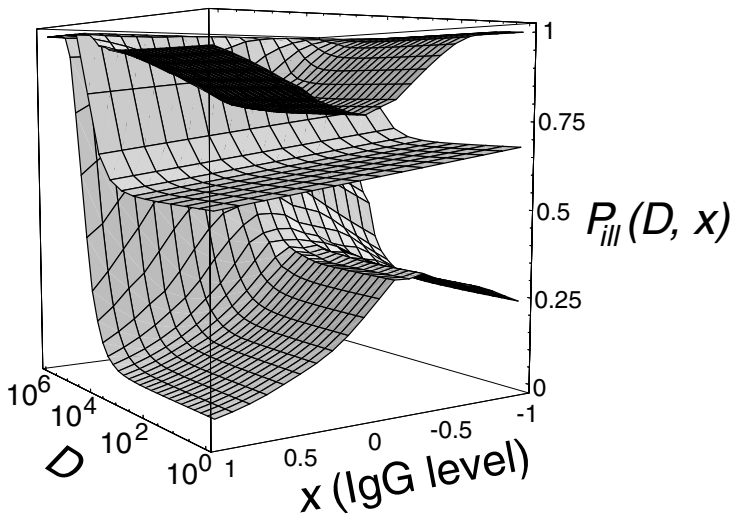


Fig. 6. Bivariate illness dose response for the Iowa strain of *Cryptosporidium*. Best fitting (maximum likelihood) plane and MCMC-based 95% limits.

occurred with oocyst production below the level of IFA sensitivity (10,000 oocysts/ml). This possibility is enhanced by the finding of low numbers of oocysts when flow cytometry was used to assay the samples. Approximately 75% of the IFA-negative samples tested in this way were positive. Earlier studies indicated that flow cytometry of human stool samples has a detection limit of approximately 1,000 oocysts per ml, although fewer oocysts could be detected in some samples. Interestingly, however, this left 25% of the samples negative by both methods. Third, the asexual stages of infection could have occurred, but the immune response may have interrupted the cycle before the oocysts were formed. The factors necessary and sufficient for the development of diarrhea are not understood at present, but it is possible that parasite replication is needed to provide sufficient stimulus to initiate diarrhea. Likewise, the exact immune mechanisms governing clearance of the infection are also still unknown, although evidence suggests that $CD4^+$ T-cells and interferon gamma are essential components. Nevertheless, if the response is directed toward either the merozoite or gametocyte stages, oocyst production would be prevented. Further, it is also possible that a combination of these possibilities may be responsible for the lack of detectable oocysts.

5.1. Susceptibility to Infection and Illness

Current understanding of the factors influencing susceptibility comes from a combination of studies, most of which utilize animal models. Thus, the relevance of this data for human infections is not always clear. Neonatal animals have long been recognized as being especially susceptible to *Cryptosporidium* infection. For example, neonatal mice can be readily infected just after birth and remain so until they reach the point of gut maturation, when they self-cure. In contrast, adult mice given large *C. parvum* doses may become infected, but only transiently for one to two days (Harp *et al.*, 1988; Griffiths *et al.*, 1998). (Interestingly, neither age group exhibits a diarrheal illness.) These observations extend to many naturally infected herbivorous species. For example, calves and lambs, often contract the infection, excrete large numbers of oocysts, and have significant morbidity and mortality from diarrhea (Tzipori *et al.*, 1981, 1982; Ongerth and Stibbs, 1989; Harp *et al.*, 1990; Olson *et al.*, 1997). Adult animals may also be infected, but excrete few oocysts and have no symptoms (Henriksen and Krogh 1985; Harp *et al.*, 1990). In the human population, the largest number of recognized cases of cryptosporidiosis are found in children and decrease after age two (Wiedermann *et al.*, 1985; Zu *et al.*, 1994; Agnew *et al.*, 1998; Guerrant *et al.*, 1999). Unlike other species, however, adults can also become infected, excrete large numbers of oocysts, and experience a diarrhea, which can be profuse (DuPont *et al.*, 1995; Okhuysen *et al.*, 1998, 1999; Chappell *et al.*, 1996, 1999).

Age is inextricably tied to immune status since the gastrointestinal tract in neonatal animals is immature and undergoes important developmental changes after birth. Likewise, the mucosal immune

system is immature and comparatively inactive at birth and undergoes significant functional changes as stimuli are encountered, processed, recognized, and responded to. These processes of gut and immune maturation vary with species. Indeed, many anatomical, physiological, and immunological differences are known to exist between humans and the animal models currently used to study cryptosporidiosis. Perhaps the most relevant models for human illness are the porcine models since this species appears to be closest to humans in gut anatomy and physiology. However, chronic infections beyond the neonatal period require significant manipulation of the immune system, either by direct drug-induced suppression (Rasmussen and Healey, 1992) or by artificially delaying immune maturation via maintaining the animal in a gnotobiotic environment (Tzipori *et al.*, 1982; Yuan *et al.*, 1996). The immuno-suppressed pig model is roughly analogous to the immuno-compromised state seen in humans. Thus, the porcine model may serve as a useful system for studying different genotypes and/or nonparvum species of *Cryptosporidium* and to test new therapeutic agents for activity against the organism.

Although all adult humans are potentially susceptible to *Cryptosporidium*, individuals who have compromised immune systems are at increased risk of developing chronic and/or progressive disease, particularly if they have low concentrations ($<180/mm^3$) of CD4 T-cells. AIDS patients or patients receiving cancer chemotherapy or other drug-induced immuno-suppression may fall into this category. Chronic cryptosporidiosis can be reversed by giving antiretroviral therapy (for HIV), which increases CD4 counts, or by discontinuing immunosuppressive drugs until CD4 counts recover (Meisel *et al.*, 1976; Smith *et al.*, 1998; Dionisio *et al.*, 1998). Likewise, individuals with certain immuno-deficiencies, such as for IFNγ (interferon gamma) (Pollok *et al.*, 2001; Gomez Morales *et al.*, 1996) or IgA (Weisburger *et al.*, 1979), also are at increased risk for prolonged illness. Currently, there are no recognized curative therapies available for *Cryptosporidium* infection. A combination of azithromycin and paromomycin has been shown to decrease the number of excreted oocysts and provide some therapeutic benefit for AIDS patients (White Jr. *et al.*, 1994).

Other factors affecting the immune response may also contribute to the severity and duration of *C. parvum* infection. For example, malnourished individuals may have a decrease in immune responsiveness as evidenced by decreased CD4 T-cell levels. Malnutrition in children is associated with prolonged *Cryptosporidium* infections (Zu *et al.*, 1994; Guerrant 1997; Agnew *et al.*, 1998). Furthermore, there is evidence to suggest that *Cryptosporidium* infection, although self-limited in nature, may play a role in the future nutritional status of children (Checkley *et al.*, 1998). Little is known about the role of genetic factors in the susceptibility of persons to *Cryptosporidium* infection or illness. There is some evidence, however, that different strains of inbred mice have different susceptibilities to infection (Griffiths *et al.*, 1998; Aguirre *et al.*, 1998; Tarazona *et al.*, 1998). It is possible that the same phenomenon exists in other species, but this hypothesis has not been confirmed.

5.2. Health Effects

In the past, studies of human cryptosporidiosis depended primarily on outbreaks, travelers to developing countries, and *Cryptosporidium*-infected AIDS patients. These sources revealed an illness in the immuno-competent population that had an incubation period of seven days and a usual duration of 10–13 days. Symptoms included diarrhea, abdominal pain and cramping, weight loss, nausea, vomiting, and occasional low grade fever (Jokipii and Jokipii, 1986; Pohjola *et al.*, 1986; Jongwutiwes *et al.*, 1990; Tangermann *et al.*, 1991; Richardson *et al.*, 1991). While diarrhea could be profuse (1–2 liters/day), those requiring hospitalization were few in number and typically involved the very young or very old, both of whom are more susceptible to dehydration. Mortality has been documented in rare instances in malnourished children in developing countries (Wiedermann *et al.*, 1985; Agnew *et al.*, 1998). In contrast, serious, life-threatening infections in developed countries have been limited to individuals with profoundly depressed immune responses (Hoxie *et al.*, 1997). In these patients, especially before antiretroviral therapy was available, chronic infections with 3–6 liter fluid losses were not uncommon. Diarrhea may persist for weeks or months and occasionally improves without specific therapy. A more fulminant course can also be seen with fluid losses in the range of 17–20 liters per day and death occurring rapidly.

In humans with intact immune systems, the parasite is thought to be in highest concentration in

the ileal region (Harp *et al.*, 1996). In contrast, immuno-suppressed individuals often experience a contiguous spread of infection into the colon, the proximal portions of the bowel; infection can also be found in the biliary tree and gall bladder epithelium (Vakil *et al.*, 1996; Chen *et al.*, 1998). In some cases, infection has been documented in the gastric mucosa, esophagus, and the respiratory tract (Hojlyng and Jensen, 1988; Current and Garcia, 1991). There are even reports of *Cryptosporidium* sinusitis (Davis and Heyman, 1988; Dunand *et al.*, 1997).

Differences in the severity of illness seen between the volunteer studies and reports from naturally acquired infections may be due to differences in *Cryptosporidium* isolates, the ability to collect accurate data from recall surveys, or other factors. The major difference, however, may lie with the general health status of the individual at the time of challenge. Persons with naturally acquired infections represent all segments of the population, i.e., young and old, in all states of health, previously exposed and naïve. Also, those reporting to the medical establishment are self-selected for the more severe symptoms. In contrast, the experimentally infected volunteers were typically between the ages of 25 and 35 and were selected for their robust health. These subjects generally had milder illnesses of shorter duration compared to the more general population.

5.3. Usefulness for Quantitative Risk Assessment

The dose-response information assembled here gives a characterization of the occurrence of human health effects caused by *Cryptosporidium* that may be more complete than that of any other pathogen. This is because of the human feeding studies, which have been going on uninterrupted since 1993. Here we have concentrated on variation between hosts with different preexisting antibody levels against *Cryptoporidium*, both for infection and illness (given infection). These results can be used for different purposes.

The infectivity (and probability of illness given infection) appear to correlate with preexisting IgG levels. These can be measured in any human population. Therefore, the dose-response information provided here can be used to assess the distribution of susceptibility to infection (illness) by *Cryptosporidium* oocysts among human subjects with known anti-crypto-IgG levels.

REFERENCES

Agnew DG, Lima AAM, Newman RD, Wuhib T, Moore RD, Guerrant RL, Sears CL. Cryptosporidiosis in northeastern Brazilian children: association with increased diarrhea morbidity. *Journal of Infectious Diseases* 1998;177:754–760.

Aguirre SA, Perryman LE, Davis WC, McGuire TC. IL-4 protects adult C57BL/6 mice from prolonged *Cryptosporidium parvum* infection: analysis of CD4(+)alpha beta+IFN-gamma(+) and CD4(+)alpha beta+IL-4(+) lymphocytes in gut-associated lymphoid tissue during resolution of infection. *Journal of Immunology* 1998;161(4):1891–1900.

Auranen K, Eichner M, Kayhty H, Takala AK, Arjas E. A hierarchical Bayesian model to predict the duration of immunity to *Haemophilus influenzae* type b. *Biometrics* 1999;55(4):1306–1313.

Chappell CL, Okhuysen PC, Sterling CR, DuPont HL. *Cryptosporidium parvum*: intensity of infection and oocyst excretion patterns in healthy volunteers. *Journal of Infectious Diseases* 1996;173(1):232–236.

Chappell CL, Okhuysen PC, Sterling CR, Wang C, Jakubowski W, DuPont HL. Infectivity of *Cryptosporidium parvum* in healthy adults with preexisting anti-*C. parvum* serum immunoglobulin G. *American Journal of Tropical Medicine and Hygiene* 1999;60(1):157–164.

Checkley W, Epstein LD, Gilman RH, Black RE, Cabrera L, Sterling CR. Effects of *Cryptosporidium parvum* infection in Peruvian children: growth faltering and subsequent catch-up growth. *American Journal of Epidemiology* 1998;148(5):497–506.

Chen XM, Levine SA, Tietz P, Krueger E, McNiven MA, Jefferson DM, Mahle M, LaRusso NF. *Cryptosporidium parvum* is cytopathic for cultured human biliary epithelia via an apoptotic mechanism. *Hepatology* 1998;28(4):906–913.

Current WL, Garcia LS. Cryptosporidiosis. *Clinical Microbiology Reviews* 1991;4(3):325–358.

Davis JJ, Heyman MB. Cryptosporidiosis and sinusitis in an immunodeficient adolescent. *Journal of Infectious Diseases* 1988;158(3):649.

Dionisio D, Orsi A, Sterrantino G, Meli M, Di Lollo S, Ibba Manneschi LL, Trotta M, Pozzi M, Sani L, Leoncini F. Chronic cryptosporidiosis in patients with AIDS: stable remission and possible eradication after long-term, low dose azithromycin. *Journal of Clinical Pathology* 1998;51(2):138–142.

Dunand VA, Hammer SM, Rossi R, Poulin M, Albrecht MA, Doweiko JP, DeGirolami PC, Coakley E, Piessens E, Wanke CA. Parasitic sinusitis and otitis in patients infected with human immunodeficiency virus: report of five cases and review. *Clinical Infectious Diseases* 1997;25(2):267–272.

DuPont HL, Chappell CL, Sterling CR, Okhuysen PC, Rose JB, Jakubowski W. The infectivity of *Cryptosporidium parvum* in healthy volunteers. *New England Journal of Medicine* 1995;332(13):855–859.

Gomez Morales MA, Ausiello CM, Guarino A, Urbani F, Spagnuolo MI, Pignata C, Pozio E. Severe, protracted intestinal cryptosporidiosis associated with interferon gamma deficiency: pediatric case report. *Clinical Infectious Diseases* 1996;22(5):848–850, Comment in *Clinical Infectious Diseases* 1996;23(6):1336–1337.

Griffiths JK, Theodos C, Paris M, Tzipori S. The gamma interferon gene knockout mouse: a highly sensitive model for evaluation of therapeutic agents against *Cryptosporidium parvum*. *Journal of Clinical Microbiology* 1998;36(9):2503–2508.

Guerrant DI, Moore SR, Lima AA, Patrick PD, Schorling JB, Guerrant RL. Association of early childhood diarrhea and cryptosporidiosis with impaired physical fitness and cognitive function four-seven years later in a poor urban community in

northeast Brazil. *American Journal of Tropical Medicine and Hygiene* 1999;61:707–713.

Guerrant RL. Cryptosporidiosis: an emerging, highly infectious threat. *Emerging Infectious Diseases* 1997;3(1):51–57.

Haas CN. Estimation of risk due to low doses of microorganisms: a comparison of alternative methodologies. *American Journal of Epidemiology* 1983;118(4):573–582.

Harp JA, Fayer R, Pesch BA, Jackson GJ. Effect of pasteurization on infectivity of *Cryptosporidium parvum* oocysts in water and milk. *Applied and Environmental Microbiology* 1996;62(8):2866–2868.

Harp JA, Wannemuehler MW, Woodmansee DB, Moon HW. Susceptibility of germfree or antibiotic-treated adult mice to *Cryptosporidium parvum*. *Infection and Immunity* 1988;56(8):2006–2010.

Harp JA, Woodmansee DB, Moon HW. Resistance of calves to *Cryptosporidium parvum*: effects of age and previous exposure. *Infection and Immunity* 1990;58(7):2237–2240.

Henriksen SA, Krogh HV. Bovine cryptosporidiosis in Denmark. 1. Prevalence, age distribution, and seasonal variation. *Nordic Veterinary Medicine* 1985;37(1):34–41.

van Herck K, Beutels P, van Damme P, Beutels M, van den Dries J, Briantais P, Vidor E. Mathematical models for assessment of long-term persistence of antibodies after vaccination with two inactivated hepatitis A vaccines. *Journal of Medical Virology* 2000;60(1):1–7.

Hojlyng N, Jensen BN. Respiratory cryptosporidiosis in HIV-positive patients. *Lancet* 1988;1:590–591.

Hoxie NJ, Davis JP, Vergeront JM, Nashold RD, Blair KA. Cryptosporidiosis-associated mortality following a massive waterborne outbreak in Milwaukee, Wisconsin. *American Journal of Public Health* 1997;87(12):2032–2035.

Jokipii L, Jokipii AM. Timing of symptoms and oocyst excretion in human cryptosporidiosis. *New England Journal of Medicine* 1986;315(26):1643–1647.

Jongwutiwes S, Kraivichian P, Kulkumthorn M, Sitthichareonchai P, Jaroenkorn M. Cryptosporidiosis among orphanage children in Thailand: a one year prospective study. *Southeast Asian Journal of Tropical Medicine and Public Health* 1990;21(3):458–464.

McCullagh P, Nelder JA. Generalized linear models, volume 37 of *Monographs on statistics and applied probability*. Chapman and Hall, London, 1989.

Meisel JL, Perera DR, Meligro C, Rubin CE. Overwhelming watery diarrhea associated with *cryptosporidium* in an immunosuppressed patient. *Gastroenterology* 1976;70(6): 1156–1160.

de Melker HE, Versteegh FGA, Conyn van Spaendonck MAE, Elvers LH, Berbers GAM, van der Zee A, Schellekens JFP. Specificity and sensitivity of high levels of immunoglobulin G antibodies against pertussis toxin in a single serum sample for diagnosis of infection with *Bordetella pertussis*. *Journal of Clinical Microbiology* 2000;38(2):800–806.

Okhuysen PC, Chappell CL, Crabb JH, Sterling CR, DuPont HL. Virulence of three distinct *Cryptosporidium parvum* isolates for healhty adults. *Journal of Infectious Diseases* 1999;180(4):1275–1281.

Okhuysen PC, Chappell CL, Sterling CR, Jakubowski W, DuPont HL. Susceptibility and serologic response of healthy adults to reinfection with *Cryptosporidium parvum*. *Infection and Immunity* 1998;66(2):441–443.

Olson ME, Guselle NJ, O'Handley RM, Swift ML, McAllister TA, Jelinski MD, Morck DW. *Giardia* and *Cryptosporidium* in dairy calves in British Columbia. *Canadian Veterinary Journal* 1997;38(11):703–706.

Ongerth JE, Stibbs HH. Prevalence of *Cryptosporidium* infection in dairy calves in western Washington. *American Journal of Veterinary Research* 1989;50(7):1069–1070.

Pohjola S, Oksanen H, Jokipii L, Jokipii AM. Outbreak of cryptosporidiosis among veterinary students. *Scandinavian Journal of Infectious Diseases* 1986;18(2):173–178.

Pollok RC, Farthing MJ, Bajaj-Elliott M, Sanderson IR, McDonald V. Interferon gamma induces enterocyte resistance against infection by the intracellular pathogen *Cryptosporidium parvum*. *Gastroenterology* 2001;120(1):99–107.

Rasmussen KR, Healey MC. Experimental *Cryptosporidium parvum* infections in immunosuppressed adult mice. *Infection and Immunity* 1992;60(4):1648–1652.

Reed LJ, Muench H. A simple method of estimating fifty per cent end points. *American Journal of Hygiene* 1938;27(3):493–497.

Richardson AJ, Frankenberg RA, Buck AC, Selkon JB, Colbourne JS, Parsons JW, Mayon-White RT. An outbreak of waterborne cryptosporidiosis in Swindon and Oxfordshire. *Epidemiology and Infection* 1991;107(3):485–495.

Smith NH, Cron S, Valdez LM, Chappell CL, White Jr AC. Combination drug therapy for cryptosporidiosis in AIDS. *Journal of Infectious Diseases* 1998;178(3):900–903, Comment in *Journal of Infectious Diseases* 1998;178(3):915–916.

Tangermann RH, Gordon S, Wiesner P, Kreckman L. An outbreak of cryptosporidiosis in a day-care center in Georgia. *American Journal of Epidemiology* 1991;133(5):471–476.

Tarazona R, Blewett DA, Carmona MD. *Cryptosporidium parvum* infection in experimentally infected mice: infection dynamics and effect of immunosuppression. *Folia Parasitologica* 1998;45(2):101–107.

Teunis PFM, Havelaar AH. The Beta Poisson model is not a single hit model. *Risk Analysis* 2000;20(4):511–518.

Teunis PFM, Nagelkerke NJD, Haas CN. Dose response models for infectious gastroenteritis. *Risk Analysis* 1999;19(6):1251–1260.

Tzipori S, Angus KW, Gray EW, Campbell I, Allan F. Diarrhea in lambs experimentally infected with *Cryptosporidium* isolated from calves. *American Journal of Veterinary Research* 1981;42:1400–1404.

Tzipori S, Smith M, Makin T, Halpin C. Enterocolitis in piglets caused by *Cryptosporidium* sp. purified from calf faeces. *Veterinary Parasitology* 1982;11:121–126.

Vakil NB, Schwartz SM, Buggy BP, Brummitt CF, Kherellah M, Letzer DM, Gilson IH, Jones PG. Biliary cryptosporidiosis in HIV-infected people after the waterborne outbreak of cryptosporidiosis in Milwaukee. *New England Journal of Medicine* 1996;334(1):19–23.

Weisburger WR, Hutcheon DF, Yardley JH, Roche JC, Hillis WD, Charache P. Cryptosporidiosis in an immunosuppressed renal-transplant recipient with IgA deficiency. *American Journal of Clinical Pathology* 1979;72(3):473–478.

White Jr AC, Chappell CL, Hayat CS, Kimball KT, Flanigan TP, Goodgame RW. Paromomycin for cryptosporidiosis in AIDS: a prospective, double-blind trial [see comments]. *Journal of Infectious Diseases* 1994;170(2):419–424, Comment in *Journal of Infectious Diseases* 1995;171(4):1070; Comment in *Journal of Infectious Diseases* 1995;172(4): 1169–1170.

Wiedermann BL, Kaplan SL, Marino B. Prevalence and significance of cryptosporidiosis in children. *Pediatric Infectious Diseases* 1985;4(3):292–293.

Yuan L, Ward LA, Rosen BI, To TL, Saif LJ. Systemic and intestinal antibody-secreting cell responses and correlates of protective immunity to human rotavirus in a gnotobiotic pig model of disease. *Journal of Virology* 1996;70(5):3075–3083.

Zu SX, Li JF, Barrett LJ, Fayer R, Shu SY, McAuliffe JF, Roche JK, Guerrant RL. Seroepidemiologic study of *Cryptosporidium* infection in children from rural communities of Anhui, China and Fortaleza, Brazil. *American Journal of Tropical Medicine and Hygiene* 1994;51(1):1–10.