

# Title: Understanding the Occurrence of *Cryptosporidium* and *Giardia* in Water

Jerry E. Ongerth<sup>1</sup>

*<sup>1</sup>Civil, Mining, & Environmental Engineering, University of Wollongong, Wollongong NSW  
2522, Australia*

**FINAL DRAFT w/ FIGURES**

\*Corresponding Author. Mailing Address: Prof. J. E. Ongerth, CME Bldg 4 EEC, University of Wollongong, Wollongong NSW 2522, Australia. Tel: ++61 0408 548 427. Fax: ++61 2 4210 6163. Email: [jongerth@uow.edu.au](mailto:jongerth@uow.edu.au). 27/10/2014 10:59:25 AM

27/10/2014 10:59:25 AM

## 1. Introduction

Monitoring sources of drinking water for *Cryptosporidium* and *Giardia* has become a common element of water quality management for public water suppliers. Yet few suppliers monitor on a self-motivated basis seeking to understand the characteristics of these water quality parameters but respond almost exclusively to the motivation of external regulation. Too often, despite the very significant expense of collecting and analyzing source water samples for *Cryptosporidium* and *Giardia*, the result is virtually all zero analytical results, i.e., no organisms found. The practical result is that no information of value to understanding the occurrence of these organisms is produced and often the incorrect inference is made that the organisms are simply not present in that particular source (Hansen & Ongerth, 1991; Ongerth & Saaed, 2012).

Monitoring for these organisms has unique features that impair the ability of water quality managers to develop and implement a monitoring plan that will serve their needs for information apart from simply meeting the minimum regulatory requirement. The purpose of this paper is to provide a compilation of information essential to understanding the occurrence of *Cryptosporidium* and *Giardia* in surface waters and to provide a basis for development and implementation of monitoring plans essential to producing data on which rational watershed and treatment management can be based. Essential information includes the following:

1. Sources of the *Cryptosporidium* oocysts and *Giardia* cysts including their universal distribution, and the processes that cause the organisms to be transported to and distributed in surface water;

2. The means by which *Cryptosporidium* oocysts and *Giardia* cysts can be found in representative water samples, including information essential to understanding the limitations of sampling and analysis that must be taken into account in interpreting analytical results whether positive or negative;
3. Implementation of a monitoring plan including expression and interpretation of typical monitoring data for both *Cryptosporidium* and *Giardia*; and
4. Independent means of checking occurrence information specific to a sampling site.

### **Sources and Distribution of *Cryptosporidium* and *Giardia***

*Cryptosporidium* oocysts and *Giardia* cysts originate in the feces of infected animals including the human population. Virtually every animal population that has ever been examined, both wild and domestic, from the smallest (e.g. mice and voles) to the largest (e.g. bear, elk, cattle) has been found to be a source of one or the other or both of these organisms (Yoder et al, 2010a & 2010b). A significant proportion of the wide range of species of both *Cryptosporidium* and of *Giardia* has been shown to be associated with human infection (Thompson, 2004; Xiao and Fayer, 2008). The practical implication of this is that human and animal sources of *Cryptosporidium* and *Giardia* exist, wide-spread, in every watershed world-wide and must be considered continuous sources of oocysts and cysts.

The means by which the organisms arrive in sources of water supply begins with deposition of feces to the land surface. Weather and hydrologic processes distribute and transport fecal remnants across the land surface to water, and progressively downstream. These are the same natural processes that affect other particulate and microbiological contaminants in surface water producing the characteristic uniformity of concentrations characteristic of lakes and streams. Systematic variations occur in each portion of the generation,

distribution, and transport processes that will contribute ultimately to systematic and characteristic variations in the concentrations of oocysts and cysts that are the object of any water quality monitoring. The literature commonly describes shedding of both organisms preferentially by infants of any species. Natural or managed reproduction in any animal population will have relatively high and low parts of a typical annual cycle.

Earliest literature on *Giardia* in water (USEPA, 1978) suggested that the fecal contribution of a single animal (e.g. beaver) could account for the atypical presence of cysts/oocysts (Shaw, 1977; Lippy, 1978). This idea occurred in the period of crude and inefficient analytical procedure before current methods were developed. It was assumed that the normal condition was absence rather than presence. Data developed more with the benefit of improved analytical ability have produced a more realistic understanding (Hansen and Ongerth, 1991). Animal populations, ranging from smallest to largest, inhabit every watershed of water supply significance in numbers sufficient and at sufficient levels of infection by *Cryptosporidium* and *Giardia* that make the contribution of a single animal completely insignificant.

Weather patterns vary geographically with dryer and wetter periods in the annual cycle. Fecal deposits will tend to accumulate in dry or low runoff periods including periods of winter snow accumulation. Periods of higher runoff are well established as major contributors to sediment and microorganism transport to surface waters. Although published information on *Cryptosporidium* and *Giardia* concentrations are relatively weak, mainly owing to the lack of recovery efficiency information, available data presented below support the occurrence of typical annual cycles in the concentrations of these organisms at any selected sampling location.

Once oocysts and cysts enter surface water they are distributed by natural mixing processes that affect all dissolved and particulate inputs. Minimum energy processes contributing to mixing include molecular diffusion and Brownian motion. In lakes and reservoirs mixing also occurs as a product of bulk flow through the system and due to wind and thermal currents. The size and specific gravity of oocysts and cysts is sufficiently small that settling in the complete absence of mixing would be only a few cm/day. The uniformity of indicators of dissolved and particulate distribution strongly suggests that *Cryptosporidium* and *Giardia* will also be uniformly distributed. In a flowing stream dissolved and particulate inputs become incorporated as a product of natural mixing that causes essentially homogeneous distribution within a few equivalent stream widths travel downstream.

An indication of the magnitude of *Cryptosporidium* and *Giardia* occurrence can be gained by assessing organism source activity in the watershed of interest. Intuitively, the farthest upstream remote watershed areas having the least animal activity should produce the lowest levels that may be found in water. Again intuitively, in farther downstream areas, as human activity increases ultimately including wastewater discharges, and as domestic animal activity increases, contributions to surface water concentrations should increase, perhaps reaching a plateau suggesting a balance between increasing inputs and losses due to settling, predation, and decomposition. Some data have been reported supporting these intuitive suggestions. *Cryptosporidium* concentrations reported for upstream and downstream reaches of a Pacific Northwest river (Hansen and Ongerth, 1991) were expressed in terms of oocyst production rate per unit area per day, ranging from  $0.2 \times 10^7$  oocysts/mi<sup>2</sup>/day from a low activity watershed area to ca.  $10^8$  oocysts/mi<sup>2</sup>/day from a watershed area including dairy farming and community wastewater discharge. *Giardia*

concentrations, watershed areas, and flowrates for three rivers from protected Pacific Northwest watersheds (Ongerth, 1989) provide the basis for calculating *Giardia* cyst production rates. They ranged from  $0.05 \times 10^7$  cysts/mi<sup>2</sup>/day to  $0.84 \times 10^7$  cysts/mi<sup>2</sup>/day from protected public water supply watersheds having no unsupervised human activity.

## **Detection and Monitoring for *Cryptosporidium* and *Giardia* in Water Samples**

The problem of finding *Cryptosporidium* and *Giardia* in a water sample can aptly be described as that of finding a needle in a haystack. Oocysts and cysts that have been transported to water occur at levels reported in the literature ranging from 0.01/L to as much as 10/L. They occur in surface waters among more than  $10^6$ /L of other naturally occurring particles in the cyst-oocyst size range i.e. 1 to 20  $\mu$ m consisting of silt, detritus, and other microorganisms. To enable finding the small number of target organisms in water samples of practical volume, i.e. 10 to 50 L, requires several steps including application of sophisticated technology that must be implemented skilfully accompanied by control procedures sufficient to confirm the proper functioning of the overall procedure. Overall, an effective analytical procedure must include five essential components:

1. Collection of a representative sample of sufficient volume to permit detection;
2. A means of collecting all particles in the target size range from the water sample;
3. A means of selectively separating the target organisms from extraneous particles;
4. A means of identifying the target organisms in the final particle assemblage; and

- 131 5. Both negative control to assure the lack of contamination and positive control to  
132 measure the efficiency of recovering the target organisms from the specific water  
133 being processed, i.e., the “matrix”.

134 Beginning in the early 1980’s (Ongerth and Stibbs, 1987), prompted by waterborne  
135 outbreaks of both giardiasis and cryptosporidiosis, a 15 year period of technological  
136 development and evolution resulted in the current most widely used and standardized  
137 analytical method, USEPA Method 1623. The laboratory procedure, accompanied by  
138 rigorous QA/QC, g consists of the following steps:

- 139 1. Filtration, elution, and centrifugation to form an initial particle assemblage including  
140 the target organisms;  
141 2. Immunomagnetic separation (IMS) to preferentially concentrate the oocysts and  
142 cysts; and,  
143 3. Immunofluorescence staining (IFA) and epi-illumination microscopy for identification  
144 and counting.

145 The method requires a variety of quality control procedures designed to assure  
146 effectiveness of applying the method and to assure lack of contamination. But, Method  
147 1623 does not require measurement of recovery efficiency relevant to 95% of samples  
148 analysed. The method as written requires at least one “matrix spike” sample to be analysed  
149 for every 20 samples analysed. Typical practice among commercial and water utility labs is  
150 to analyse one spiked duplicate “matrix” sample for every 20 field samples analysed. Given  
151 the wide range of recovery efficiency observed at individual sampling locations over an  
152 annual cycle and even more so between different sampling locations, the few matrix spike

analyses simply do not permit defining the concentration of cysts and oocysts from the raw numbers (Ongerth, submitted and under review, 2012).

## ***Cryptosporidium* and *Giardia* in Water-- Data and Interpretation**

Data on the occurrence of *Cryptosporidium* and *Giardia* are available from a variety of sources in the literature and posted on the internet present significant challenges for interpretation. In reviewing data from the literature it is essential to understand the context in which they were generated. Data are essentially of two types: 1) results of single samples collected from a variety of disparate sites designed to survey the occurrence of *Cryptosporidium* and *Giardia* across a broad geographic area; and 2) results of multiple samples collected at one or more sites in one or more geographically related areas. Data generated from analytical methods prior to 2002 and the general adoption of Method 1623 must be viewed with caution and are most likely biased to the low side due to the generally lower recovery efficiencies of prior method components.

An example of *Cryptosporidium* and *Giardia* occurrence data of the first type resulted from a survey sponsored by the AWWA RF (LeChevallier et al, 1991a, 1991b). Single samples of ca. 100 L collected from 66 surface water locations in 14 US states and 1 Canadian province were analysed by the ASTM/ICR method. *Giardia* cysts were found in 69 of 85 samples ranging from 0.04 to 66 cysts/L (raw numbers not adjusted for recovery). *Cryptosporidium* oocysts were found in 74 of 85 samples, ranging from 0.07 to 484 oocysts/L (raw numbers not adjusted for recovery). These data are important in describing the virtually universal distribution of *Cryptosporidium* and *Giardia* in surface water supplies across the United



States. However, they are of little value to the needs of an individual water utility to understand features of organism occurrence at their specific water source locations.

An example of *Giardia* occurrence data collected at an individual sample location over time is available from data posted on the New York City Department of Protection (NYC DEP) website ([http://www.nyc.gov/html/dep/html/drinking\\_water/pathogen.shtml](http://www.nyc.gov/html/dep/html/drinking_water/pathogen.shtml)). The NYC DEP analysed 50 L samples weekly at three sampling sites for the calendar years 2009 and 2010. A chronological plot of the data, Figure 1, shows a pattern of relatively high occurrence beginning in mid winter, declining to relatively low levels through the summer and autumn,

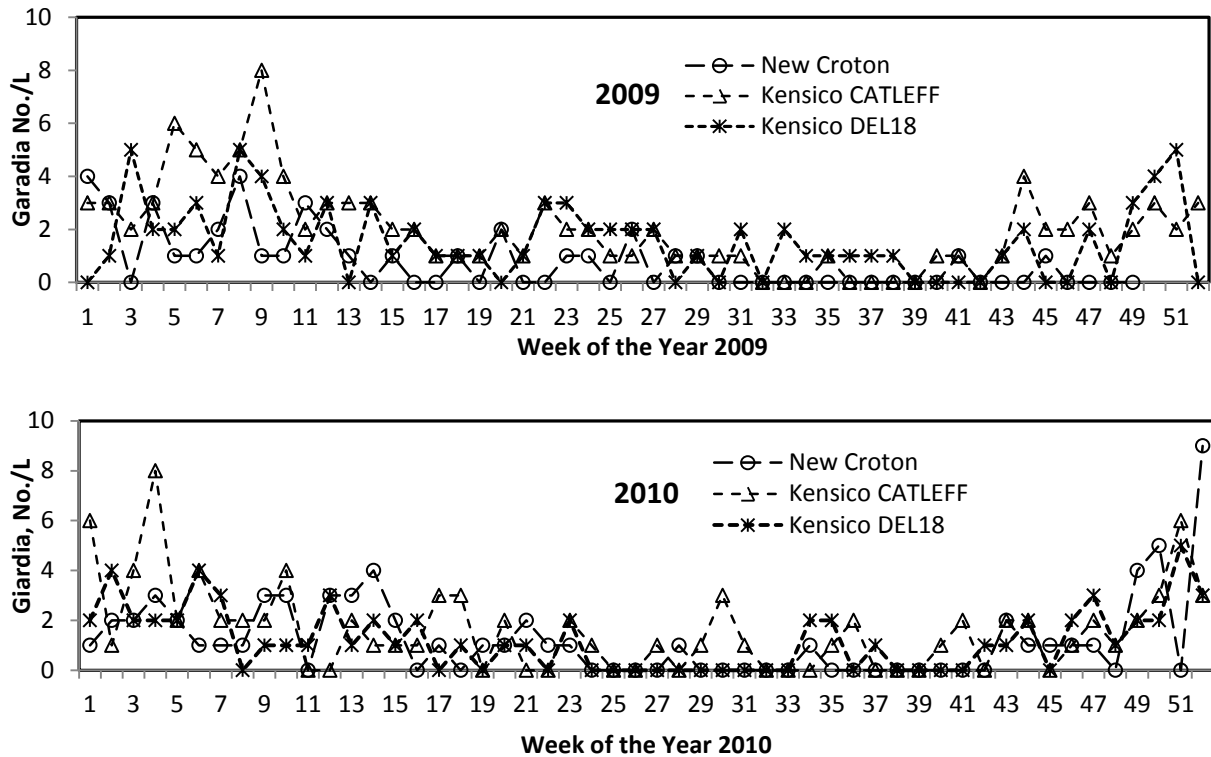


Figure 1a & b. Chronological occurrence *Giardia* raw no's vs time measured by EPA Method 1623 in 50 L samples from three NYC DEP locations over annual cycles, 2009 & 2010,.

increasing again to highest levels again in mid winter. The patterns of occurrence in the two years of data are similar but differ. The patterns observed for the three sampling sites are

also similar but with apparent differences that should be relatable to differences in watershed characteristics between the three sampling sites.

Another way of looking at annual data sets is in terms of cumulative frequency plots that can describe graphically both the relative magnitude, e.g. median values, and the degree of variability, e.g. slope. The NYC DEP *Giardia* data for 2009 and 2010 presented in the form of log-probability plots, Figures 2a and 2b, lend themselves to comparison of essential features of *Giardia* occurrence between the three sampling sites and between the observations in

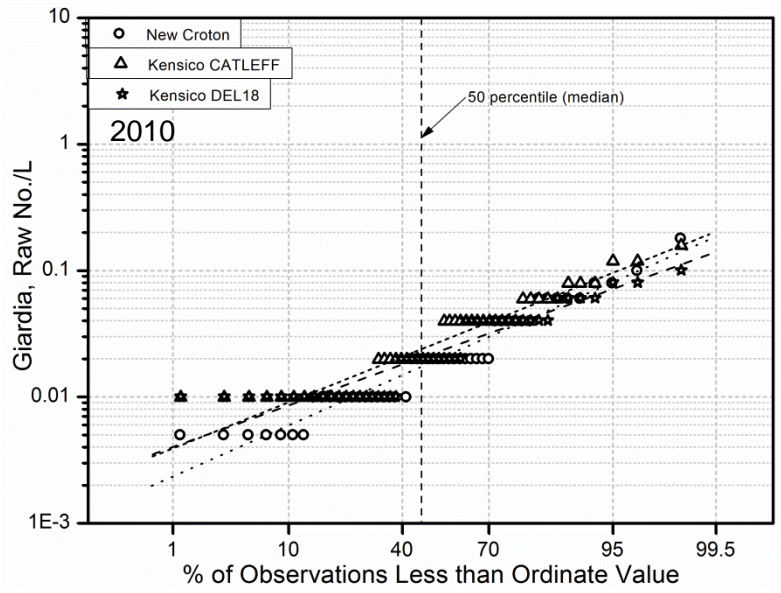
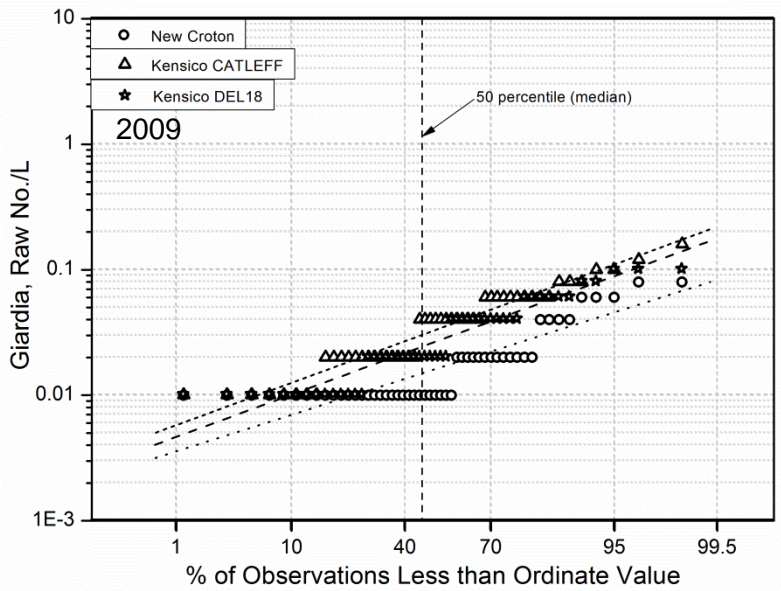


Figure 2a & b. Log-probability plots of NYC DEP *Giardia* raw numbers measured by EPA Method 1623 in 50 L samples from New Croton; Kensico CATLEFF; & Kensico DEL18, 2009 & 2010.

2009 and 2010. The median occurrence at the New Croton site was consistently lowest and levels at all three sites appear to have been marginally lower in 2010 compared to 2009.

An example of *Cryptosporidium* and *Giardia* occurrence data from a Pacific Northwest river draining a mountain watershed having little human activity other than logging, Figure 3.

These data are expressed as true concentration with recovery efficiency having been

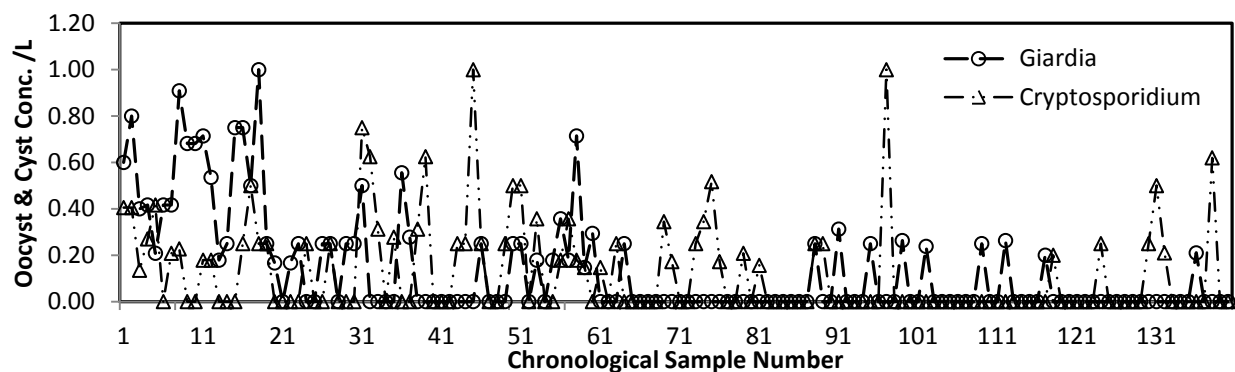


Figure 3. Chronological plot of *Cryptosporidium* and *Giardia* concentrations (No./L) in a Pacific Northwest River from a low activity watershed, July 1992 to September 1993.

measured and taken into account. The data show a pattern of relatively high concentrations through the summer and autumn of the first year with lower concentrations persisting through the winter, continuing through the second summer. The pattern of *Giardia* concentrations appears to differ from that of the *Cryptosporidium* concentrations. The same data when presented as log probability plots provide a means of comparison between the concentrations observed for both organisms. Overall, the *Giardia* (○) concentrations were marginally higher than the *Cryptosporidium* (△) concentrations. Additional lines have been added to Figure 4, representing data on both *Cryptosporidium* and *Giardia* published previously for Pacific Northwest river locations. The *Giardia* concentrations on Figure 4

cannot be compared directly to those of the NYC DEP on Figures 2a and 2b because they are reported as raw numbers. An approximate adjustment to the Figure 2 distributions could be made assuming an average recovery efficiency of 40% for *Giardia*. The distributions would

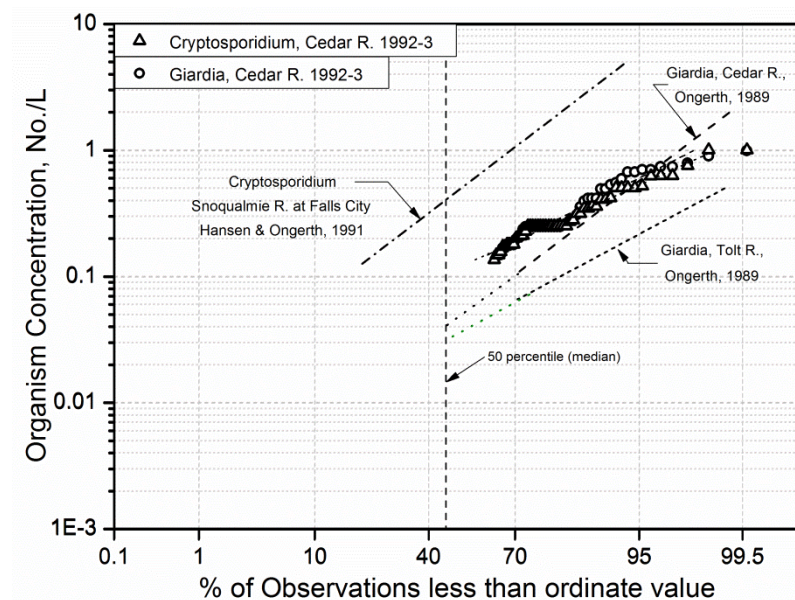


Figure 4. Log-probability plots of *Cryptosporidium* and *Giardia* concentrations for Pacific Northwest river sampling locations.

accordingly be higher than shown by a factor of  $1/0.4$  or  $\times 2.5$ . At the higher adjusted levels the NYC DEP data would be at similar levels to the late 1980's Pacific Northwest data, Ongerth, 1989.

The usefulness of data presented in this form was illustrated using LT2 data recently posted by the USEPA (USEPA, 2012), Figure 5 (Ongerth, 2012), summarizing *Cryptosporidium* occurrence (raw numbers unadjusted for recovery efficiency) from representative locations throughout the USA reporting positive (non zero) field sample results. In this form the important features of *Cryptosporidium* occurrence can be readily compared between the sampling locations. The data for each individual sampling site describe both the median level of occurrence (the ordinate value at the median or 50th percentile) and the degree of variability indicated by the slope of each distribution. The degree of risk due to

*Cryptosporidium* associated with any sampling site will be proportional to both the level and variability. An important feature of the data summarized on Figure 5 is the difference between the minimum occurrence levels measured at sampling sites for which 10 L samples were analysed and those sites

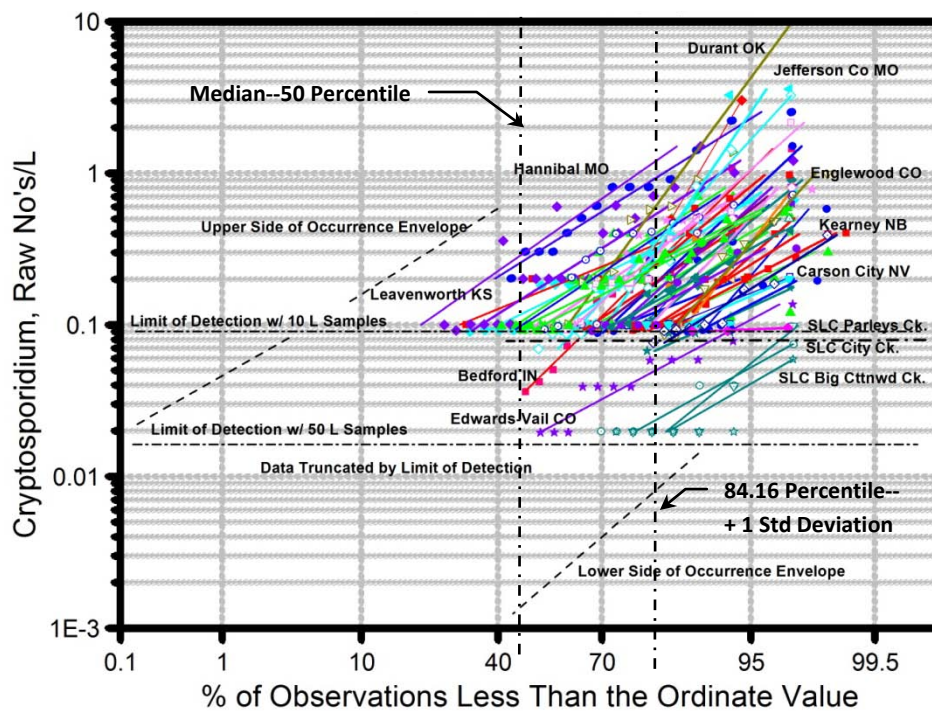


Figure 4. Log-probability plots of USEPA LT2 *Cryptosporidium* data for 50 river sampling locations representative of every region of the USA.

from which larger samples, 30 L and 50 L, were analysed. The limits of detection, not accounting for recovery efficiency, would be 0.1/L, 0.033/L and 0.02/L for samples of 10 L, 30 L, and 50 L samples respectively. The presence of *Cryptosporidium* at lower occurrence levels found by analyzing larger samples clearly illustrates that occurrence must be thought of as continuous, with occurrence below the limit of detection only hidden from observation due to the limited sample volume analysed. Finally, the data encompassed on Figure 5 appears to describe the entire spectrum of *Cryptosporidium* occurrence in surface water anywhere in the USA. Ultimately, as more data of this type are developed with



249 accompanying information on watershed area, flowrates at the time of sampling and  
250 summary information on *Cryptosporidium* and *Giardia* source activities in the watershed,  
251 calculation of watershed production rates for oocysts and cysts can be compiled and related  
252 to actual concentrations and loading rates. These data will assist in estimating likely levels at  
253 previously unsurveyed locations, and as a rough check on the quality of data produced  
254 through sampling and analysis.

## 255 **Summary and Conclusions**

256 The record of waterborne outbreak occurrence and evidence of universal distribution of  
257 *Cryptosporidium* and *Giardia* in surface water are ample motivation for public water supply  
258 water quality managers and for public health regulatory professionals to have a clear  
259 understanding the occurrence and distribution of these organisms. Tools are available to  
260 permit collection of essential data on the occurrence characteristics of *Cryptosporidium* and  
261 *Giardia* at any individual sampling location and to provide a rational understanding of  
262 features essential to their management for protection of public health. An effective  
263 sampling plan should be developed following an assessment of likely sources in the  
264 watershed combined with knowledge of water quality and typical annual cycles at the  
265 sampling point. Essential factors that will provide for useful data include: 1) sampling at a  
266 minimum of 4 to 6 times representative of typical water quality periods in the year; 2)  
267 analysis of sufficient sample volumes at each sampling time to find at least one target  
268 organism; 3) measurement of recovery efficiency with each set of samples and use of  
269 recovery efficiency measurements to calculate oocyst and cyst concentrations. An initially  
270 modest sampling program designed principally to establish the approximate concentration  
271 range provides a basis for beginning to understand *Cryptosporidium* and *Giardia* occurrence

at an individual sampling location. Evaluation of the resulting data should include comparison to indications of occurrence in other watersheds having similar characteristics. Once approximate concentration levels are known planning for more detailed monitoring is practical. The volume of additional samples should be based on the preliminary sample results with the goal of achieving on the order of 75% positive analytical results. The goal of overall data collection should be to permit clear definition of “typical” (median) concentrations, the degree of variability...whether relatively stable or not..., and the period of the year, with associated water quality characteristics, in which concentrations are typically highest. A routine program of monthly sampling and analysis should produce data to satisfy these goals. Depending on findings additional detail can be pursued as needed. If concentrations are relatively high and/or highly variable, identifying the source may be of value, particularly if the operating agency has ability to manage watershed conditions. If the agency has no control over watershed conditions the information on likely annual cycles in concentration provide important information for management of water treatment systems. Attention to the quality of treatment and operation for maximum particulate removal and achievement of minimum finished water turbidity in high concentration and high variability periods is important.

Past inclinations to view negative (zero) results of monitoring as attractive must be set aside along with past interpretations of the simple presence as being a major problem. Spending a minimum of \$450 per sample (for analysis alone) to produce no information is not only useless, it serves no useful purpose, the results are misleading, and it is a waste of public resources.

## References

1. Hansen, J. and J.E. Ongerth, 1991. Effects of time and watershed characteristics on the concentration of *Cryptosporidium* oocysts in river water. *Appl. Environ. Microbiol.*, 57(10):2790-2795.
2. Ongerth, JE, and Saaed, FMA, 2012. Distribution of *Cryptosporidium* oocysts and *Giardia* cysts in water above and below the normal limit of detection. *Parasitol Res. On-line*, <http://www.springerlink.com/openurl.asp?genre=article&id=doi:10.1007/s00436-012-3155-8>, Oct. 14, 2012
3. Shaw, PK et al. 1977. A community-wide outbreak of giardiasis with evidence of transmission by a municipal water supply. *Ann. Intern. Med*, 87:426-432.
4. Lippy, EC, 1978. Tracing a giardiasis outbreak at Berlin, New Hampshire, *J Am Water Works Assoc*, 70:512-520.
5. Ongerth, J.E. *Giardia* cyst concentrations in river water, 1989. *J. Am. Water Wks. Assoc.* 81(9): 81-86.
6. Yoder, JS, C Harral, MJ Beach, 2010a. *Cryptosporidiosis* Surveillance --- United States, 2006-2008 *Morbidity & Mortality Weekly Report*, 59(SS06); 1-14, June 11, 2010.
7. Yoder, JS, C Harral, MJ Beach, 2010b. *Giardiasis* Surveillance --- United States, 2006-2008 *Morbidity & Mortality Weekly Report*, 59(SS06); 15-25, June 11, 2010.
8. Thompson RC., 2004. The zoonotic significance and molecular epidemiology of *Giardia* and giardiasis. *Vet Parasitol*, 126:15--35.
9. Xiao L, Fayer R., 2008. Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. *Int J Parasitol*, 38:1239-55.



- 318 10. Ongerth, JE, Stibbs, HH, 1987 Identification of Cryptosporidium in river water. Appl.  
319 Environ. Microbiol. 53: 672-676.
- 320 11. Ongerth, JE, 2012. Cryptosporidium occurrence, variations, and matrix spike recovery.  
321 Presentation to USEPA LT2 ESWTR: Monitoring data analysis, occurrence forecasts,  
322 binning and the microbial toolbox; Public Meeting, Washington DC, Nov. 15, 2012
- 323 12. USEPA, 1979. Waterborne Transmission of Giardiasis. EPA-600/9-79-001  
324