

**NEW YORK CITY DEPARTMENT OF ENVIRONMENTAL PROTECTION  
BUREAU OF WATER SUPPLY**

**DEP Pathogen Mid-Term Surveillance Report on  
*Giardia* spp., *Cryptosporidium* spp., and Human Enteric Viruses**

*This report presents the six month mid-term status of DEP's watershed surveillance results for *Giardia* spp., *Cryptosporidium* spp. and human enteric viruses for waste water treatment plants, upstream source water, Kensico perennial streams, pathogen source origin, and method updates. This report provides information primarily for the period of July 1 through December 31, 2010.*

**January 31, 2011**

*Prepared in accordance with Section 5.1 of the 2007 United States Environmental Protection Agency Filtration Avoidance Determination*

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## Acknowledgments

This report summarizes results of the protozoan and virus samples collected and analyzed by the New York City Department of Environmental Protection (DEP) Watershed Water Quality Operations (WWQO) staff in the last six months of 2010 that are not already included in the current weekly and monthly DEP reports. These data are results from *Cryptosporidium*, *Giardia*, and human enteric virus samples collected from eleven Wastewater Treatment Plants (WWTP), five source water keypoints, eight Kensico perennial streams, and eight pathogen source indicator sites. In addition, this report provides updates related to pathogen method research performed during this time period. This report was produced by the Watershed Water Quality Science and Research (WWQSR) Watershed Impacts and Pathogen Assessment section, with a contribution from the WWQO Kingston Pathogen Laboratory.

The DEP WWQO field groups, both East and West of the Hudson River, collected all samples during this period. The DEP Kingston Pathogen Laboratory analyzed all *Cryptosporidium* and *Giardia* samples, and the Environmental Associates Ltd. contract laboratory analyzed all human enteric virus samples.

## **Glossary**

BSTP	Brewster Sewage Treatment Plant
DEP	New York City Department of Environmental Protection
WQ	Water Quality Directorate
EOH	East-of-Hudson
FAD	Filtration Avoidance Determination
HEV	Human Enteric Virus
HV	High Volume (50 Liters)
ICR	Information Collection Rule
L	Liter
L <sup>-1</sup>	per Liter
SDWA	Safe Drinking Water Act
USEPA	United States Environmental Protection Agency
WOH	West-of-Hudson
WMP	Watershed Water Quality Monitoring Plan
WWQO	Watershed Water Quality Operations
WWTP	Wastewater Treatment Plant
WWQSR	Watershed Water Quality Science and Research

## Executive Summary

The New York City Department of Environmental Protection (DEP) performs compliance and surveillance monitoring for *Cryptosporidium*, *Giardia* and human enteric viruses in the New York City Watershed as outlined in the Watershed Water Quality Monitoring Plan (WMP) (DEP, 2009). Routine samples (n = 152) were collected by DEP for protozoan and viral analyses during this six month reporting period (July 1<sup>st</sup> to December 31st, 2010). One additional sample was collected as part of a special investigation of an elevated *Giardia* result, but no other enhanced or storm event samples were collected during this timeframe. This report includes the sampling results not included in the current weekly and monthly reports that are already written and distributed by DEP. Routine sampling was performed weekly, monthly, bimonthly or quarterly depending on the WMP requirements.

Specifically, this document provides an update on four of the objectives outlined in the WMP: Waste Water Treatment Plants (WWTP), upstream source waters, Kensico Reservoir perennial streams, and watershed pathogen source origin. In addition, an update on laboratory in-house genotyping work is provided.

A shift in the monitoring of WWTPs by DEP was continued in 2010. Previously, the same ten plants were monitored West of Hudson (WOH) and one plant East of Hudson (EOH) since 2002. Starting in 2009, six of the ten WOH plants from the previous monitoring plan were discontinued and replaced with four WOH plants and two EOH facilities. All WWTPs were sampled at the required sampling frequency during this period and no samples were positive for *Cryptosporidium* oocysts. Of the 27 WWTP samples collected, three samples were positive for *Giardia* WOH, and three samples were positive for *Giardia* EOH (all three at the Brewster plant) this sampling period. All 15 WWTP samples collected for Human Enteric Virus (HEV) analysis were negative for this period; however, it should be noted that HEV sampling was discontinued in November 2010 at ten of the WWTPs (all sites except BSTP).

DEP monitors five reservoir effluents upstream of Kensico monthly for *Cryptosporidium* and *Giardia*. These sample sites are located at the effluents of the Cannonsville, Neversink, Pepacton, and Rondout reservoirs in the Delaware District, and the Schoharie Reservoir in the Catskill District. All samples were collected as scheduled and only one sample was positive for *Cryptosporidium* oocysts (Schoharie 12/1/2010 - 2 oocysts 50L<sup>-1</sup>) during this sampling period. *Giardia* was detected relatively frequently at the four Delaware district locations (54% positive) and at concentrations ranging from 0 to 8 cysts 35.9 L<sup>-1</sup>; however, as was seen last year, it was recovered more frequently at the Schoharie effluent (83% positive), where concentrations ranged from 0 to 31 cysts 38.9 L<sup>-1</sup>.

The eight Kensico perennial streams are sampled monthly for *Cryptosporidium* and *Giardia* (oo)cysts. *Cryptosporidium* oocysts were detected in 18.8% of the samples with results ranging from 0 to 2 oocysts per sampled volume. *Giardia* was detected in 70.8% of the Kensico stream samples with a range of 0 to 75 cysts per sampled volume. Results are generally reported per volume collected since, on occasion, sample filters clogged and the target 50 liters could not always be collected. Streams E9 and BG9 had the two highest maximum results per sample at

75 cysts  $15 \text{ L}^{-1}$  and 40 cysts  $50 \text{ L}^{-1}$ , respectively; however, the August sample at E9 had the second highest per liter *Giardia* concentration with 31 cysts found in a 13 L sample.

To satisfy the objective of monitoring for protozoan source origin, the 2009 WMP includes monthly monitoring at eight stream sites in the Delaware and Catskill districts where protozoan concentrations have historically been found to be the highest. A regular review of data from these streams is performed to further target sampling in order to identify point sources of protozoans. Site S7I was selected for targeted upstream sampling in 2010. Sampling at two of the other streams sites, at the low end of the mean protozoan concentration scale (ABCG and PMSB), was discontinued to allow for the enhanced sampling upstream of S7I. During the latter six month period of 2010, *Cryptosporidium* was found infrequently in samples at the routine source indicator sites with only 9 positive results out of 48 samples (19%) and a maximum concentration of 1 oocyst in a 15.8 L sample. *Giardia*, unlike *Cryptosporidium*, was found in all 48 samples taken at routine indicator sites, demonstrating the ubiquitous nature of *Giardia* in the WOH streams. Mean cyst concentrations for this period were found to be as high as 183.5 cysts  $50 \text{ L}^{-1}$ , heavily influenced by a maximum sample result of 251 cysts  $15.8 \text{ L}^{-1}$  at stream site S4. This sample was taken on December 1<sup>st</sup>, 2010 when over an inch of rain was received for the day. Samples targeted for identifying the source of protozoa found at S7I were rotated among 4 sites upstream of the S7I site in 2010, with 3 of these upstream sites monitored during the last six months of 2010. Results from the most current upstream sites have been relatively high, and have closely resembled S7I values, indicating a potential source in the sub-basin. Upstream sampling will likely continue in this sub-basin for several additional months.

DEP continues to investigate genotyping methods to develop an in-house genotyping program. As a follow up to the last report, after many trials and difficulties using the Invitrogen Kit and dealing with the manufacturer, it was decided that this method would be dropped and other methods investigated. It was at this time that AWWA was offering a hands-on genotyping workshop in Savannah, Georgia, and DEP sent a representative. Specifically this workshop provided training on a simple and reliable method for the genotyping of *Cryptosporidium*. After this workshop, DEP decided to work towards using this method in its genotyping efforts. One of the first steps necessary was to replace the existing mounting media used in slide preparation with a non-formalin based mounting media due to the fact that formalin damages DNA and; therefore, interferes with genotyping. Currently the lab is testing different mounting media and purchasing the appropriate supplies for this new genotyping method.

## 1.0 Introduction

Since July of 2002, all *Cryptosporidium* and *Giardia* samples (50-liter volumes, unless specified) have been collected and analyzed by DEP following USEPA Method 1623HV (USEPA, 2005). Human enteric virus sampling and analyses follow general procedures listed in the Information Collection Rule (ICR) Microbial Laboratory Manual (USEPA, 1996) and have been processed by Environmental Laboratory Associates Ltd. While references may be made to historical data, previous semi-annual reports or the DEP website should be reviewed to assess historical data.

## 2.0 Surveillance Monitoring

This section addresses four of the objectives outlined in the Watershed Water Quality Monitoring Plan (WMP). The purpose of the first subsection is to present results of the long-term monitoring of (oo)cysts and human enteric viruses at WWTPs in order to monitor treatment upgrades. The second and third subsections are a summary of data collected from the upstate keypoint source water monitoring and the surveillance data from the perennial tributaries of Kensico Reservoir. The fourth section is an update on DEP's sampling program at stream indicator sites attempting to identify any potential point source or non-point source origins of protozoa in the watershed.

### 2.1 Long-term (oo)cyst and Human Enteric Virus Monitoring at WWTPs

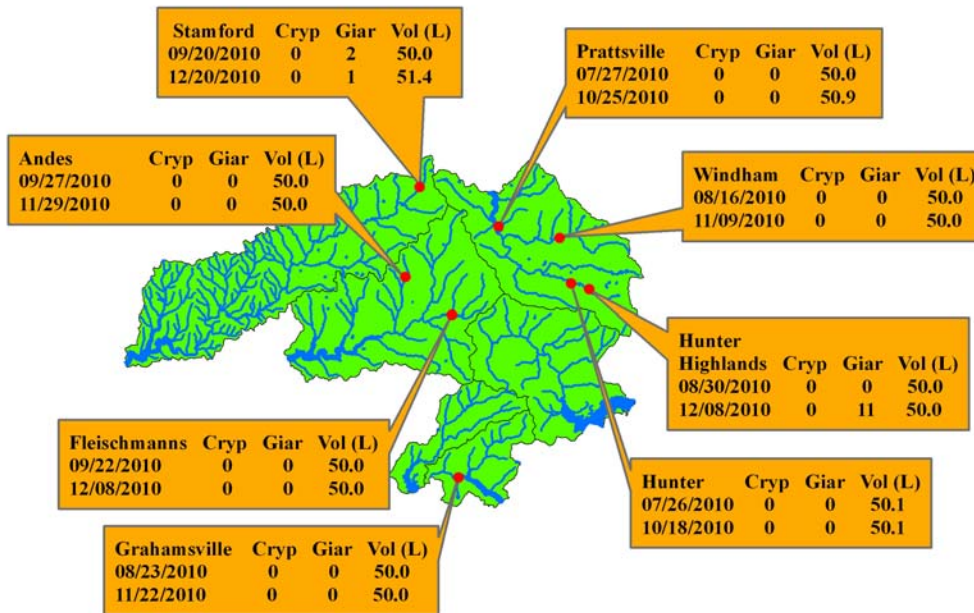
Sampling was conducted quarterly at 10 of the plants as required by the WMP; therefore, they were monitored at least twice during this six month reporting period. The exception was the Brewster WWTP, which is monitored monthly for protozoan pathogens and bi-monthly for human enteric viruses (HEVs), as specified by the Croton Consent Decree. In total, there are 11 wastewater plants currently being monitored; eight WOH and three EOH. DEP proposed discontinuing monitoring for viruses at WWTPs in 2010 and this request was approved by NYS DOH and took effect on November 1, 2010.

#### *West of Hudson*

Of the 16 WWTP samples collected West of Hudson during the latter half of 2010, there were no positive samples for *Cryptosporidium*, and there were three positive samples for *Giardia*. Two of the detections occurred at Stamford WWTP, and the other detection was at Hunter Highlands WWTP (Figure 1). Both Stamford and Hunter Highlands WWTPs had past *Giardia* detections which were the basis for continued monitoring at these sites as part of the new monitoring plan. As mentioned in previous reports, it was suspected that wildlife may have had access to open areas (chlorine contact tank, etc...) of the treatment process at the Grahamsville and Hunter Highland sites. As a result, DEP decided to move the sample collection sites from post open chlorine contact tank, to a locations prior to the open areas. While the change in sampling site



may have helped at the Grahamsville location (no detects this period) it may not have made as much of an impact at the Hunter Highlands plant with 11 *Giardia* detected in the December sample.



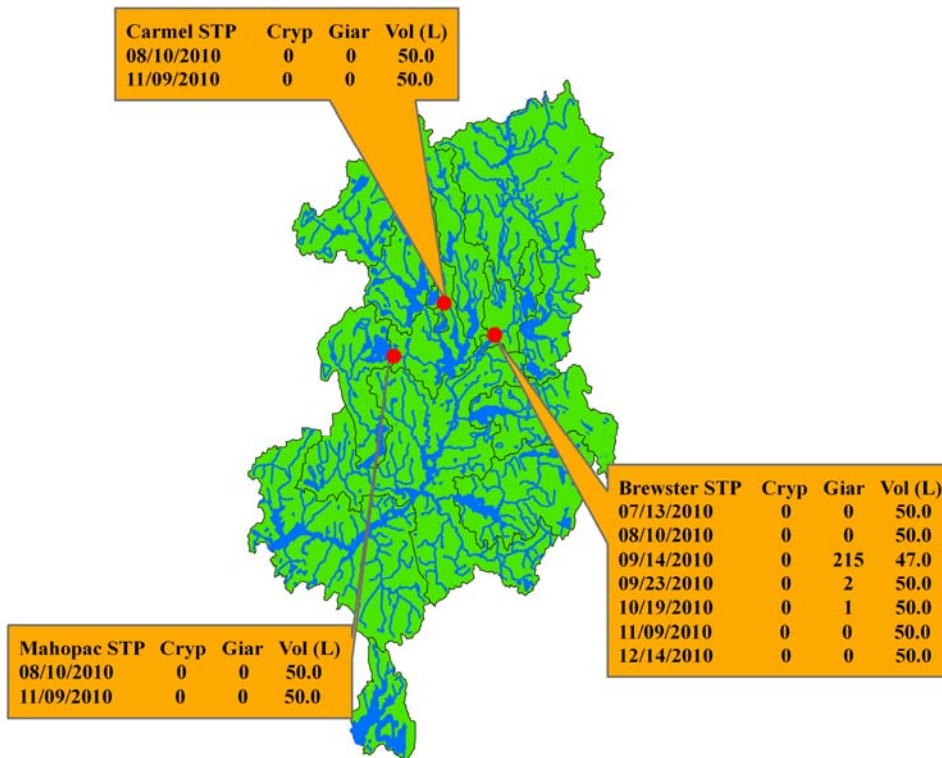
**Figure 1 - *Cryptosporidium* and *Giardia* monitoring results and sample volumes at WOH WWTPs: July 1<sup>st</sup> - December 31<sup>st</sup>, 2010.**

All HEV results for samples collected at these WOH WWTPs were negative for the latter half of 2010.

### ***East of Hudson***

EOH WWTP monitoring included monthly protozoan monitoring and bimonthly HEV monitoring at the Brewster WWTP, and quarterly monitoring for protozoa and HEV at the Carmel and Mahopac WWTPs. No *Cryptosporidium* oocysts were found in any of the samples taken at the three EOH treatment plants during this six month period; however, *Giardia* was detected. A high concentration of *Giardia* cysts was detected in the September 14<sup>th</sup> Brewster sample (215 cysts 47L<sup>-1</sup> sample) [BSTP had 3 positive *Giardia* samples last year during the same six month period; however, all were only 1 cyst]. A follow up sample was collected on September 23 with a result of 2 cysts 50L<sup>-1</sup>, and subsequently 1 cyst 50L<sup>-1</sup> was detected in the routine October sample. Upon returning to collect the September 23<sup>rd</sup> sample, DEP discovered that prior to the first positive sample, Brewster WWTP was bypassing its microfiltration step due to an operational issue with the compressor. It is believed that the elevated result found during this reporting period is related to the bypass, and the subsequent detections appear to have

tapered off as time progressed, returning to zero cysts for both the November and December samples. All samples taken at the Carmel and Mahopac plants during this period were negative for *Giardia* cysts (Figure 2).



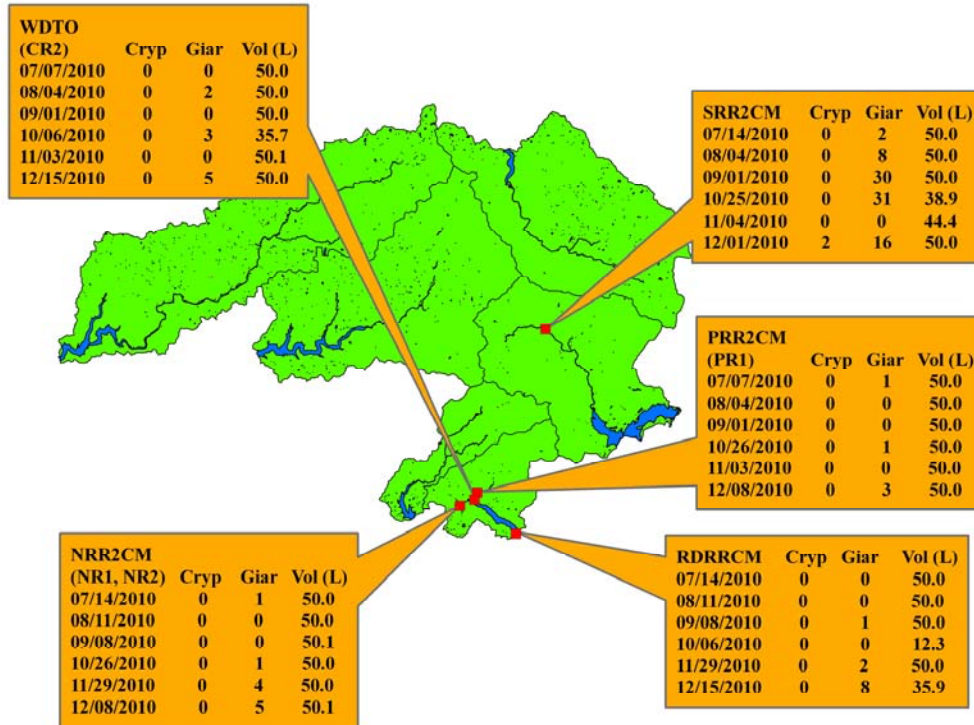
**Figure 2 - *Cryptosporidium* and *Giardia* monitoring results and sample volumes at EOH WWTPs: July 1<sup>st</sup> - December, 2010.**

No HEV were recovered from any of the EOH treatment plant samples for this sampling period.

## 2.2 Keypoint Monitoring Upstream of Source Water

DEP conducts monthly monitoring at five upstate reservoir effluents. Four of these effluents are in the Delaware district - Cannonsville, Pepacton, Neversink, and Rondout (WDTO, PRR2CM, NRR2CM, RDRRCM, respectively) and one reservoir effluent in the Catskill district, Schoharie Reservoir (SRR2CM), is monitored at the outlet of the Shandaken Tunnel.

During the past six months each site was sampled six times (Figure 3). As part of this objective, DEP is also required to sample the Catskill and Delaware source waters just prior to their entry to Kensico Reservoir. However, the Catskill and Delaware influent sample results (CATALUM and DEL17, respectively) are already reported monthly according to FAD requirements, hence those results have not been repeated in this report.



**Figure 3** - Upstream keypoint *Cryptosporidium* and *Giardia* monitoring results and sample volumes: July 1<sup>st</sup> - December 31<sup>st</sup>, 2010.

All four Delaware system upstream source water sites were negative for *Cryptosporidium* during this period (Figure 3, Table 1). The Catskill system had one *Cryptosporidium* detection (2 oocysts 50 L<sup>-1</sup>) in December at the outlet of the Shandaken tunnel; however, the annual mean at this site remains very low at 0.17 oocysts 50 L<sup>-1</sup> for 2010.

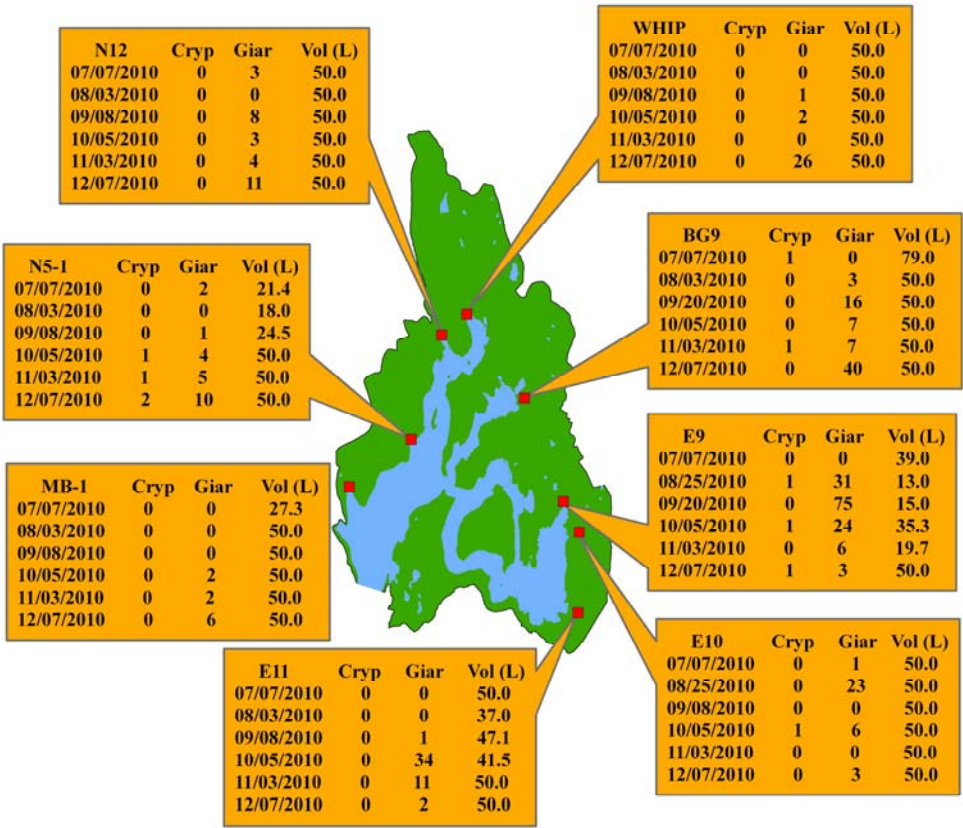
*Giardia* results at the four Delaware sites were similar to those during the same period in 2009, with an overall average of approximately 1.5 cysts for this six month period, and an average detection rate of 54%, (Figure 3). The Catskill Schoharie Reservoir effluent site; however, had a slightly higher average this year (14.5 cysts) compared to last year (9.2 cysts) and an 83% detection rate. The two highest results for the period were both at the Schoharie effluent and occurred in the September and October samples of 2010.

**Table 1** - Upstream reservoir effluent detection rates and maximum counts for *Cryptosporidium* and *Giardia* during the sampling period July 1<sup>st</sup> – December 31<sup>st</sup> 2010.

		Neversink	Pepacton	Rondout	Schoharie	Cannonsville
<i>Cryptosporidium</i>	% Positive	0%	0%	0%	16.7%	0%
	Maximum	0	0	0	2 (50.0 L <sup>-1</sup> )	0
<i>Giardia</i>	% Positive	66.7	50.0%	50.0%	83.3%	50.0%
	Maximum	5 (50.1 L <sup>-1</sup> )	3 (50.1 L <sup>-1</sup> )	8 (35.9 L <sup>-1</sup> )	31 (38.9 L <sup>-1</sup> )	5 (50.1 L <sup>-1</sup> )

### 2.3 Evaluation of Kensico Reservoir Stream Inputs

Eight perennial streams representing the major watershed inputs of Kensico Reservoir were monitored monthly during this period. These eight streams represent a very small proportion, approximately 0.5%, of Kensico Reservoir’s annual flow budget (Pace *et al.*, 2006) in relation to the Catskill and Delaware influents (over 1 billion gallons per day). However, these streams are monitored for pathogens due to their proximity to Kensico Reservoir effluents and their potential for an abrupt, negative impact on those effluents. During the July through December sampling period, 48 samples were collected and analyzed for *Cryptosporidium* and *Giardia* (Figure 4). The target sample volume was always 50 liters; however, 12 of the 48 samples (25%) (compared to 19 out of 48 samples during the same period last year) were less than 50 liters when filters became clogged with suspended particles during collection. Due to a field error, the July BG9 sample resulted in 79 liters filtered, regardless, both *Giardia* and *Cryptosporidium* results were low.



**Figure 4** - Kensico perennial streams, *Cryptosporidium* and *Giardia* monitoring results and sample volumes: July 1<sup>st</sup> - December 31<sup>st</sup>, 2010.

Analysis of Kensico stream protozoan samples generally demonstrated the very low occurrence of *Cryptosporidium* found in these streams, as only 9 of 48 (18.8%) samples were positive for *Cryptosporidium* (matching 2009 period) and the highest individual sample count was 1 oocyst in a 13-L sample at E9 in August (not all volumes were 50L – Figure 4). Positive *Cryptosporidium* samples did not seem to center on one stream or area of the reservoir, but was dispersed among four of the eight streams in the watershed with a relatively infrequent overall occurrence. Percent detection and maximum counts per stream are provided (Table 2).

**Table 2** - Kensico perennial stream percent detection and maximum counts for *Cryptosporidium* and *Giardia* during the sampling period July 1<sup>st</sup> – December 31<sup>st</sup> 2010.

Site	<i>Cryptosporidium</i>		<i>Giardia</i>	
	% Detection	MAX*	% Detection	MAX*
BG9	33%	1	83%	40
E10	17%	1	67%	23
E11	0%	0	67%	34
E9	50%	1	83%	75
MB-1	0%	0	50%	6
N12	0%	0	83%	11
N5-1	50%	2	83%	10
WHIP	0%	0	50%	26

\* Maximum values are per sample, regardless of individual sample volumes, see Fig. 4 for volumes.

*Giardia* was detected in 34 of 48 (70.8%) Kensico tributary samples taken during this period, with results ranging from 0 to 75 cysts per sample with varying sample volumes below 50L, suggesting actual concentrations were likely higher. Notably, 12 of the 48 samples taken had volumes less than 50 liters (Figure 4); however, this seemed to have little effect on the overall rate of *Giardia* detection as the rate for 50L (or greater) samples at all Kensico streams was 72.2% compared to 70.8% for samples under 50L. Thirty-seven of the 48 samples had values of less than 10 cysts per volume sampled.

## 2.4 Watershed Pathogen Source Origin

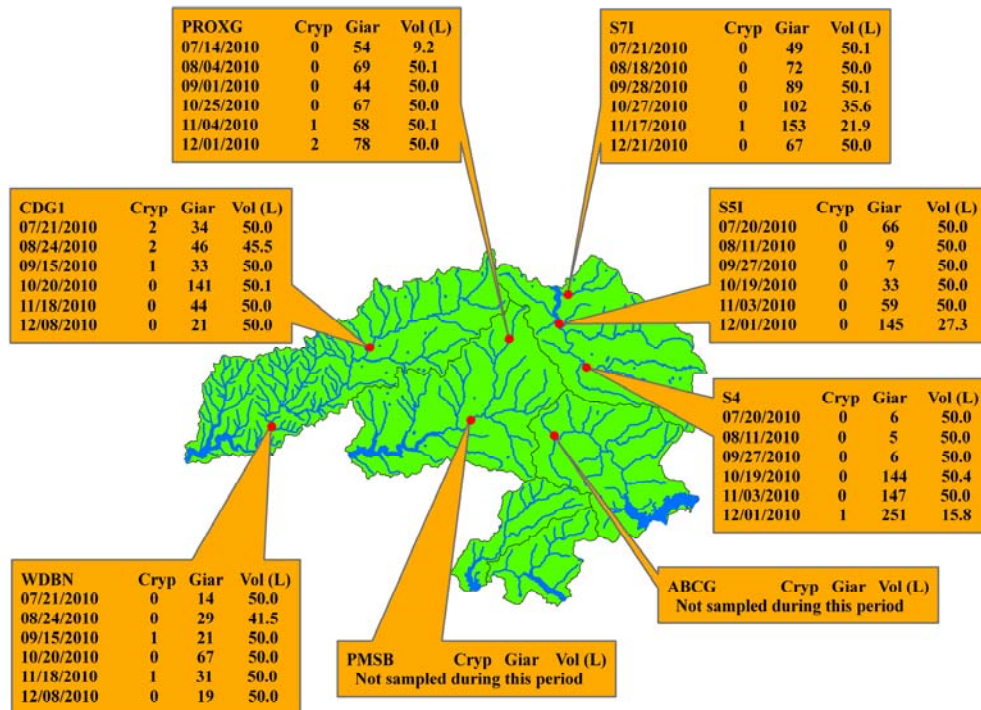
To satisfy the objective of monitoring WOH stream sites with higher mean protozoan concentrations relative to other previously monitored sites, the WMP includes monthly monitoring at a total of eight stream sites located in the Delaware and Catskill districts.

The purpose of this objective is to assess the eight indicator streams and use the data to target additional sampling upstream in order to identify potential point sources of protozoa. To accomplish this goal, the data must be reviewed regularly for unusual occurrences for the purpose of responding to changes in the streams. As reported previously, analysis of historical data up through June 2009 indicated no concerns regarding *Cryptosporidium* concentrations at the eight sites; however, there were some interesting *Giardia* results. Of the eight sampling locations, the one with the highest mean *Giardia* concentration was S7I (Manorkill), which is located on the Schoharie Creek (Figure 5). The *Giardia* mean at S7I was 183 cysts 50L<sup>-1</sup>. Site PROXG in Pepacton was the second highest, with 128 cysts 50L<sup>-1</sup>, while the mean results at the other six sites ranged between 22 and 64 cysts 50L<sup>-1</sup>.



In order to investigate two new sites upstream of S7I, DEP discontinued sampling at the two sites that had the lowest *Giardia* means, which were PMSB in Pepacton and ABCG in Ashokan. As a result, neither PMSB nor ABCG were sampled during 2010, as DEP rotated through several sites upstream of S7I.

*Cryptosporidium* data were, again, not remarkable for the six routine sites monitored during this reporting period, with no more than 2 oocysts detected per volume sampled and a 25% occurrence rate (Figure 5). Mean concentrations of *Cryptosporidium* remained relatively stable with a few small increases compared to last year's results during the same reporting period. As with other sampling sites in the NYC watershed, *Cryptosporidium* was found at very low levels, and very near the detection limit of the method, hence any increases in mean concentrations are generally within the expected variability of the data. *Giardia*, on the other hand, were detected 100% of the time at the six routine sites, demonstrating the ubiquitous nature of *Giardia* in WOH streams, and were recovered at a range of 5 to 251 cysts per volume sampled (Figure 5). The mean values per 50 liter volume ranged from 39 at WDBN, to 184 at site S4. Site S7I had the second highest mean *Giardia* value of 128 cysts 50L<sup>-1</sup> (Table 3). Sites S4 and S7I both showed increases in mean *Giardia* concentration compared to the same period in 2009 when the means were 32 and 30 cysts 50L<sup>-1</sup>, respectively. Overall, these results support historical data showing that *Giardia* is generally one to two orders of magnitude higher in concentration than *Cryptosporidium* in the watershed.



**Figure 5** – Watershed pathogen source indicator site *Cryptosporidium* and *Giardia* monitoring results and sample volumes: July 1<sup>st</sup> - December 31<sup>st</sup>, 2010.

**Table 3** - Mean concentrations and maximum counts for *Cryptosporidium* and *Giardia* at routine WOH indicator streams. July 1<sup>st</sup> – December 31<sup>st</sup> 2010. (NS= not sampled)

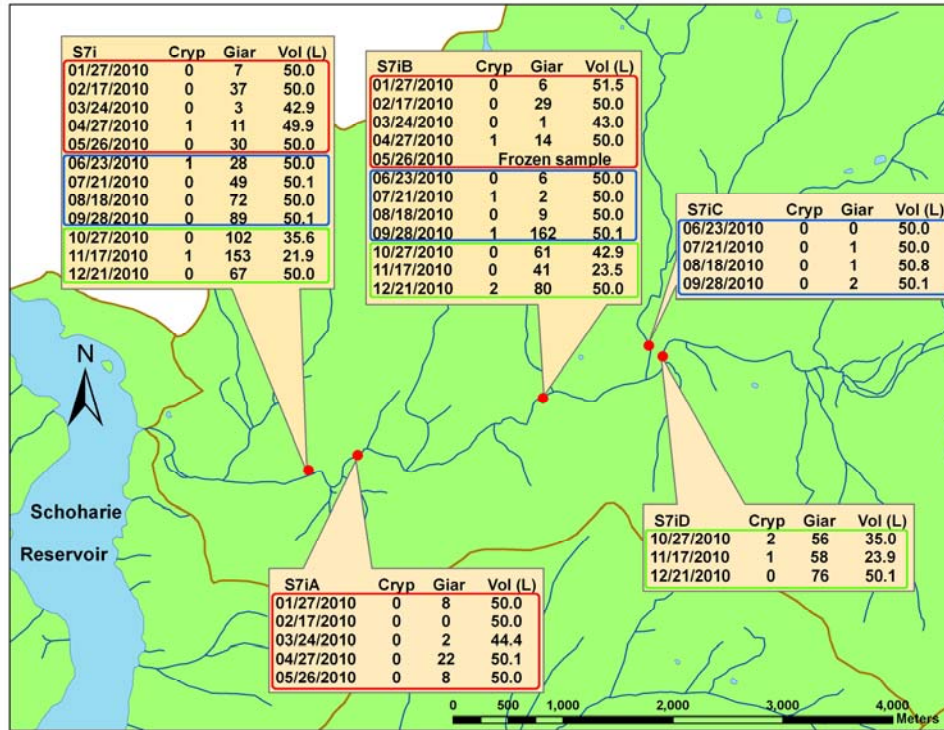
Site	<i>Cryptosporidium</i>		<i>Giardia</i>	
	MEAN <sup>†</sup> (50 L <sup>-1</sup> )	MAX*	MEAN <sup>†</sup> (50 L <sup>-1</sup> )	MAX*
ABCG	NS	NS	NS	NS
CDG1	0.87	2	53.88	141
PMSB	NS	NS	NS	NS
PROXG	0.50	2	101.54	78
S4	0.53	1	183.53	251
S5I	0.00	0	73.26	145
S7I	0.38	1	128.22	153
WDBN	0.33	1	39.00	67

<sup>†</sup>All samples were standardized to 50L volumes before calculation of mean concentrations.

\*Maximum values are per sample, regardless of individual sample volumes, see Fig. 5 for volumes.

Targeted sampling upstream of site S7I began in January 2010 with sites S7IA and S7IB (Figure 6). While S7IA sampling occurred outside of this reporting period, results are included here to provide insight into to further site selection in the latter half of the year. Site S7IA was selected for the reason that it is located just downstream from a confluence that drains farmland, and Site S7IB was chosen since it is just downstream of a moderately sized tributary. Also considered during site selection, was the accessibility of the site (terrain), and whether outside permission was needed prior to entry of the site. These sites were sampled monthly from January through May on the same day that S7I was sampled. Generally, and unexpectedly, Site S7IA, which is the closest to S7I, had more discordant results when compared to S7I, than S7IB (Figure 6, Table 4). While two of the five S7IA results were similar to S7I, all four of the samples collected at S7IB were similar to S7I.





**Figure 6** – Map of targeted upstream sampling sites in the Manorkill watershed.

**Table 4** - Mean concentrations and maximum counts for *Cryptosporidium* and *Giardia* at S7I and targeted upstream sites, July 1<sup>st</sup> – December 31<sup>st</sup> 2010. (NS= not sampled)

Site (sample period)	<i>Cryptosporidium</i>		<i>Giardia</i>	
	MEAN <sup>†</sup> (50 L <sup>-1</sup> )	MAX*	MEAN <sup>†</sup> (50 L <sup>-1</sup> )	MAX*
S7I (Jan-Dec)	0.38	1	128.22	153
S7IA (Jan-May)	0.00	0	8.05	22
S7IB (Jan-Dec)	0.67	2	68.50	162
S7IC (Jun-Sep)	0.00	0	1.33	2
S7ID (Oct-Dec)	1.65	2	92.40	76

<sup>†</sup>All samples were standardized to 50L volumes before calculation of mean concentrations.

\*Maximum values are per sample, regardless of individual sample volumes, see Fig. 5 for volumes.

After this data assessment was completed, it was decided to keep S7IB and replace S7IA with a site further upstream. Approximately one mile upstream of S7IB the creek splits into two branches. Once again, farmland known to have livestock was the reason for selecting the left branch of the creek (Bearkill) for S7IC (Figure 6). The three sites were sampled monthly from June through September, and not only were no oocysts recovered, but the maximum result for *Giardia* at S7IC was 2 cysts 50L<sup>-1</sup> in September. Conversely, September samples for S7IB and

S7I were 162 and 89 cysts, respectively. Subsequently, S7IC was replaced with site S7ID, which is located on the right branch of the creek (Figure 6).

To date, results from S7ID samples collected from October through December have been very similar to those collected at S7IB, and comparable to two of the three samples collected at S7I. The remaining S7ID sample (November, 121 cysts  $50L^{-1}$ ) was less than half the S7I result (349 cysts  $50L^{-1}$ ); however, there may at times be an additive effect of S7ID with S7IB (87 cysts  $50L^{-1}$ ), and other streams, that may explain the data. Unless further analysis is performed on the cysts recovered from these sites it will be difficult to be certain how much of an effect, additive or otherwise, these upstream sites have on S7I. Additionally complicating the assessment, is the fact that not all samples are the same volume due to filter clogging and collection pressure and that travel time varies between the sites. Therefore, extrapolation needs to be performed to estimate concentrations when comparing sites, and this can be misleading if cysts are not homogeneously distributed in the stream. In any event, future plans include additional sampling at S7ID, as well as further assessment of the land use adjacent to the site.

### **3.0 Pathogen Projects and Method Updates**

This section provides updates on pathogen related projects or method research conducted by DEP during the course of this reporting period.

#### **3.1 In-House Genotyping**

The NYC DEP Analytical Pathogen Laboratory staff has been researching and practicing the skills necessary to perform genotyping on *Cryptosporidium* oocysts from its water samples.

The Pathogen Laboratory began working with Invitrogen's™ *Cryptosporidium* Genotyping Kit (series A10387) in 2009. This kit was designed to provide a simple molecular method for detecting *Cryptosporidium* oocysts captured during water testing following immunomagnetic separation techniques that are already utilized in the DEP laboratory. However, the kit posed some problems that were unable to be explained by Invitrogen™.

After many trials using the Invitrogen Kit, it was decided that this method would be dropped and other methods investigated. It was at this time that AWWA was offering a hands-on workshop in Savannah, Georgia, and DEP allowed one individual to attend. Specifically this workshop provided training on a simple and reliable method for the genotyping of *Cryptosporidium*. After this workshop, DEP decided to work towards using this method in its genotyping efforts. One of the first steps necessary was to replace the existing mounting media used in slide preparation with a non-formalin based mounting media due to the fact that formalin damages DNA. Currently the lab is testing different mounting media and purchasing the appropriate supplies for this new genotyping method.

Ultimately, the goal is to acquire the ability to successfully genotype *Cryptosporidium* oocysts recovered in the NYC watershed from the Method 1623 microscopy slides. As a note, DEP is a participating utility in a Water Research Foundation grant for selecting and standardizing the most appropriate tool for regulatory *Cryptosporidium* genotyping, for which some initial work will begin in the upcoming year.

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