

**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. and *Cryptosporidium* spp. for wastewater 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, 2011.*

**January 31, 2012**

*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 2011 that are not already included in the current weekly and monthly DEP reports. These data are results from *Cryptosporidium* and *Giardia* samples collected from 11 Wastewater Treatment Plants (WWTP), 5 source water keypoints, 8 Kensico perennial streams, and 8 pathogen source indicator sites. In addition, this report provides protozoan and human enteric virus (HEV) results for the Brewster WWTP, and updates related to the process of implementing molecular and HEV methods in the DEP laboratory 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.

## List of Acronyms

|                 |  |
|-----------------|--|
| BSTP            | Brewster Sewage Treatment Plant                      |
| DEP             | New York City Department of Environmental Protection |
| WQD             | 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  |
| MPN             | Most Probable Number                                 |
| SDWA            | Safe Drinking Water Act                              |
| EPA             | United States Environmental Protection Agency        |
| WOH             | West of Hudson                                       |
| WWQMP           | 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 (HEV) in the New York City Watershed as outlined in the Watershed Water Quality Monitoring Plan (WWQMP) (DEP 2009). Routine samples (n = 150) were collected by DEP for protozoan and viral analyses during this six-month reporting period (July 1 to December 31, 2011). Two additional samples were collected as part of the response to Tropical Storms Irene and Lee. 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 WWQMP requirements.

Specifically, this document provides an update on four of the objectives outlined in the WWQMP: Wastewater Treatment Plants (WWTPs), upstream source waters, Kensico Reservoir perennial streams, and watershed pathogen source origin. In addition, an update on laboratory in-house method training is provided.

All WWTPs were sampled at the required sampling frequency during this period and no samples were positive for *Cryptosporidium* oocysts. Of the 26 WWTP samples collected, two samples were positive for *Giardia* WOH, and no samples were positive for *Giardia* EOH this sampling period. Of the three samples collected at BSTP for HEV analysis, one was positive during this period. As reported previously, HEV sampling at the ten quarterly sampled WWTPs was discontinued with NYS DOH approval in November 2010.

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. Of the 25 routine samples, only one sample was positive for *Cryptosporidium* oocysts (Schoharie 11/17/2011 – 1 oocyst 30.4L<sup>-1</sup>) during this sampling period. *Giardia* was detected relatively frequently at the four Delaware district locations (40% positive) and at concentrations ranging from 0 to 4 cysts 9.0 L<sup>-1</sup>. As was seen last year, *Giardia* was recovered more frequently at the Schoharie effluent (80% positive), where concentrations ranged from 0 to 19 cysts 31.8 L<sup>-1</sup>. One non-routine sample was taken at the outlet of Ashokan Reservoir (EARCM) after Tropical Storm Irene on August 31. This 50 L sample was positive for *Giardia* (one cyst) and *Cryptosporidium* (two oocysts).

The eight Kensico perennial streams are sampled monthly for *Cryptosporidium* and *Giardia* (oo)cysts. *Cryptosporidium* oocysts were detected in 6.1% of the samples, with results ranging from 0 to 2 oocysts 50.1L<sup>-1</sup>. *Giardia* was detected in 93.9% of the Kensico stream samples, with a range of 0 to 143 cysts 50.1L<sup>-1</sup>. Stream E9 had the two highest *Giardia* results per sample, at 143 cysts 50.1L<sup>-1</sup> and 72 cysts 50 L<sup>-1</sup>; however, the October sample at E11 had the second highest per liter *Giardia* concentration, with 54 cysts found in a 27 L sample.

To satisfy the objective of monitoring for a geographic source origin for protozoa, the 2009 WWQMP 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 identified site S7I for targeted upstream sampling beginning in January 2010. During the latter six-month period of 2011, *Cryptosporidium* was found in 14 of the 36 samples (38.9%) collected at the six routine source indicator sites, with a maximum concentration of 5 oocysts in a 25.1 L sample found in the East Branch Delaware River site at Roxbury (PROXG). *Giardia* was found in all but one of the 36 samples taken at the six routine indicator sites, demonstrating the ubiquitous nature of *Giardia* in WOH streams. Mean cyst concentrations for this period were found to be as high as 89.76 cysts 50 L<sup>-1</sup> at stream site PROXG. Samples targeted for identifying the geographic source of protozoa found at S7I were rotated among four sites upstream of the S7I site in 2011, with three of these upstream sites monitored during the last six months of 2011. Results from the most current upstream sites have continued to closely resemble those results from the downstream S7I site, indicating a potential source in the sub-basin. Upstream sampling will likely continue in this sub-basin for several additional months.

DEP has continued to investigate genotyping methods to develop an in-house genotyping program. As a follow up to the last report, a new mounting medium was selected that would not interfere with polymerase chain reaction (PCR) testing. The new medium was validated in-house, and approved for use in January 2011. Additional trials of PCR throughout the year indicated that a new thermal cycler was needed. DEP researched and purchased a new thermal cycler and the result was improved recovery of oocysts from Method 1623 slides. Preliminary results have demonstrated the ability to genotype a single *Cryptosporidium* oocyst retrieved from the microscope slide after quantitative analysis has been completed. In addition to this work, DEP has begun the process of bringing HEV analysis in-house as well.

## 1.0 Introduction

Since July 2002, all *Cryptosporidium* and *Giardia* samples (50 L volumes, unless specified) have been collected and analyzed by DEP following EPA 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. Appendix A, displaying mean concentrations of protozoa per liter, is provided at the end of the document in order to simplify a comparison of data among the non-WWTP sites.

## 2.0 Surveillance Monitoring

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

### 2.1 Long-term Protozoan Monitoring at WWTPs

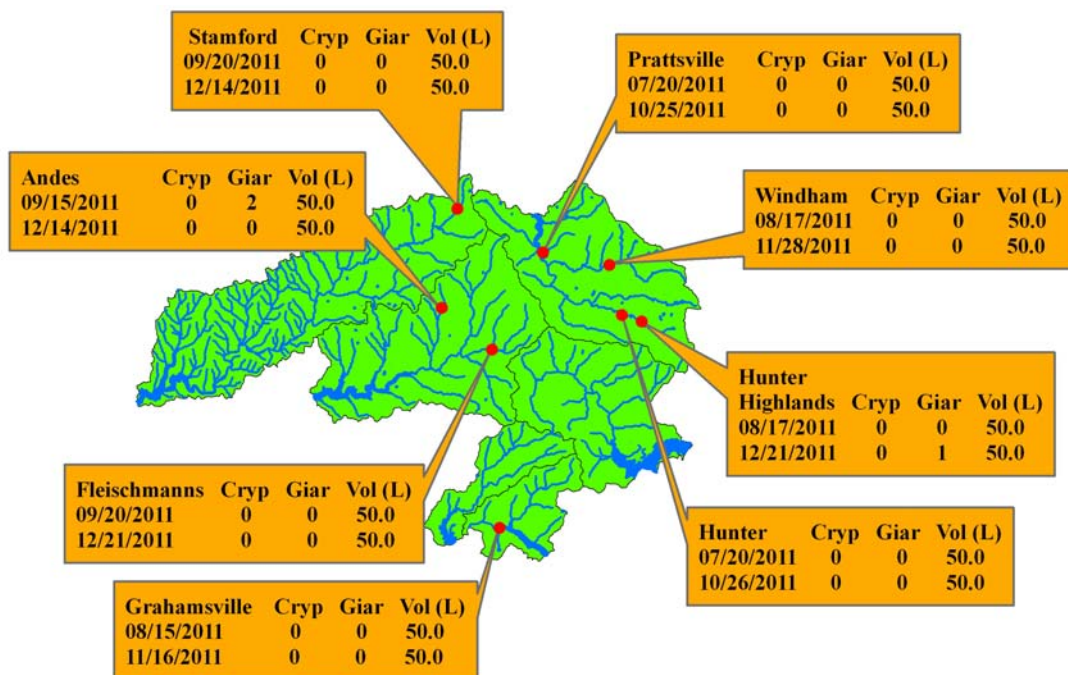
Sampling was conducted quarterly at 10 plants as required by the WWQMP; therefore, they were monitored at least twice during this six-month reporting period. The exception was the Brewster WWTP, which is monitored monthly for protozoan pathogens and bi-monthly for human enteric viruses (HEVs), as specified by the Croton Consent Decree (CCD). In total, there are 11 WWTPs currently being monitored: eight WOH and three EOH. In 2010, DEP proposed discontinuing monitoring for viruses at non-CCD WWTPs, 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 2011, there were no positive samples for *Cryptosporidium*, and there were two positive samples for *Giardia* (Figure 1). One detection occurred at the Andes WWTP, and this was the first detection on record for this plant. A potential explanation for the detection may have been the bypassing of some treatment steps (SBRs, sand, microfiltration) in order to prevent an overflow at the plant in the wake of Tropical Storm Lee on September 7. Plant operators resumed treatment steps on September 8, with sand and microfiltration coming back on-line September 9 at 8:00 AM. After the plant resumed normal operations on September 9, the post-aeration tank, ultraviolet lights and trough, and the effluent meter pit were cleaned. All of this activity, in addition to continued drainage from the storms, may have provided a possible source for the 2 *Giardia* cysts found in the sample collected on September 15.



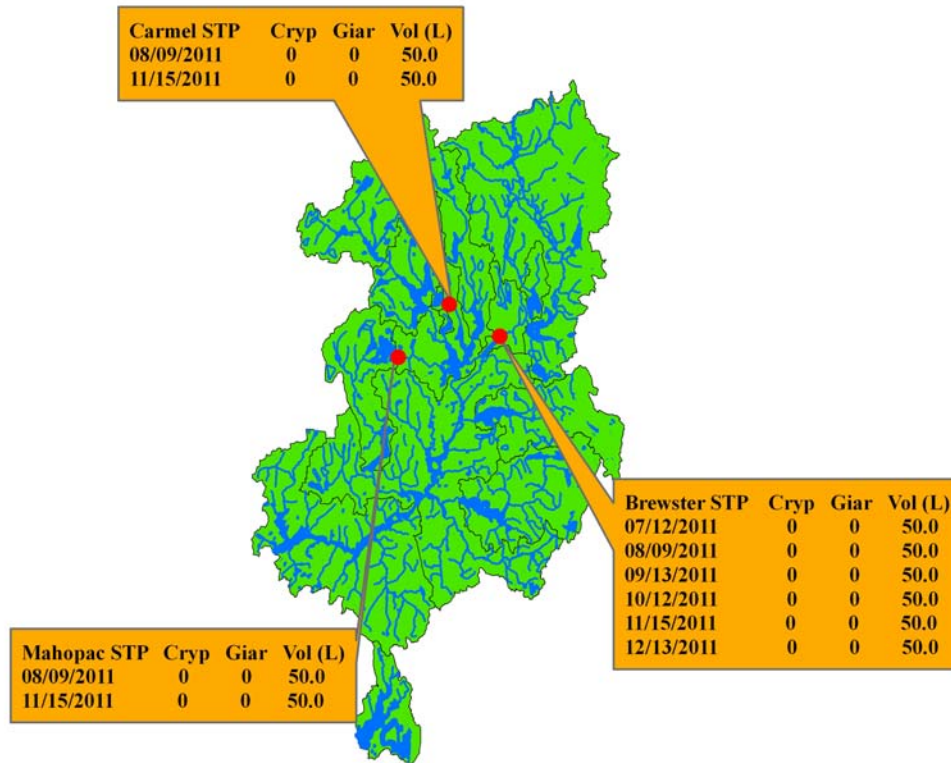
The other *Giardia* detection was at the Hunter Highlands WWTP. The Hunter Highlands WWTP has had past *Giardia* detections and, as mentioned in previous reports, it was suspected that wildlife may have had access to open areas of the treatment process (chlorine contact tank, etc.). As a result, DEP decided to move the sample collection site from the post-open chlorine contact tank to a location prior to the open areas. While the change in sampling site may have helped decrease the frequency of detections at the Hunter Highlands plant, the new sample location is not at the effluent of the plant. Consequently, the December 21 sample (1 cyst 50 L<sup>-1</sup>) occurred during a recirculation process where the sampled water was being sent back to the headworks of the plant, and therefore did not represent the plant's actual effluent. DEP will be coordinating with operators to avoid sampling under these conditions in the future.



**Figure 1.** *Cryptosporidium* and *Giardia* monitoring results and sample volumes at WOH WWTPs, July 1–December 31, 2011.

### East of Hudson

EOH WWTP monitoring included monthly protozoan monitoring with bi-monthly HEV monitoring at the Brewster WWTP, and quarterly monitoring for protozoa at the Carmel and Mahopac WWTPs. No *Cryptosporidium* oocysts or *Giardia* cysts were found in any of the samples taken at the three EOH treatment plants during this six-month period (Figure 2). This is unlike the prior two years when there were three positive *Giardia* detections at the Brewster WWTP in the final six months of the year. For the first time since September 2006, there was a positive HEV detection at the Brewster WWTP, occurring on November 15, 2011 (1.03 MPN 100 L<sup>-1</sup>). Plant operators did not indicate any abnormal circumstances or special conditions which might explain the virus detection.

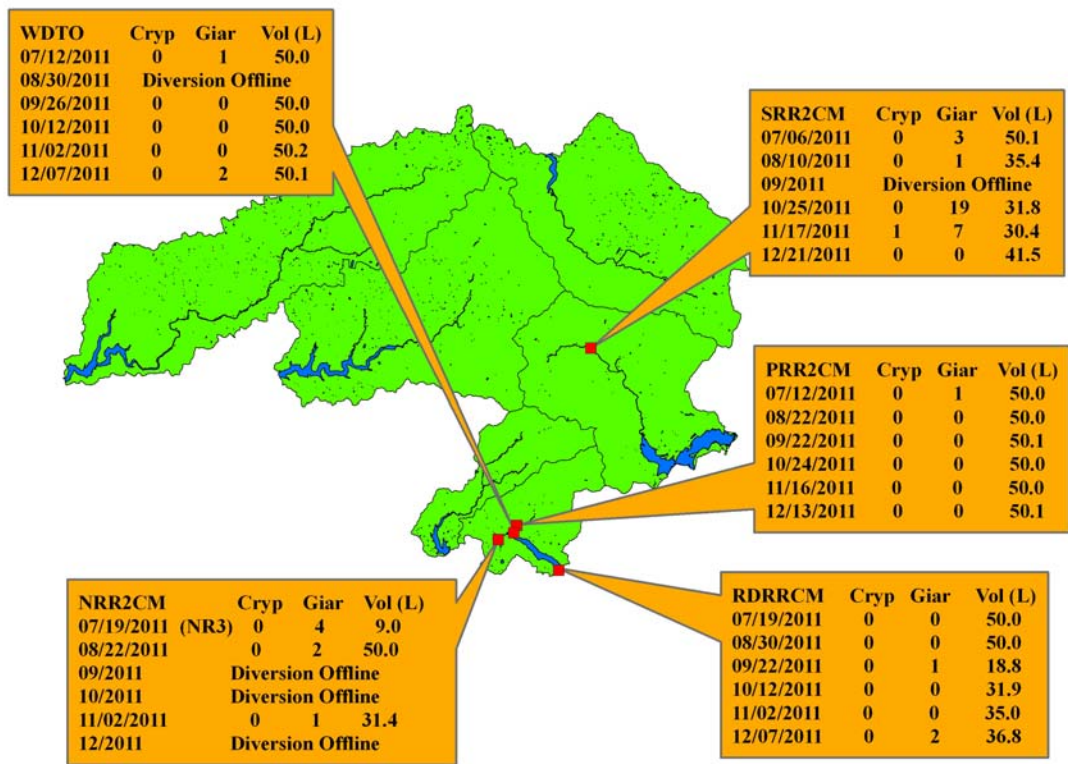


**Figure 2.** *Cryptosporidium* and *Giardia* monitoring results and sample volumes at EOH WWTPs, July 1 –December 31, 2011.

## 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 (Schoharie Reservoir (SRR2CM)) is in the Catskill district and is monitored at the outlet of the Shandaken Tunnel. 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. As of September 12, 2011, DEP discontinued sampling at alternate upstream sample taps when the reservoir diversions are not actively in use. For this reason, five monthly samples (three from Neversink and one each from Cannonsville and Schoharie) were not scheduled since the reservoir diversions were off-line.

All four Delaware System upstream source water sites were negative for *Cryptosporidium* during this period (Figure 3). The Catskill System had one *Cryptosporidium* detection (1 oocyst 30.4 L<sup>-1</sup>) in November at the outlet of the Shandaken Tunnel (Figure 3); however, the annual mean at this site remains low at 0.33 oocysts 50 L<sup>-1</sup> for 2011.



**Figure 3.** Upstream keypoint *Cryptosporidium* and *Giardia* monitoring results and sample volumes, July 1–December 31, 2011.

*Giardia* results at the four Delaware sites were similar to those during the same period in 2010, with an overall average of approximately 1.76 cysts for this six-month period, and an average detection rate of 40% (Figure 3). However, the Schoharie Reservoir effluent site; located in the Catskill System, had a slightly lower average result for this period (9.16 cysts) compared to last year (15.97 cysts), and an 80% detection rate. The two highest *Giardia* results for the period were both at the Schoharie effluent and occurred in the 2011 October and November samples, perhaps demonstrating a delayed consequence of Tropical Storms Irene and Lee (Figure 3, Table 1).

Table 1. *Giardia* concentrations ( $L^{-1}$ ) found in individual samples taken at upstream reservoir keypoint sites (July 1–December 31, 2011).

| Sites  | July       | August | September | October | November | December |
|--------|------------|--------|-----------|---------|----------|----------|
| NRR2CM | 0.44 (NR3) | 0.04   | NS        | NS      | 0.03     | NS       |
| PRR2CM | 0.02       | 0.00   | 0.00      | 0.00    | 0.00     | 0.00     |
| RDRRCM | 0.00       | 0.00   | 0.05      | 0.00    | 0.00     | 0.05     |
| SRR2CM | 0.06       | 0.03   | NS        | 0.60    | 0.23     | 0.00     |
| WDTO   | 0.02       | NS     | 0.00      | 0.00    | 0.00     | 0.04     |

On August 27 and 28, Tropical Storm Irene brought as much as 14 inches of rain to areas of the Catskill and Delaware watersheds, causing widespread damage as well as the potential for water quality issues. *Giardia/Cryptosporidium* and HEV samples were taken as routinely scheduled on August 29, with an additional round of samples taken on August 31. Due to operational changes, the CATALUM site, upstream of Kensico Reservoir, was not sampled and was replaced with the next keypoint sample site upstream along the Catskill Aqueduct (EARCM). The results from the routinely sampled sites have been reported previously, and the results from EARCM are provided below (Table 2).

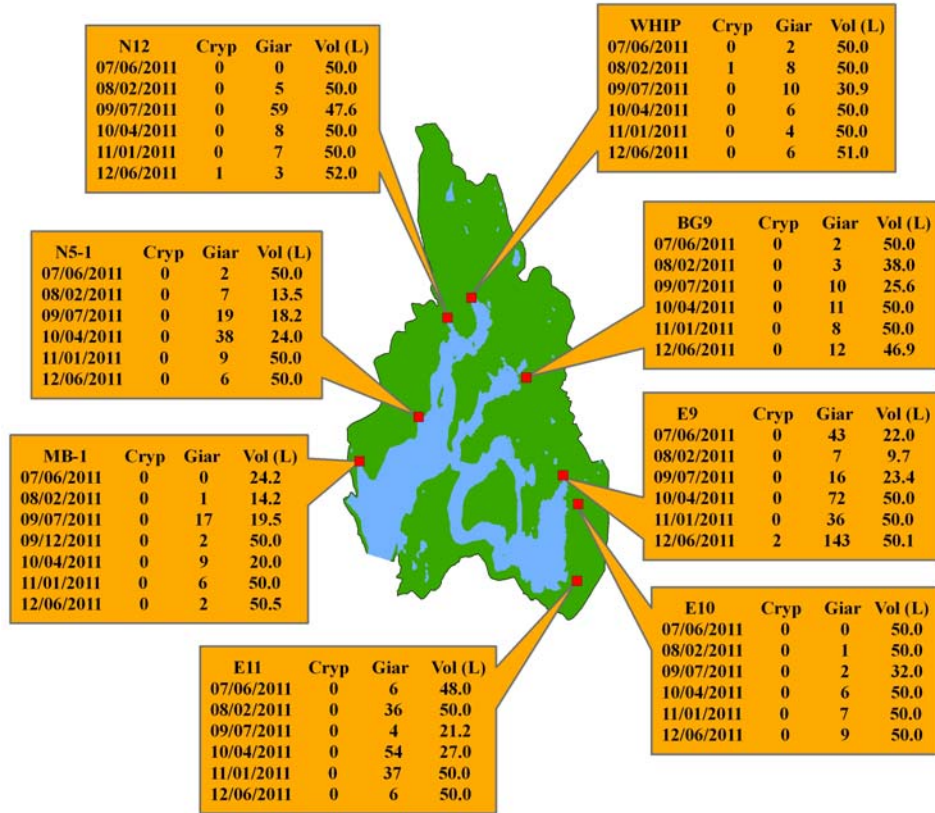
**Table 2.** Sample results from Ashokan Reservoir effluent after Tropical Storm Irene.

| Site  | Date      | Results                |                | Sample Volume (L) |
|-------|-----------|------------------------|----------------|-------------------|
|       |           | <i>Cryptosporidium</i> | <i>Giardia</i> |                   |
| EARCM | 8/31/2011 | 2                      | 1              | 50.0              |

### 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) compared to the amount contributed by the Catskill and Delaware influents (over 1 billion gallons per day). However, these streams are monitored for pathogens due to their proximity to the Kensico Reservoir effluents and their potential for an abrupt, negative impact on those effluents.

During the July through December sampling period, 49 samples (48 routine and 1 additional sample from Malcolm Brook after Tropical Storms Irene and Lee) were collected and analyzed for *Cryptosporidium* and *Giardia* (Figure 4). The target sample volume was always 50 L; however, 19 of the 49 samples (39%) (compared to 12 out of 48 samples during the same period last year) were less than 50 L because filters became clogged with suspended particles during collection.



**Figure 4.** *Cryptosporidium* and *Giardia* monitoring results and sample volumes for Kensico perennial streams, July 1–December 31, 2011.

Analysis of Kensico Reservoir tributary samples demonstrated a low occurrence of *Cryptosporidium*, as 3 of 49 samples (6.1%) were positive for *Cryptosporidium* (lower than 2010 and 2009 during the same period) and the highest individual sample count was 2 oocysts in a 50.1 L sample at E9 in December (Figure 4). Positive *Cryptosporidium* samples did not center on one stream or area of the reservoir, but were dispersed among three of the eight streams in the watershed.

*Giardia* was detected in 46 of 49 Kensico tributary samples (93.9%) taken during this period, with results ranging from 0 to 143 cysts per sample, with many sample volumes below 50 L. Notably, 19 of the 48 samples taken had volumes less than 50 L (Figure 4); however, this seemed to have little effect on the overall rate of *Giardia* detection, as the rate for 50 L samples at all Kensico streams was 93.3% compared to 94.7% for samples under 50 L. Thirty-three of the 49 samples (68%) had values of less than 10 cysts per volume sampled. For ease of comparison between individual samples, concentrations of *Giardia* per liter are provided below (Table 3).

**Table 3.** *Giardia* concentrations ( $L^{-1}$ ) found in individual samples taken at Kensico perennial stream sites (July 1–December 31 2011).

| Sites | Sample Dates |          |          |           |           |           |           |
|-------|--------------|----------|----------|-----------|-----------|-----------|-----------|
|       | 7/6/2011     | 8/2/2011 | 9/7/2011 | 9/12/2011 | 10/4/2011 | 11/1/2011 | 12/6/2011 |
| BG9   | 0.040        | 0.026    | 0.391    | NS        | 0.220     | 0.160     | 0.256     |
| E10   | 0.000        | 0.020    | 0.063    | NS        | 0.120     | 0.140     | 0.180     |
| E11   | 0.125        | 0.720    | 0.189    | NS        | 2.000     | 0.740     | 0.120     |
| E9    | 1.955        | 0.722    | 0.684    | NS        | 1.440     | 0.720     | 2.854     |
| MB-1  | 0.000        | 0.070    | 0.872    | 0.040     | 0.450     | 0.120     | 0.040     |
| N12   | 0.000        | 0.100    | 1.239    | NS        | 0.160     | 0.140     | 0.058     |
| N5-1  | 0.040        | 0.519    | 1.044    | NS        | 1.583     | 0.180     | 0.120     |
| WHIP  | 0.040        | 0.160    | 0.324    | NS        | 0.120     | 0.080     | 0.118     |

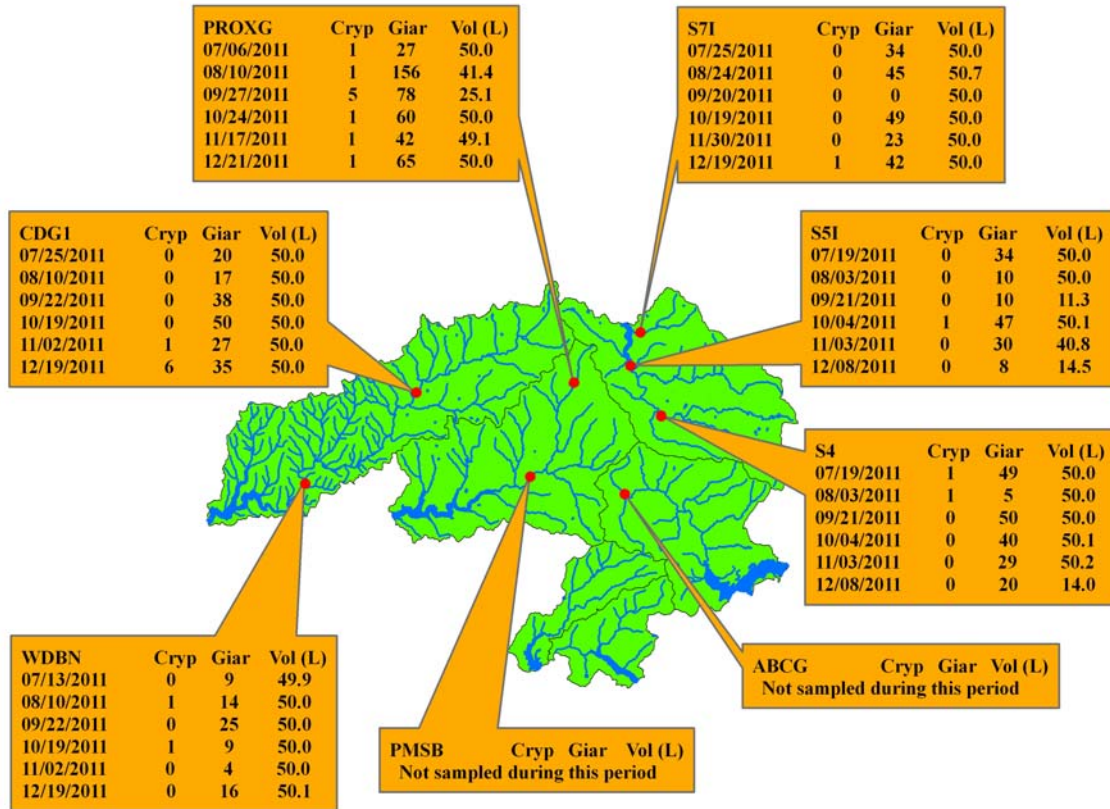
NS = no sample

## 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 WWQMP 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 geographic point sources of protozoa. 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 and stream site S7i, in the Manorkill watershed, was selected for upstream sampling. 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 2011, as DEP rotated through four sites upstream of S7i.

*Cryptosporidium* data were unremarkable at most of the routine sites monitored during this reporting period, with no more than 1 oocyst detected per volume sampled and a 25% occurrence rate at four of the six sites (Figure 5). The other two routine sites, PROXG and CDG1, had a result of 5 and 6 oocysts, respectively, per sample volume, which exceeded the historical 95<sup>th</sup> percentile calculated for each site, and mean concentrations of 1.17 and 2.53 oocysts  $50 L^{-1}$  for the six-month period. *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 156 cysts per volume sampled (Figure 5). The mean values per liter for this period are provided in Table 4 for comparison purposes. All sites exhibited decreases in mean *Giardia* concentration compared to the same period in 2010 when the means ranged from 39 to 184 cysts  $50L^{-1}$ . 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.



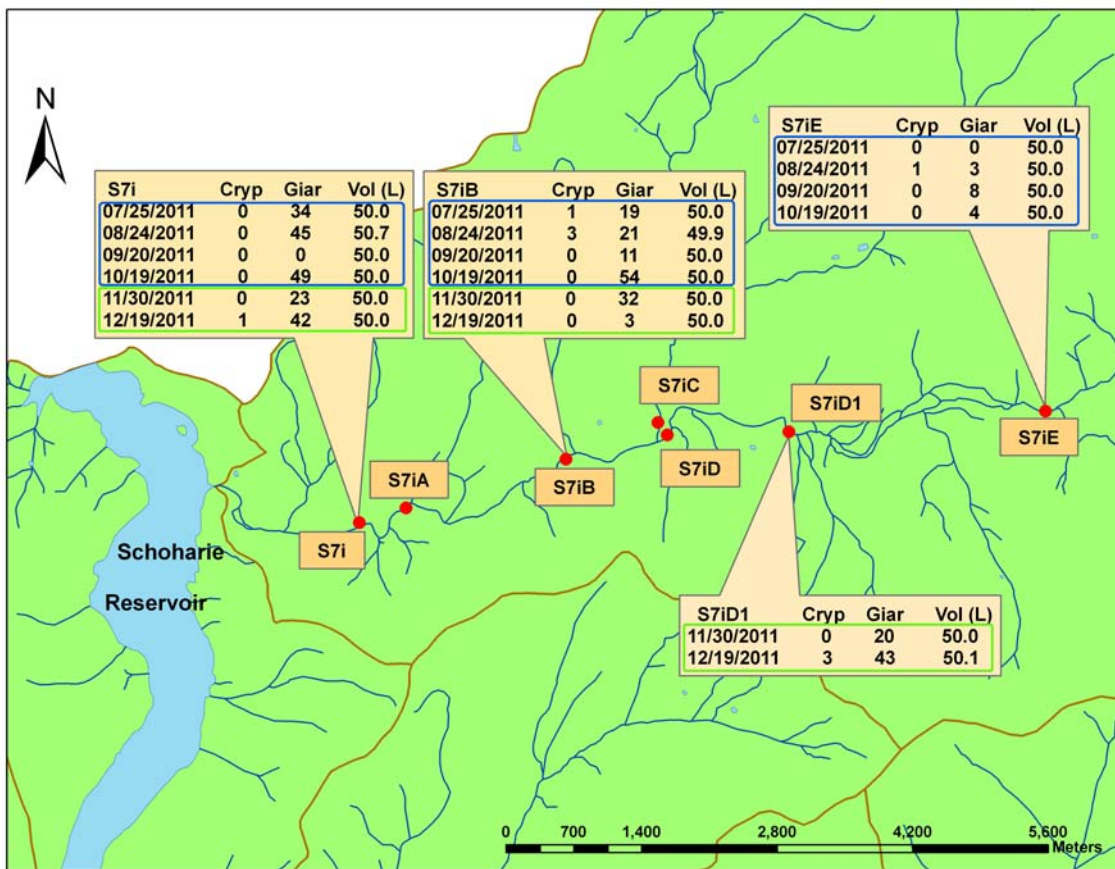
**Figure 5.** Watershed pathogen source indicator site *Cryptosporidium* and *Giardia* monitoring results and sample volumes, July 1–December 31, 2011.

**Table 4.** *Giardia* concentrations ( $L^{-1}$ ) found in individual samples taken at pathogen source origin sites (July 1–December 31, 2011).

| Sites | July  | August | September | October | November | December |
|-------|-------|--------|-----------|---------|----------|----------|
| ABCG  | NS    | NS     | NS        | NS      | NS       | NS       |
| CDG1  | 0.400 | 0.340  | 0.760     | 1.000   | 0.540    | 0.700    |
| PMSB  | NS    | NS     | NS        | NS      | NS       | NS       |
| PROXG | 0.540 | 3.768  | 3.108     | 1.200   | 0.855    | 1.300    |
| S4    | 0.980 | 0.100  | 1.000     | 0.798   | 0.578    | 1.429    |
| S5i   | 0.680 | 0.200  | 0.885     | 0.938   | 0.735    | 0.552    |
| S7i   | 0.680 | 0.888  | 0.000     | 0.980   | 0.460    | 0.840    |
| S7iB  | 0.380 | 0.421  | 0.220     | 1.080   | 0.640    | 0.060    |
| S7iE  | 0.000 | 0.060  | 0.160     | 0.080   | NS       | NS       |
| S7iD1 | NS    | NS     | NS        | NS      | 0.400    | 0.858    |
| WDBN  | 0.180 | 0.280  | 0.500     | 0.180   | 0.080    | 0.319    |

NS = no sample

Targeted monthly sampling upstream of site S7i began in January 2010 with sites S7iA and S7iB, progressing upstream to S7iC and S7iD by the end of 2010 (Figure 6). Sampling continued at S7iB and S7iD from January through May 2011, until collection was moved upstream again to try and further refine the geographic location of a potential source. Sites S7iB and S7iE were sampled from June through October 2011 (along with S7i), during which time it was determined that the source was most likely downstream of S7iE due to its low results. For the last two months of 2011, samples were taken at S7iB and at a new site between S7iD and S7iE called S7iD1. Results from these last two sets of samples show reasonable comparability between upstream and downstream pathogen results (highlighted in Table 4). Additional months of sampling are expected as DEP attempts to narrow down the source between S7iD1 and S7iE.



**Figure 6.** Map of targeted upstream sampling sites in the Manorkill watershed and data since July 2011.

In addition to upstream sampling, DEP had the opportunity to have molecular analysis performed on some of the *Giardia* cysts recovered from these sites. Preliminary results have not identified a specific source, although they have provided valuable information. *Giardia duodenalis* assemblage B was isolated from many of the samples, and they were identical (Alderisio, 2011) suggesting one source. Additionally, while *G. duodenalis* has been found in humans and many other animals, they are less commonly found in wildlife – with the exception of beavers, muskrats and rabbits, where they are frequently found. Interestingly, there are many beaver



lodges and dams along the stretch of the S7i stream being studied, as well as muskrats and rabbits. Moreover, this molecular testing has identified two new genotypes of *Giardia* not seen before; therefore, they cannot yet be associated with a source.

### **3.0 Pathogen Method Updates**

This section provides updates on the progress of the implementation of molecular and HEV methods in-house at DEP during the course of this reporting period.

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

The DEP Pathogen Laboratory staff has been continuing their work on genotyping *Cryptosporidium* oocysts from Method 1623 slides.

As a follow-up to the November 2010 workshop in Savannah, Georgia, where it was learned that the current mounting medium interfered with PCR testing, DEP obtained a new medium and performed initial demonstration of capability tests. The new medium was approved and implemented in January 2011. In April 2011, DEP staff attended a hands-on workshop that centered around the *Cryptosporidium* slide genotyping method that was developed under Water Research Foundation Projects 4099 and 4284. Additional PCR testing was carried out throughout the year and during these tests it was determined that a new thermal cycler was needed, and has been purchased. A standard operating procedure for genotyping has been developed and skills are being fine tuned to make this an available lab procedure when requested. Preliminary results have demonstrated that the Pathogen Lab now has the ability to genotype a single *Cryptosporidium* oocyst retrieved from the microscope slide after quantitative analysis has been completed.

#### **3.2 In-House Human Enteric Virus (HEV) Analysis**

Early in 2011, the Pathogen Lab began taking on the HEV filter housing sterilizations. This helped to greatly reduce the cost to New York City of HEV testing. As time progressed, the lab also began looking into taking on in-house virus testing. Several months were spent examining health and safety concerns. After these concerns were resolved, the laboratory staff moved forward with learning the various parts of the ICR method that is currently in use by the contract laboratory. DEP's goal is to bring all HEV testing in house during 2012.

## 4.0 References

**Alderisio, K.** 2011. Source Tracking *Giardia* spp. in a New York City Watershed Stream: Sampling Considerations. NYWEA Annual Watershed Science and Technical Conference Proceedings. Sept. 15-16, Hotel Thayer, West Point, New York.

**DEP. 2008.** [http://www.nyc.gov/html/dep/html/drinking\\_water/pathogen.shtml](http://www.nyc.gov/html/dep/html/drinking_water/pathogen.shtml)

**DEP. 2009.** 2009 Watershed Water Quality Monitoring Plan. New York City Department of Environmental Protection, Valhalla, New York.

**Pace, C., K. Alderisio.** 2009. Calculation of a Protozoan Budget for Kensico Reservoir. NYWEA, Annual Watershed Science and Technical Conference Proceedings, Hotel Thayer, West Point, New York.

**USEPA.** 2005. US EPA Method 1623: *Cryptosporidium* and *Giardia* in Water by filtration/IMS/FA. EPA/821-R-01-025.

**USEPA.** 1996. ICR Laboratory Microbial Manual. EPA 600/R-95/178. Office of Research and Development. Washington D.C. Government Printing Office.

## Appendix A

Mean concentrations per liter for *Cryptosporidium* and *Giardia* at upstream reservoir keypoints Kensico perennial streams, and pathogen source origin objective sites during the sampling period July 1–December 31 2011.

| Objective                       | Site   | Mean<br><i>Cryptosporidium</i> L <sup>-1</sup> | Mean<br><i>Giardia</i> L <sup>-1</sup> |
|---------------------------------|--------|--|--|
| Upstream Reservoir<br>Keypoints | NRR2CM | 0.000  | 0.172                                  |
|                                 | PRR2CM | 0.000  | 0.003                                  |
|                                 | RDRRCM | 0.000  | 0.018                                  |
|                                 | SRR2CM | 0.007  | 0.183                                  |
|                                 | WDTO   | 0.000  | 0.012                                  |
| Kensico<br>Perennial Streams    | BG9    | 0.000  | 0.182                                  |
|                                 | E10    | 0.000  | 0.087                                  |
|                                 | E11    | 0.000  | 0.649                                  |
|                                 | E9     | 0.007  | 1.396                                  |
|                                 | MB-1   | 0.000  | 0.227                                  |
|                                 | N12    | 0.003  | 0.283                                  |
|                                 | N5-1   | 0.000  | 0.581                                  |
|                                 | WHIP   | 0.003  | 0.140                                  |
| Pathogen<br>Source Origin       | ABCG   | NS   | NS                                     |
|                                 | CDG1   | 0.023  | 0.623                                  |
|                                 | PMSB   | NS   | NS                                     |
|                                 | PROXG  | 0.051  | 1.795                                  |
|                                 | S4     | 0.007  | 0.814                                  |
|                                 | S5i    | 0.003  | 0.665                                  |
|                                 | S7i    | 0.003  | 0.641                                  |
|                                 | S7iB   | 0.013  | 0.467                                  |
|                                 | S7iE   | 0.005  | 0.075                                  |
|                                 | S7iD1  | 0.030  | 0.629                                  |
|                                 | WDBN   | 0.007  | 0.257                                  |