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Cryptosporidium source tracking in the Wissahickon Creek Watershed

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Cryptosporidium
Source Tracking in
the Wissahickon
Creek Watershed

May 2008

Cryptosporidium Source Tracking in the
Wissahickon Creek Watershed

by

Amy Elizabeth Lynch

A Thesis

Presented to the Graduate and Research Committee

of Lehigh University

in Candidacy for the degree of

Master of Science

in Environmental Engineering

in

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April 25, 2008

Certificate of Approval

Approved and recommended for acceptance as a thesis in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering

April 25, 2008

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Abstract

Cryptosporidium is a parasitic protozoa of concern for drinking water treatment. With the Philadelphia Water Department, the Wissahickon Creek watershed was studied because of it a source for Philadelphia's drinking water. The purpose of this study is to determine the species and genotypes of *Cryptosporidium* found in the watershed for source identification. Two water locations, three wastewater effluents, and animal fecal sampling occurred from August 2006 through March 2008. The samples were processed with IMS and PCR before sequencing and phylogenetic analysis to identify genotypes. About 20 % of the water samples and 10 % of the wastewater effluents were positive for *Cryptosporidium*. It was also detected in 9 geese, a calf, a deer, a raccoon, and an unknown animal. A total of 12 genotypes were found, with mostly human and wildlife sources. Human health risk is present with *C. hominis* and *C. parvum*, found in 37.5% of the sequences, with geese as a main source of these two genotypes. Some seasonal distribution can be seen. All *C. hominis* occurred after reported outbreak in Montgomery County in the summer of 2007. In addition, the method for preparing fecal samples was optimized for best recovery of oocysts by testing three procedures: the current protocol of settling and removing middle suspension, adding Tween 80 and kaolin to the current protocol, and filtering through gauze. All procedures tested had low recoveries (less than 15%), but the current protocol or adding Tween 80 and kaolin were best. More testing with varying parameters is needed to find which method is best.

I. Wissahickon Creek Source Tracking

1. Background

1.1 Objective

To determine the species and genotypes of *Cryptosporidium* found in the Wissahickon watershed for source identification and evaluation of human health risk.

1.2 *Cryptosporidium*

Cryptosporidium is a parasitic protozoa which causes cryptosporidiosis, a diarrheal disease that is especially dangerous for immunocompromised people.

Cryptosporidiosis infections range from being asymptomatic to resulting in mortality.

Common symptoms include watery diarrhea, dehydration, nausea, vomiting, fever, and weight loss. There is no medical cure for cryptosporidiosis. *Cryptosporidium* has been found all over the world and in a growing list of animal hosts. (Center for Disease Control) The largest outbreak recorded was in Milwaukee, Wisconsin in 1993, when 403,000 people were infected and 100 people died. (Fayer 2008)

Cryptosporidium's infectious form is an oocyst, a thick walled spore stage of its life-cycle. Oocysts are released into the environment by the feces of infected hosts and can survive in this form for long periods of time before ingested by a new host.

Treating drinking water for *Cryptosporidium* is a growing concern for human health.

Waterborne transmission of *Cryptosporidium* is the focus of this project, but zoonotic, person-to-person, and foodborne transmission also leads to infection. (Xiao & Ryan 2004)

Cryptosporidium is a pathogen of concern in terms of drinking water because it is resistant to chlorine disinfection. The small size of an oocyst also eliminates filtration as a sole treatment option. Ultra-violet (UV) light is a proven disinfectant for *Cryptosporidium*, but installation of UV treatment systems is prohibitively expensive for many drinking water treatment plants. (Clancy 2008)

1.3 Known Genotypes

The main species that infect humans are *C. hominis*, *C. parvum*, *C. mealegridis*, *C. felis*, and *C. canis*. The most prevalent species in humans cases though are *C. hominis* and *C. parvum*. (Jiang 2005). Many other genotypes have been found, including host-adapted species and genotypes. There are 16 valid species and over 40 genotypes. Several criteria need to be met before a genotype can be classified as a new species. *Cryptosporidium* species cannot be differentiated by their morphology, making species identification difficult. Genotypes are isolates that are distinct on a molecular level. Additional investigation is necessary before a novel genotype can be classified as a species. The classification of valid species and genotypes used in this study was referenced from “*Cryptosporidium* and Cryptosporidiosis”. (Fayer 2008)

The host specificity varies among different species and genotypes. Some genotypes only infect one host, called host-adapted genotypes, such as goose genotype I. Other types infect a range of hosts, such as *C. parvum* which infects cattle, sheep, goats, deer, and horses. (Xiao and Ryan 2004) Most genotypes though only infect one class of vertebrates (e.g. birds, mammals) with exceptions such as *C. meleagridis* which is associated with birds but also infects humans.

1.4 Project justification

USEPA's Long Term 2 Enhanced Surface Water Treatment Rule

(abbreviated LT2ESWT or LT2 rule), enacted in 2006, requires water treatment plants serving more than 100,000 people to monitor their source water for *Cryptosporidium* for 24 months, after which the average concentration found will determine further action. (EPA 816-F-06-005)

In response to the LT2 rule, the Philadelphia Water Department (PWD) wanted to investigate the factors and sources contributing to *Cryptosporidium* contamination in the drinking water for the city of Philadelphia. With a better picture of what the sources of *Cryptosporidium* are in the watershed, the PWD can enact preventative watershed management strategies to protect a water source for Philadelphia's drinking water, which may be a better solution than the end-of-pipe strategy of additional treatment at the water treatment plants.

The detection methods for *Cryptosporidium* regulated by the EPA do not include genotyping data, only enumeration data. Since only a few species are known to infect humans, the health risk to humans is overestimated. A study of the types of *Cryptosporidium* in the watershed gives a better estimate of the human health risk present.

1.5 Wissahickon Creek

Wissahickon Creek, shown in Figure 1, was chosen because the mouth empties into the Schuylkill River right before the intake for the Queen Lane Water Treatment Plant, causing it to significantly contribute to the influent water going into the treatment plant. Additionally, the Wissahickon Creek watershed includes a mix of urban, suburban, and agricultural land.

The occurrence of *Cryptosporidium* in one of the City of Philadelphia's water sources can be more easily characterized by looking at the Wissahickon watershed than the other water sources because of the extensive size of the other sources, the Delaware River and Schuylkill River.

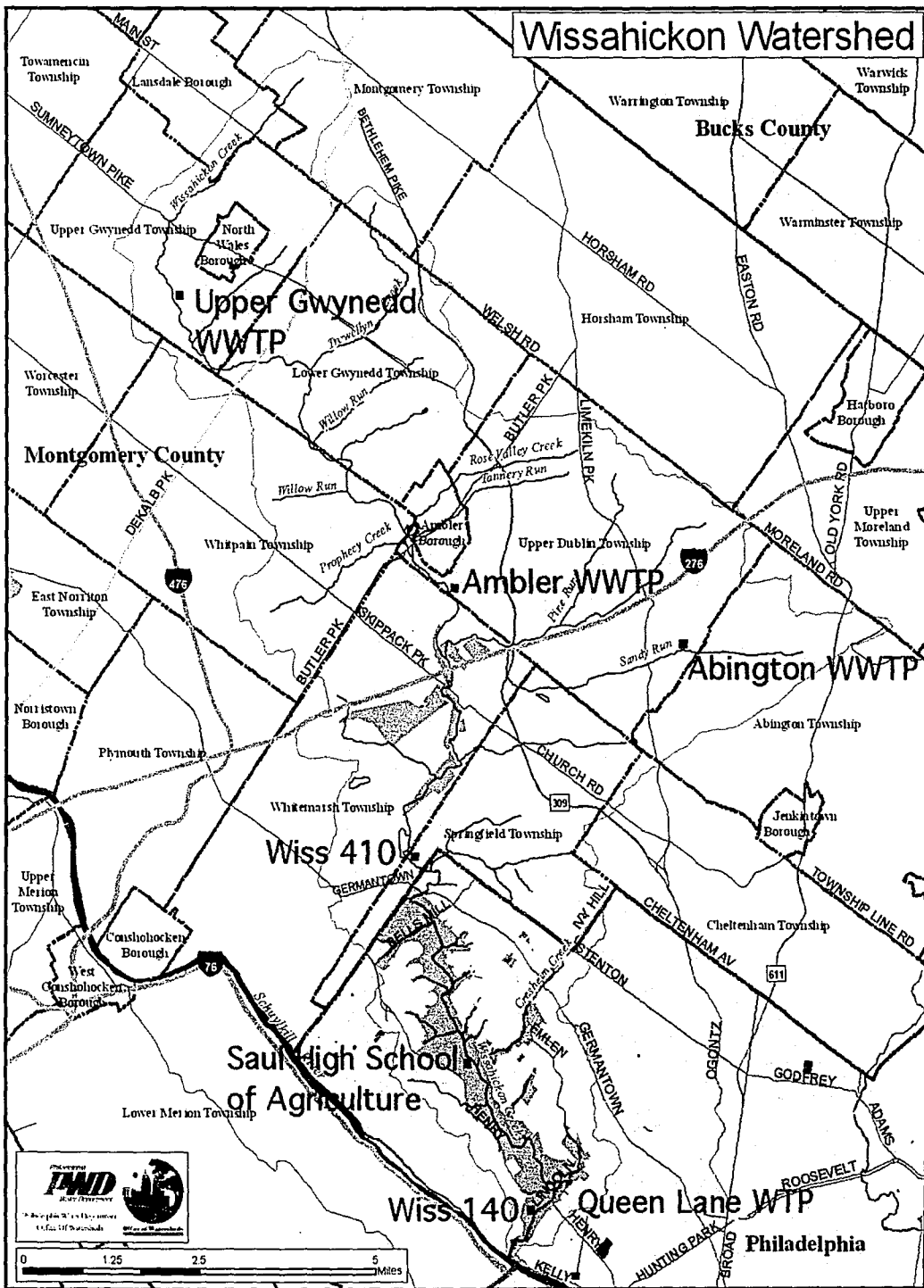


Figure 1: Map of Wissahickon Creek watershed and sampling locations

1.6 Type of sampling

The possible sources to contaminate the drinking water include human waste from wastewater treatment plant effluents, wild animal wastes, and agriculture wastes from anywhere within the Delaware and Schuylkill River watersheds, which includes the Wissahickon Creek watershed. Three different types of samples are looked at in this study to find *Cryptosporidium* from all of these sources: Wissahickon Creek water, wastewater treatment plant effluents, and animal fecal samples. The water samples show what *Cryptosporidium* genotypes are present in the creek and entering into the water treatment plant. The wastewater effluents are a source into the creek and also an indicator of human sources. The animal fecal sampling is direct source sampling that supplements the genotype data from the environmental samples from unknown hosts.

2. Methods

2.1 Sampling locations

The water sampling sites, Wiss 140 and Wiss 410, are located near the mouth of the creek and approximately 6 miles upstream, respectively. The map of the watershed in Figure 1 displays all of the water sampling locations. The wastewater effluent sampling sites at the wastewater treatment plants located in the towns of Abington, Ambler, and Upper Gwynedd are spread out across the watershed.

Fecal samples were collected at various locations in the watershed from a number of different animals. The animals sampled include deer, geese, ducks, dogs, donkeys, sheep, calves, cows, horses, lamb, heifers, woodchucks, opossums, a raccoon, and a skunk. The locations include Saul Agriculture High School, Fairmount Park, Upper Gwynedd, Militia Hill Park, Kelly Drive on the Schuylkill, Valley Green Road on the Wissahickon, Schuylkill Center for Environmental Education, and Morris Arboretum (at location of Wiss 410).

2.1.1 Sampling timeline

The project has been continued year-round for three years (since May 2005) to check for seasonal trends within the watershed. Water samples are collected on the first and last Monday of every month. Wastewater samples were collected on the same schedule, with Abington starting on January 23, 2006, Ambler on February 5, 2007, and Upper Gwynedd on February 26, 2007. Monthly fecal sampling began in January 2007, on the third Monday of the month. Previously to this schedule, one fecal sampling event occurred in August 2006. The data included here is from August 2006 to March 2008.

2.1.2 Sampling protocol

PWD personnel collected all of the water samples. The samples were then filtered using Gelman Envirocheck Sampling Capsules (Pall Gelman Sciences, Inc., Ann Arbor, MI) and eluted in the PWD lab. Up through January 2008, the volume of water filtered was determined by however much it took to clog one filter. Starting in

January 2008, a standard volume of 10 liters of water were filtered for all samples, with more than one filter used for especially turbid samples. The sample pellets were shipped with ice overnight to Lehigh University for the rest of the processing.

PWD personnel collected all of the fecal samples, with the exception of one sampling event in August 2006. The samples were shipped overnight to the Lehigh lab packed in ice. About 1.5 - 2 grams of each sample was suspended in 40 milliliters of distilled water and vortexed thoroughly. After settling for about one minute, 20 milliliters was removed from the middle suspension. The fecal samples and water samples were treated the same for the rest of the processing.

2.2 IMS

Sample processing continued with immunomagnetic separation (IMS) using the Aureon Biosystems Crypto Kit (Vienna, Austria). Immunomagnetic separation uses magnetic beads with *Cryptosporidium* antibodies attached to pull out with a magnet any *Cryptosporidium* oocysts present in an environmental sample. As the manufacturer recommends, one IMS tube with 100 microliters of beads is used for every 1 milliliter of pelleted material. 0.5 M of hydrochloric acid is used to dissociate the beads from the oocysts.

2.3 DNA Extraction

The deoxyribonucleic acid (DNA) of the oocysts is extracted using phenol chloroform with an initial overnight incubation with Proteinase K and SDS solution.

2.4 PCR

The DNA extracted from the sample is put directly into nested polymerase chain reactions (PCRs). The first PCR is a 50 microliter reaction using GoTaq Flexi DNA Polymerase (Promega Corporation, Madison, WI) and primers KLJ1 and KLJ 2. The result is a strand of DNA that is about 1056 base pairs long. (Jellison 2002). The second PCR uses the primers CPB-DIAGF and CPB-DIAGR and the DNA template is the first PCR product. The nested PCR amplicon is about 434 base pairs long.

Primers:

KLJ1: (5'-CCA CAT CTA AGG AAG GCA GC-3')

KLJ2: (5'-ATG GAT GCA TCA GTG TAG CG-3')

CPB-DIAGF: (5'-CAA TTG GAG GGC AAG TCT GGT GCC AGC-3')

CPB-DIAGR: (5'-CCT TCC TAT GTC TGG ACC TGG TGA GT-3')

The PCR cycle consisted of initial denaturation of 5 minutes at 80°C and 30 seconds at 98°C, 40 cycles of amplification (denaturation for 30 seconds at 94°C, annealing for 30 seconds at 53°C, and extension for 1 minute at 72°C), and a final extension of 10 minutes at 72°C.

A detection limit test shows that the PCR reactions are sensitive enough to detect one oocyst. Figure 2 is the gel electrophoresis image with clearly visible bands for a concentration of DNA equivalent to 10000 oocysts down to 1 oocyst.

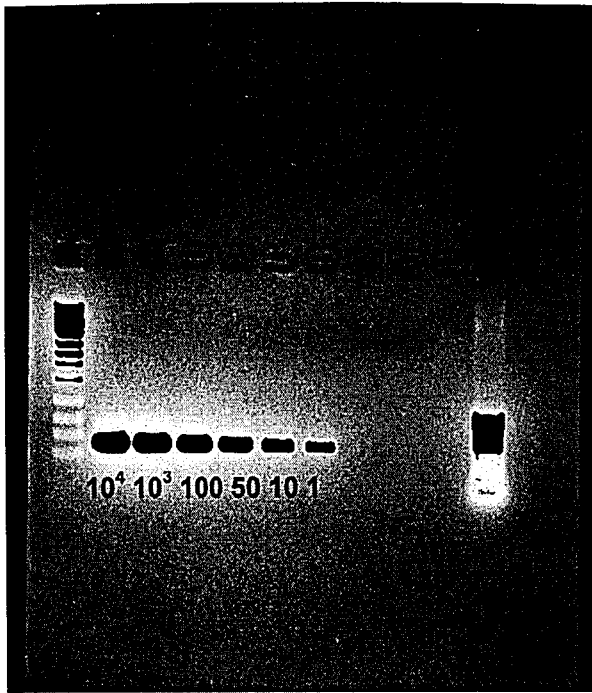


Figure 2: Detection limit test for PCR reactions

The nested PCR product was processed by gel electrophoresis to confirm the presence of *Cryptosporidium* DNA by the length of the DNA fragments (~433 base pairs). If a sample has the correct length DNA, it is cleaned up with the QIAquick PCR Purification Kit (QIAGEN Sciences, Germantown, MD). The QIAquick Gel Extraction Kit was used instead if there are multiple bands visible on the gel for a given sample.

2.5 Quality Control

A positive and negative control for each step of the procedure ensures quality control for each environmental sample. Oocysts from Waterborne, Inc. (New Orleans, LA) were used for all positive controls, with approximately 5000 oocysts used for the IMS control, 500 oocysts for the DNA extraction control, and 10000 oocysts for the PCR



Figure 2: Detection limit test for PCR reactions

The nested PCR product was processed by gel electrophoresis to confirm the presence of *Cryptosporidium* DNA by the length of the DNA fragments (~433 base pairs). If a sample has the correct length DNA, it is cleaned up with the QIAquick PCR Purification Kit (QIAGEN Sciences, Germantown, MD). The QIAquick Gel Extraction Kit was used instead if there are multiple bands visible on the gel for a given sample.

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controls. The correct results for the controls validate any positive samples detected within the environmental samples. A positive result for a negative control means contamination was present somewhere in the procedure. A negative result for a positive control means the procedure may not detect oocysts in the sample even if they were present.

2.6 Cloning

The Promega pGEM-T Easy Vector System I (Promega Corporation, Madison, WI) inserted the nested PCR product into a plasmid. The plasmid was transformed into the z-competent *E. coli* strain DH5 α (Zymo Research Corporation, Orange, CA). The *E. coli* cells are grown and the ones containing the PCR products were isolated. The plasmids from six isolates were removed with the QIAprep Spin Miniprep Kit (QIAGEN Germantown, MD). Multiple isolates were screened to recover more than one genotype of *Cryptosporidium* possibly present within the sample. The presence of the *Cryptosporidium* DNA was confirmed with restriction enzyme *NotI* and the heterogeneity, or sequence differences, among the isolates with restriction enzyme *NdeI*. At least three isolates were chosen to be sent out for sequencing, depending on the heterogeneity found.

2.7 Sequencing

The plasmids from the screened isolates were sent to University of Pennsylvania's DNA Sequencing Facility in Philadelphia, PA and sequenced using ABI (Applied

Biosystems) 96-capillary 3730XL sequencer. The primers added at the facility were SP6 and T7.

2.8 Alignment

The software program MacClade Version 4.06 was used to view and align the sequences. The 18S rRNA section of *Cryptosporidium* DNA of isolated is about 433 base pairs long. The structures of the two variable helixes found within this segment of DNA were determined using the program Vienna RNA Secondary Structure Prediction <<http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi>>. The alignments of these helixes are included in Appendix B. Spaces were included to align the bump-outs of the helixes. If there was any uncertainty with respect to where a base pair should be aligned within the helix or the rest of the sequence, those spots were blocked out throughout all of the sequences. Additionally, the variable loops of the two helixes were also blocked out. The final sequence alignments and masked out base pairs for the complete sequences are shown in Appendix C.

2.9 Phylogenetic Analysis

The final alignment of all of the samples and the GenBank sequences was input into the software program PAUP Version 4.0b10 to create the phylogenetic trees. The method chosen for the phylogenetic analysis was a parsimony tree using heuristic tree searching strategy. The bootstrap numbers represent 1000 pseudoreplicates. All other parameters were the default setting in the program.

3. Results:

Cryptosporidium was detected at both water sites and two of the three wastewater effluents. Fecal sampling resulted in *Cryptosporidium* detection in nine geese, a deer, a calf, a raccoon, and an unknown animal, as shown in Table 1. A full listing of all positive samples and characteristics can be found in Appendix A. The sequences of all the genotypes found in these positive samples, arranged in the final alignment input into PAUP, are listed in Appendix C.

3.1 Scope of results

The purpose of this study was to evaluate qualitative data associated with *Cryptosporidium*, the species or genotypes. The quantitative data, the detection percentages, listed below do not enumerate the amount of *Cryptosporidium* that is in the watershed. The detection percentages only show how often *Cryptosporidium* was detected with this method. A negative water sample does not mean there weren't any oocysts in the water that day, just that none were captured. The types of *Cryptosporidium* present in the watershed, however, are just as important to know in determining the health risk as the amount present.

Additionally, a positive animal sample only confirms the occurrence of *Cryptosporidium*, not an infection in that animal. Oocysts can be ingested by an animal without causing an infection, and possibly shed from the animal intact. If an animal is infected, the likelihood of detecting *Cryptosporidium* is higher because of the large number of oocysts that are shed from an infected animal.

The genotypes detected in the water may not come from the sources that they are named after and usually associated with. For example, the detection of the cervine genotype in an environmental sample does not necessarily mean that the source is a deer. That genotype is found mostly to infect deer, but has also been found in sheep and lemurs. (Xiao and Ryan 2004) Identifying the sources of *Cryptosporidium* with molecular genotyping data from environmental samples with 100% certainty is not possible. But it is most likely that the genotype came from the sources associated with it.

3.2 Detection Percentages

The greatest detection percentages are found in both Wissahickon Creek water sites, with *Cryptosporidium* found in 18.2% of all Wiss140 samples at the mouth of the creek and 20.5% of all Wiss 410 samples approximately six miles upstream. No *Cryptosporidium* was detected at Upper Gwynedd wastewater treatment plant effluent, but about 8% of the Abington and Ambler wastewater treatment plant effluents were positive for *Cryptosporidium*.

Of the animal fecal samples collected, multiple geese were positive for *Cryptosporidium* as well as a deer, a calf, a raccoon, and an unknown animal sample. The geese had the highest detection percentage with 16.7% of the samples positive for *Cryptosporidium*. Only 5% of deer and 11% of calf samples were positive. Not

enough raccoon samples were analyzed to give a significant detection percentage.

Overall, only 10% of all animal fecal samples were positive for *Cryptosporidium*.

Several samples contained more than one genotype. Nine samples contained two different genotypes and two contained three genotypes. Therefore, the number of sequences analyzed, listed in Table 1, is greater than the number of positive samples.

Table 1: Sample totals from each source and percent detected from August 2006 to March 2008

Source	Total Samples	Positive for Crypto	Sequences Analyzed	Detection Percentage
Wiss 140	44	8	13	18.2%
Wiss 410	44	9	11	20.5%
Abington WWTP	36	3	4	8.3%
Ambler WWTP	28	2	3	7.1%
Upper Gwynedd WWTP	26	0	0	0%
Deer	20	1	1	5.0%
Calf	9	1	1	11.1%
Unknown	1	1	1	100%
Raccoon	1	1	1	100%
Goose	54	9	13	16.7%
All other animals	46	0	0	0%
Total animals	131	13	17	9.9%
Total samples	309	35	48	11.3%

3.3 Additional Water Data

For water and wastewater samples, additional data was collected for each sample on the volume of water filtered, the volume of the packed pellet after elution, and the turbidity of the initial sample. No correlation was seen with the amount of packed pellet, but there was a relationship between the volume filtered and the turbidity, shown in Figure 3. Generally, the higher the turbidity of the water is, the lower the volume of water able to be filtered. As described in the methods section, a goal is to filter ten liters but usually the water is filtered until the filter begins to clog. Higher turbidity water causes the filter to clog faster.

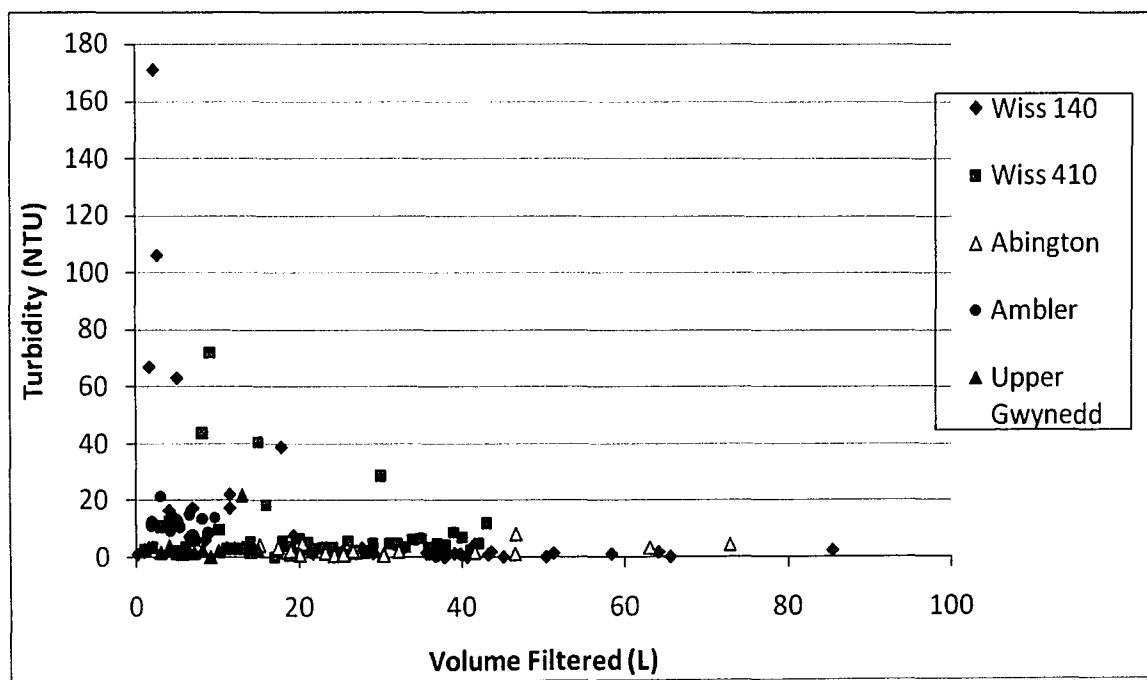


Figure 3: Turbidity of versus volume filtered

4. Discussion

4.1 Scope of genotype detection

This study is mostly a qualitative look at *Cryptosporidium* in the watershed. One drawback of the nested PCR is that while detection limits are more sensitive, quantitative data is lost in the process. The positive samples could have contained one or multiple oocysts. Several clones are screened and sequenced in hopes of finding more than one genotype within one sample. Nine samples contained two different genotypes and two contained three genotypes. The number of sequences analyzed in the phylogenetic tree is greater than the number of positive samples because of multiple genotypes found in a single sample.

4.2 Phylogenetic Tree

The species/genotypes of the sequenced samples are identified using a phylogenetic analysis. Figure 4 shows the combined tree with every *Cryptosporidium* sequence found from August 2006 to March 2008.

4.2.1 GenBank sequences

The *Cryptosporidium* genotypes found in environmental samples are unknown until compared to known, published species and genotypes of *Cryptosporidium*. All known sequences included in the phylogenetic tree are taken from GenBank <www.ncbi.nlm.nih.gov>, with the accession number listed next to the name in the tree, and have been published in one or more peer-reviewed journal articles. Only

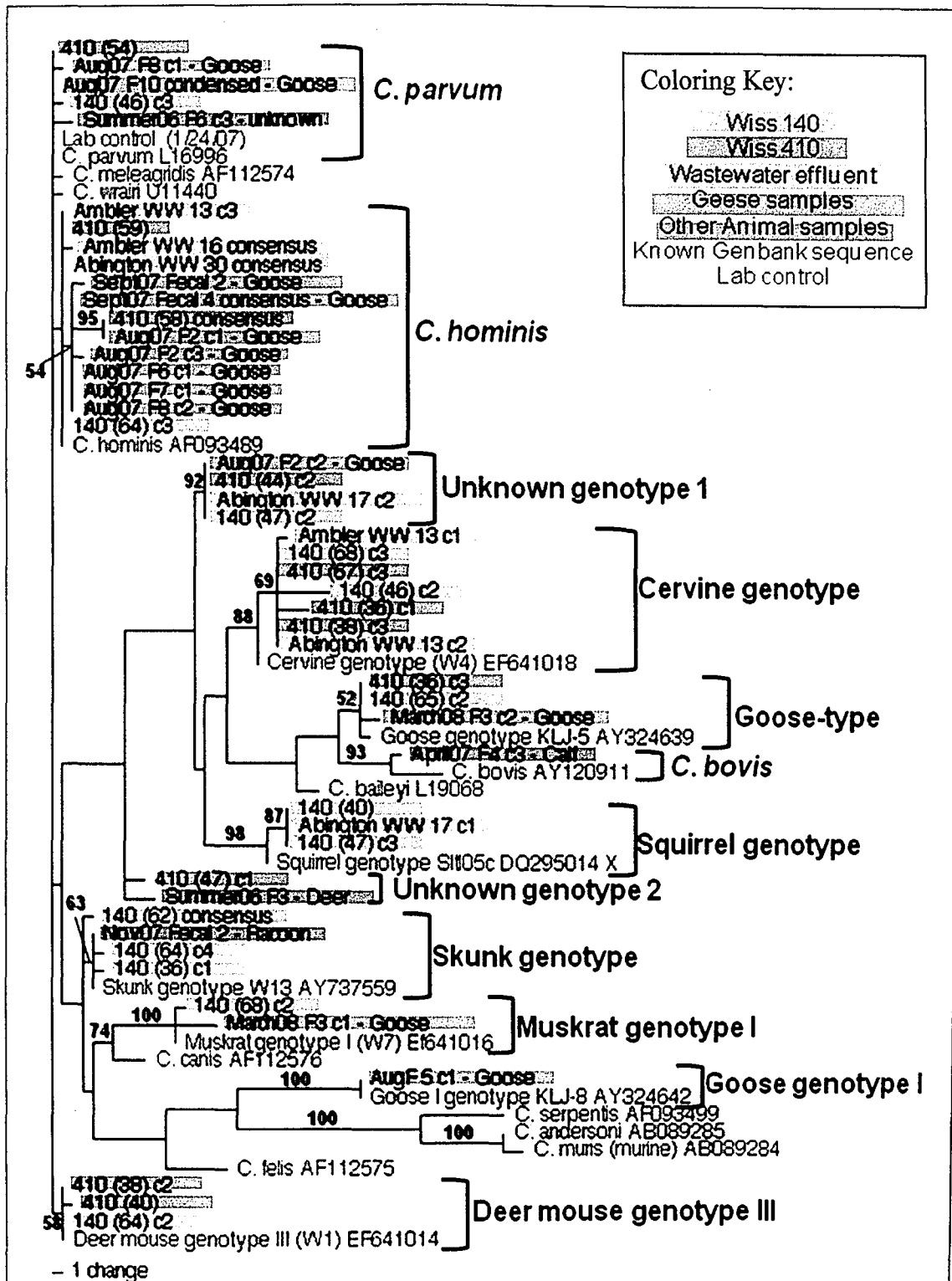


Figure 4: Parsimony phylogenetic tree for all *Cryptosporidium* sequences found in samples. (GenBank accession numbers of known genotypes are listed on the tree). Bootstraps numbers greater than 50 are shown from 1000 pseudoreplicates. The key describes the color-coding system for the types of samples.

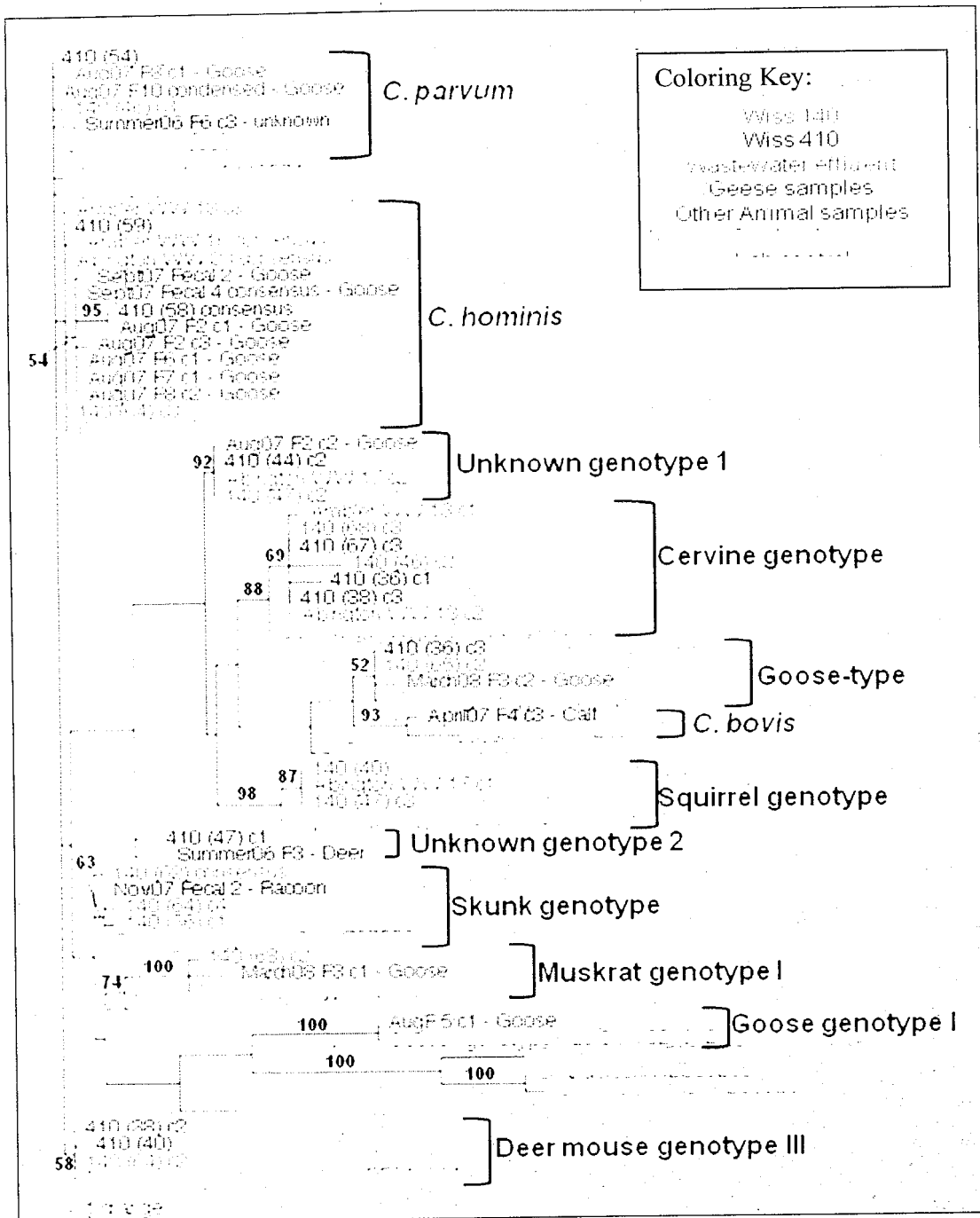


Figure 4: Parsimony phylogenetic tree for all *Cryptosporidium* sequences found in samples. (GenBank accession numbers of known genotypes are listed on the tree). Bootstraps numbers greater than 50 are shown from 1000 pseudoreplicates. The key describes the color-coding system for the types of samples.

Cryptosporidium species and genotypes accepted as valid names were included in the phylogenetic analysis. “*Cryptosporidium* and Cryptosporidiosis” served as a reference for valid classification of species and genotypes. (Fayer 2008)

4.2.2 Species/Genotype Identification

The phylogenetic comparison of all of the sequences from September 2006 to March 2008 showed 12 different species/genotype groupings. In a phylogenetic tree, a clade is every isolate that descends from the same ancestor. The species or genotype of each sample is identified by a closely related known species/genotype that is included in the same clade. All of the samples fall within 10 clades with known genotypes and two clades without any known sequence. The genotypes include *C. parvum* (5 sequences), *C. hominis* (13), *C. bovis* (1), cervine genotype (7), squirrel genotype (3), skunk genotype(4), muskrat genotype I (2), goose genotype I (1), deer mouse genotype III (3), goose-type genotype (3), unknown genotype 1 (4), and unknown genotype 2 (2).

4.2.3 Bootstraps

From the bootstrap values, some grouping relationships with known genotypes are stronger than others. Bootstrap values indicate the percentage of time that those sequences group together in 1000 different configurations of the tree. The sequences identified in the tree as unknown genotype 1, cervine genotype, *C. bovis*, squirrel genotype, muskrat genotype I, and goose genotype I all have bootstrap numbers

greater than 70 and therefore are closely related to that species or genotype. The sequences identified as goose-type genotype, skunk genotype, and deer mouse genotype III all have bootstrap numbers between 70 and 50, so their relationship with those genotypes is close but not strong. The rest of the genotypes, including *C. parvum*, *C. hominis*, and unknown genotype 2, do not have bootstrap values listed because they are less than 50 and not statistically significant. One reason for the uncertainty in these relationships could be the genetic similarity between *C. parvum* and *C. hominis*, which were considered the same species until a few years ago. (Fayer 2008)

4.2.4 Unknown Genotypes

Several sequences were not closely related to any known sequences and consequentially were labeled as an unknown genotype. The sequences labeled unknown genotype 1 are considered all one genotype based on the bootstrap value of 92. Although compared against numerous known genotypes, none were the same as this genotype. Conversely, the two sequences labeled unknown genotype 2 are not very closely related based on the bootstrap value less than 50. Goose-type genotype could also be considered an unknown. Although closely related to other isolates from geese, such as the KLJ5 isolate AY324639 (Jellison 2004), it is not one of the two accepted goose genotypes (Xiao 2008). So either this genotype is yet unnamed or is associated with a different animal host.

4.2.5 Main sources types

The dominant sources of *Cryptosporidium* spp. found in the Wissahickon Creek watershed appear to be humans and wildlife. Human associated species detected include *C. hominis* and *C. parvum*. Wildlife associated species found in the watershed include cervine genotype, squirrel genotype, skunk genotype, muskrat genotype I, deer mouse genotype III, goose genotype I, and goose-type genotype. Agriculture species, *C. bovis* and some *C. parvum* genotypes, were not identified very frequently in the watershed. *C. parvum* is categorized as a human and agriculture type because it is associated with cattle, sheep, goats, deer, and horses. (Xiao Ryan 2004)

4.2. 6 Human Health Risk Evaluation

The only species found in the watershed that are a human health risk are *C. parvum* and *C. hominis*. Unfortunately, these two species are also the most prevalent types found in the watershed, as shown in Table 2. Eighteen isolates or 37.5% of all of the *Cryptosporidium* sequences found are one of these two genotypes, broken down into 27% *C. hominis* and 10% *C. parvum*. Twenty-four isolates or 50% found carry no known human health risk. Six isolates could not be identified.

Table 2: Percent of genotypes carrying human health risk

		Number of Sequences	Percentage	
Human health risk genotypes	<i>C. hominis</i>	13	27.1 %	37.5%
	<i>C. parvum</i>	5	10.4%	
No known human health risk genotypes		24	50%	
Unknown genotypes (unknown risk)		6	12.5%	
Totals		48	(100%)	

4.2.7 Unusual genotype hosts

Of the animal samples analyzed, most of the genotypes are commonly found in the hosts sampled but there were some unexpected genotype associations. *C. hominis* and muskrat genotype I are not commonly found in geese. *C. hominis* was found in several geese on two different sampling dates and two different sampling locations on one date. Finding these genotypes in animals other than their primary hosts is proof that determining the source from an environmental sample based solely on the genotype is not a perfect science.

4.3 Distribution of genotypes by sample type

No strong distribution patterns by the type of sample can be seen in the comparison in Table 3. The two genotypes with a human health risk, *C. parvum* and *C. hominis*, are found in almost every type of sample. Geese especially appear to be a major source for these genotypes. Not surprisingly, the human genotype *C. hominis* appeared in wastewater effluents on a few dates.

Ten of the twelve genotypes found in the watershed were also found in the river water samples. Most of the genotypes found in animals and wastewater treatment plant effluents were also present in the creek. Therefore, as expected, animals and wastewater treatment plants contribute to the *Cryptosporidium* present in the source water for Philadelphia’s drinking water.

Table 3: Distribution of genotypes by sample type

Wiss 140		Wiss 410		Abington WWTP		Ambler WWTP	
Unknown genotype 1	1	Unknown genotype 1	1	Unknown genotype 1	1	Cervine genotype	1
Skunk genotype	3	Unknown genotype 2	1	Cervine genotype	1	<i>C. hominis</i>	2
Goose genotype KLJ5	1	Goose genotype KLJ5	1	Squirrel genotype	1		
Cervine genotype	2	Cervine genotype	3	<i>C. hominis</i>	1		
Deer mouse genotype III	1	Deer mouse genotype III	2				
<i>C. hominis</i>	1	<i>C. hominis</i>	2				
Muskrat genotype I	1	<i>C. parvum</i>	1				
Squirrel genotype	2						
<i>C. parvum</i>	1						
	13		11		4		3
		Geese		Other Animals (deer, raccoon, unknown, calf)			
		Unknown genotype 1	1	Unknown genotype 2	1		
		Goose genotype KLJ5	1	Skunk genotype	1		
		<i>C. hominis</i>	7	<i>C. parvum</i>	1		
		Goose genotype I	1	<i>C. bovis</i>	1		
		Muskrat genotype I	1				
		<i>C. parvum</i>	2				
			13		4		

4.4 Distribution of genotypes by seasons

A seasonal comparison of the genotypes found in the watershed shows some interesting differences in Table 4. The seasons here are defined by the official astronomical start days for that year. Certain genotypes are seen more often during certain times of the year, such as skunk genotype in the fall, *C. parvum* in the summer, muskrat genotype I in the winter, and unknown genotype 1 in the spring.

Cervine genotype however was seen year round. A lot of the genotypes though cannot be considered a trend because not enough sequences were found. One interesting observation is the appearance of *C. hominis* only in the summer and fall of 2007 more frequently than any other genotype. This doesn't seem to be a seasonal difference but a single outbreak. More evidence for this theory is examined in section 4.5. Any of the seasonal differences seen during the time period of this study could be from individual outbreaks of those genotypes. Migration, hibernation, and other seasonal behavior in the animal hosts could also cause some of these trends. Conversely though, genotypes associated with geese, who migrate south during the winter, were seen in the summer, fall, and winter. Looking at genotyping data for at least three years would be necessary to establish definite seasonal trends.

Table 4: Seasonal distribution of genotypes

Summer		Fall		Winter		Spring	
2006		2006		2007		2007	
Unknown Genotype 2	1	Skunk genotype	1	Cervine genotype	1	<i>C. bovis</i>	1
<i>C. parvum</i>	1	Goose genotype KLJ5	1	Deer mouse genotype III	1	Cervine genotype	1
		Cervine genotype	2	Squirrel genotype	1	<i>C. parvum</i>	1
		Deer mouse genotype III	1			Squirrel genotype	2
						Unknown genotype 1	3
						Unknown genotype 2	1
	2		5		3		9
2007		2007		2008			
Cervine genotype	1	Deer mouse genotype III	1	Muskrat genotype I	2		
<i>C. hominis</i>	7	<i>C. hominis</i>	6	Cervine genotype	2		
Goose genotype I	1	Skunk genotype	3	Goose genotype KLJ5	1		
<i>C. parvum</i>	3	Goose genotype KLJ5	1				
Unknown genotype 1	1						
	13		11		5		

4.5 Pennsylvania Cryptosporidiosis Outbreak

On August 16, 2007, the Pennsylvania Department of Health issued a health advisory for a cryptosporidiosis outbreak in Chester and Montgomery Counties. The majority of the Wissahickon Creek watershed is located in Montgomery County. There were over 300 cases confirmed in late June for Chester County followed by over 60 cases in Montgomery County. The source of the outbreaks was determined to be local public swimming pools. (Pennsylvania Department of Health) The seasonal distribution of genotypes shows that there was increased occurrence of *Cryptosporidium* spp. known to cause human infections in August and September 2007, possibly related to the outbreak. All *C. hominis* found in the watershed was from the summer and fall of 2007 at almost all of the sampling locations. During this period of time, *C. hominis* was detected more often than any other genotype in any season (thirteen samples total). *C. parvum*, the other species infectious to humans, also peaked during the summer of 2007 with three samples. The possibility that the observed spike in *C. hominis* and *C. parvum* is a result of the reported outbreak seems likely but needs to be corroborated with sequence data from the outbreak. The species or genotype of the *Cryptosporidium* involved in this outbreak is unknown at this time.

5. Conclusions

5.1 Summary of Findings:

Cryptosporidium was detected at every water and wastewater sampling location, with the exception of the Upper Gwynedd wastewater treatment plant. Detection at the two water sites was higher (20%) than at the two wastewater effluents (8%).

Approximately 10% of all fecal samples were positive for *Cryptosporidium*, with the most found in geese (16.7%).

Twelve different species or genotypes of *Cryptosporidium* were detected in the Wissahickon Creek watershed. Of those, the most common types were from human and wildlife sources. Agriculture sources do not seem to be a significant contributor in the watershed. There is a significant human health risk present from *C. hominis* or *C. parvum* accounting for 37.5% of all the sequences. All of the wildlife genotypes, accounting for 50% of all the samples, carry no known risk to humans. Geese, however, were shown to be a source for human infectious species.

All twelve genotypes were found in the water samples and are a risk to enter the water treatment plants downstream. No pattern was seen in the distribution of genotypes by sample type. However there were some seasonal differences in some genotypes detected. *C. hominis* was only seen in the summer and fall of 2007, which is possibly related to a reported outbreak of cryptosporidiosis in Montgomery County.

5.2 Recommendations

Some changes to the sampling plan would improve the already existing database of *Cryptosporidium*. Wastewater effluent sampling from Upper Gwynedd should be discontinued, and possibly switched to another wastewater treatment plant located within the watershed. Additional fecal sampling could improve the understanding of where the genotypes found in the water are coming from. Several, such as the cervine genotype, were not detected in any animals. More sampling of deer and agricultural sources would help the most.

Since geese were shown to be a significant source of human infectious genotypes, interventions to keep them away from the creek could be beneficial.

Additionally, it would be valuable to determine the genotype that caused the outbreak in Chester and Montgomery County. The genotype data may be already known by the health department or testing could be performed if any oocysts from infected individuals were kept.

II. Fecal Prep Optimization

1. Objective

A supplemental project to the Wissahickon source tracking project was undertaken to determine the procedure with the best recovery of oocysts from fecal samples. The goal is to optimize the amount of a fecal sample processed and the percent recovery of oocysts.

2. Project Justification

The method currently used in the Wissahickon source tracking study for preparing fecal samples for IMS has not been validated as the best method. The detection sensitivity is unknown also. Initial steps are required to reduce the amount of debris and clean up the sample before IMS without a losing any *Cryptosporidium* oocysts present in the sample.

3. Procedure

The current procedure, as previously described in section 2.1.2, mixes about 1.5 - 2 grams of each sample into 40 milliliters of distilled water. After settling for about one minute, 20 milliliters are removed from the middle suspension.

Additionally two other methods were tested: (i) adding kaolin and Tween 80 and (ii) filtering through gauze first.

The first procedure (Tween 80 and kaolin) was adapted from one used for influent wastewater samples (McCuin and Clancy 2005). All of the steps are the same as the current protocol except the addition of Tween 80, a surfactant, and Kaolin, a type of clay. A 20% Tween 80 solution (for a final concentration of 1% in the sample) is added at the beginning along with the fecal sample, followed by five minutes on a wrist-action shaker. Right before IMS, 0.5 milligrams of kaolin is added.

For the second procedure, all of the vortexed sample is filtered through gauze to remove the large particles. The sample is treated the same for rest of the procedure.

This procedure was adapted from one used for isolating oocysts from intestinal samples in Giovanni Widimer's lab at Tufts University Veterinary School in Grafton, MA.

For all of the testing 3 grams of deer fecal samples were used. ColorSeed was used to determine the oocyst recovery. ColorSeed (BTF Precise Microbiology, Inc., Sydney, Australia) contains 100 gamma-irradiated oocysts, pre-stained with Texas Red dye, to be added to each sample. Additionally, Meriflour FITC stain (Meridian Biosciences, Inc. Cincinnati, OH) was used to help identify the oocysts under the microscope.

4. Results

A test of the procedure, using only IMS with no fecal sample, found 59% oocyst recovery. The rest of the testing results for the three procedures are listed in Table 5.

Table 5: Fecal prep protocol testing results

	IMS alone (current protocol)	IMS with Tween 80 and Kaolin	Gauze and IMS
	23	15	6
	26	20*	5
	5	9	10
	2		
	18	*no kaolin added	
Average	14.800	14.667	7.000
Std Dev.	10.756	5.508	2.646

5. Discussion

None of the procedures tested had high recoveries. The protocol currently used has been shown to deliver the highest oocyst recovery. There is a lot of variability within the results, which might be attributed to different samples characteristics or slightly different settling times. The addition of Tween 80 and kaolin is a good alternative to be used instead. The oocyst recovery average is only slightly lower when it includes the one sample without any kaolin. The gauze protocol had consistently low oocyst recovery compared to the other two methods.

6. Recommendations

Further testing is required to be confident in a procedure that will best capture any oocysts present in a fecal sample. The settling time, amount of fecal sample used, and

type of fecal sample should be varied under the current protocol to determine the reason behind the variation in the recoveries. Additionally, testing with only Tween 80 and no kaolin seems promising from the one test performed. The gauze procedure does not seem promising from these results and can be disregarded.

Continuing visualization problems using the ColorSeed and the Merifluor stain under the microscope need to be worked out for future testing as well. Some additional procedures that used by other labs and worth further investigation include using a concentration gradient with Histodenz (Sigma-Aldrich, St. Louis, MO) to separate the oocysts by density (Widmer 1998) and the QIAamp DNA Stool Kit (QIAGEN Inc., Valencia, CA). (Xiao 2008)

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Appendix A - Log of All Positive Samples from 8/1/06 through 3/31/08

Sample Label	Date Sampled	Season	Description	Gel Extraction	Number of Genotypes	Final Genotype	Helix 1 Code	Helix 2 Code
SF 3	8/3/06	sum	deep fecal form Militia Hill park		1	unknown 2	A	B
SF 6	8/3/06	sum	unknown fecal from gwynedd		1	<i>C. parvum</i>	A	B
140(36)	11/27/06	fall	water sample		1	Skunk genotype	B	C
410(36)	11/27/06	fall	water sample		2	cervine genotype goose-type	C G2	D I
410(38)	12/18/06	fall	water sample	GE	2	deer mouse III cervine genotype	D C	F D
140(40)	1/22/07	win	water sample		1	squirrel genotype	E	G
410 (40)	1/22/07	win	water sample		1	deer mouse III	D	F
WT 13	1/22/07	win	Abington WWTP effluent		1	cervine genotype	C	E
IMS- (1/24)	1/22/07		unknown lab control		*1	<i>C. parvum</i>	A	B
PCR 1+ (1/24)	1/22/07		lab control		*1	<i>C. parvum</i>	A	B
410 (44)	3/26/07	spr	water sample		1	unknown 1	A3	B
WT 17	3/26/07	spr	Abington WWTP effluent		2	squirrel genotype unknown 1	E A3	G B
140 (46)	4/4/07	spr	wet weather sample		2	cervine genotype <i>C. parvum</i>	C A	E2 B
140 (47)	4/16/07	spr	wet weather sample		2	unknown 1 squirrel genotype	A3 E	B G
410 (47)	4/16/07	spr	Wet weather sample		1	unknown 2	A	A
AprilF 4	4/17/07	spr	Calf fecal from Saul HS		1	<i>C. bovis</i>	G1	H
410 (54)	7/23/07	sum	water sample	GE	1	<i>C. parvum</i> (c3 not crypto)	A	A
WB 13	8/6/07	sum	Ambler WWTP effluent		2	cervine genotype <i>C. hominis</i>	C A	E A
AugF 2	8/20/07	sum	Goose fecal from Kelly Drive		3	<i>C. hominis</i> ; <i>C. hominis</i> unknown 1	A, A2 A3	A, A B
AugF 5	8/20/07	sum	Goose Fecal from Kelly Drive		1	Goose genotype 1	H	J
AugF 6	8/20/07	sum	Goose fecal from Valley Green		1	<i>C. hominis</i>	A	A
AugF 7	8/20/07	sum	Goose fecal from Valley Green		1	<i>C. hominis</i>	A	A

AugF 8	8/20/07	sum	Goose fecal from Valley Green		2	<i>C. parvum</i> <i>C. hominis</i>	A A A	B A B
AugF 10	8/20/07	sum	Goose fecal from Valley Green		1	<i>C. parvum</i>	A	B
410(58)	9/4/06	sum	dry day water sample		1	<i>C. hominis</i>	A2	A
410(59)	9/24/07	fall	dry day water sample	GE	1	<i>C. hominis</i>	A1	A
WB 16	9/24/07	fall	Ambler WWTP effluent		1	<i>C. hominis</i>	A	A
SeptF 2	9/25/07	fall	Goose fecal from Kelley Drive		1	<i>C. hominis</i>	A	A
SeptF 4	9/25/07	fall	Goose fecal from Kelley Drive		1	<i>C. hominis</i>	A	A
WT 30	10/1/07	fall	Abbingtion WWTP effluent		1	<i>C. hominis</i>	A	A
140 (62)	11/5/07	fall	dry day water sample	GE	1	Skunk genotype	AB	F
NovF 2	11/26/07	fall	Raccoon Fecal sample		1	Skunk genotype	B	C
140 (64)	12/12/07	fall	dry day water sample		3	deer mouse III <i>C. hominis</i> , skunk genotype	D A, B	F A, C
140 (65)	12/20/07	fall	dry day water sample	GE	1	goose-type	G2	I
410 (67)	1/30/08	win	dry day water sample	GE	1	cervine genotype	C	D
140 (68)	2/4/08	win	dry day water sample		2	muskrat genotype cervine genotype	F C	F D
March08 F3	3/18/08	win	Goose fecal sample		2	muskrat genotype goose-type	F G2	F I
TOTALS					48			

Appendix B - Helix 1 alignment

Refer to Appendix A for corresponding samples

A	A1 A2 A4 A3	AB	B	C	D	E	F	G1	G2	H	
U G C U A U A A U		U A G C U A U A A U	U A G C U A U A A U	U A G C U A U A A U	U A G C U A U A A U	U A G C U A U A A U	U A G C U A U A A U	U A G C U A U A A U	U A G C U A U A A U	U A G C U A U A A U	U A G C U A U A A U
A U U A		A U U A	A U U A	A U U A	A U U A	A U U A	A U U A	A U U A	A U U A	A U U A	A U U A
A U A U U U		A U A U G U	A U A U	A U A U	A U A U G U	A U A U	A U A U	A U A U	A U A U	A U U A U	A U U A U
U A		U A	U A	U A	U A	U A	U A	U A	U A	U A	
U A A U A U A U A U	G	A U U A U A U A A U	U A A U U A U A U A	U A A U U A U A U A	A U U A U A U A U A	U A A U U A U A U A	U A A U U A U A U A	U A A U U A U G A U	A U U A U A U A A U C	A U U A U A U A U A	U A U G U C G U A U A
A U A U A U A U A U A U G U U G A		A U A U A U U A U A U G U A G A	A U A U U A U A U G U U G A	A U A U U A U A U U U U A A	A U A U U A U A U U U U A	A U G U A U A U U A U A U A U A U A U A U A U A U G U A U A U A	A U U A C G A U A U U A U U U U A U A U U A	A U U A G U C G A C	A U U A U A C G A C	A U C A U A U U A A U C G C G C G C A	

38

Masked out basepairs:
Uncertain bumpouts
variable loop

Appendix B - Helix 1 alignment

Cervine genotype EF641018	Deer mouse III EF641014	Muskrat I EF641016	Squirrel genotype DQ295014	Skunk genotype AY737559	AY120911 Goose KLJ5 AY 324639	Goose I AY120912
U A	U A	U A	U A	U A	U A	U A
G C	G C	G C	G C	G C	G C	G C
U A	U A	U A	U A	U A	U A	C G
U A	U A	U A	U A	U A	U A	U A
A U	A U	A U	A U	A U	A U	A U
A U	A U	A U	A U	A U	A U	A U
U A	U A	U A	U A	U A	U A	U A
A U	A U	A U	A U	A U	U U	A U
A U	A U	A U	A U	A U	U U	U A
U G	U	U	U	U	U A	
U	U A	U A	U A	U A	U A	U A
U A						
A U	U A	U A	U A	U A	A U	U A
U A	A U	A U	U A	A U	U A	U
A U	U A	A U	A U	A U	A U	G U
A U	A U	U A	A U	A U	A U	C G
A U	U A	A U	A U	A U	A U	A U
A U	U A	U G	A U	U A	U A	U A
A U	A U	U A	A U	A U	U	A U
U A	U	U A	U U	U	U	C
A U	A U	A U	U U	A U	A U	A U
U A	G U	A U	U A	A U	U A	A U
A U	A U	A U	C G	A U	A U	A U
U A	A U	U A	A U	U A	A U	U A
U A	A U	A U	A U	A U	C G	A U
U U	U A	A U	A U	U A	U C	C G
U U	A U	C G	A U	U A	A C	C G
U U	U A	A U	U U	U U		C G
A	U A	U U	A U	U U		A C
	U A	U		A A		
	G A					

c B - Helix 2 alignment

Appendix B - Heli

<i>C. wairi</i> U11440	<i>C. hominis</i> AF093489	<i>C. baileyi</i> L19068	<i>C. andersoni</i> AB089285	<i>C. muris</i> AB089284	<i>C. parvum</i> AF093493	<i>C. canis</i> AF112576	<i>C. serpentina</i> AF093499	<i>C. felis</i> AF112575	Cervine genotype EF641018
U A	U A	U A	U A	U A	U A	U A	U A	U A	U A
U A	U A	U A	U A	U A	U A	U A	U A	U A	U A
CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
AU	AU	AU	CG	CG	AU	AU	CG	AU	AU
UA	UA	CG	UA	UA	UA	UA	UA	UA	UA
AU	AU	AU	AU	AU	AU	AU	AU	AU	AU
UA	UA	UA	UA	UA	UA	UA	UA	UA	UA
U	U	U	U	U	U	U	U	U	U
AU	AU	AU	AU	AU	AU	AU	AU	UA	AU
								U	
								U U	
CG	CG	CG	UA	UA	CG	CG	UA	UA	CG
UA	UA	UA	AU	AU	UA	UA	AU	UA	UA
AU	AU	UA	UA	UA	AU	AU	UA	AU	AU
U U	U U	A A	UA	UA	U U	U A	UA	AU	UA
A U	U U	U U	C A	U A	A U	U U	U U	GU	AU
U U	U U	U	U	C U	U U		U	AU	U U
A U	U U				A			CG	
	U U							UA	
	U U							GU	
	U U							AU	
	U U							A U	
	U U							U U	

ix 2 alignment

Deer mouse III
EF641014

U A
U A
C G
A U
U A
A U
U A
U

A U

C G
U A
A U
U U
A U
U U
U U

Muskrat I
EF641016

U A
U A
C G
A U
U A
A U
U A
U

A U

C G
U A
A U
U U
U U
A U
U

Squirrel
DQ295014

U A
U A
C G
A U
U A
A U
U A
U

A U

C G
U A
A U
A U
G U
A U
A A
U A
A

Skunk genotype W13
AY737559

U A
U A
C G
A U
U A
A U
U A
U

A U

C G
U A
A U
U U
A U
U U
U U
A

Goose-type KL35
AY324639

U A
U A
C G
G C
C G
A U
U A
U

A U

C G
U U
U A
U

Goose I
AY120912

U A
C G
C G
G C
C G
A U
U A
U

A U

C G
C G
U C
C

C. bowis
AY120911

U A
U A
C G
A U
U A
A U
U A
U

A U

C G
U A
U U
U U

Appendix C: Final full alignment of all sequences

Alignment Notes:

Sequences aligned and masked out exactly as input into PAUP for the phylogenetic tree, including sequence names.

Masked out base pairs are darkened.

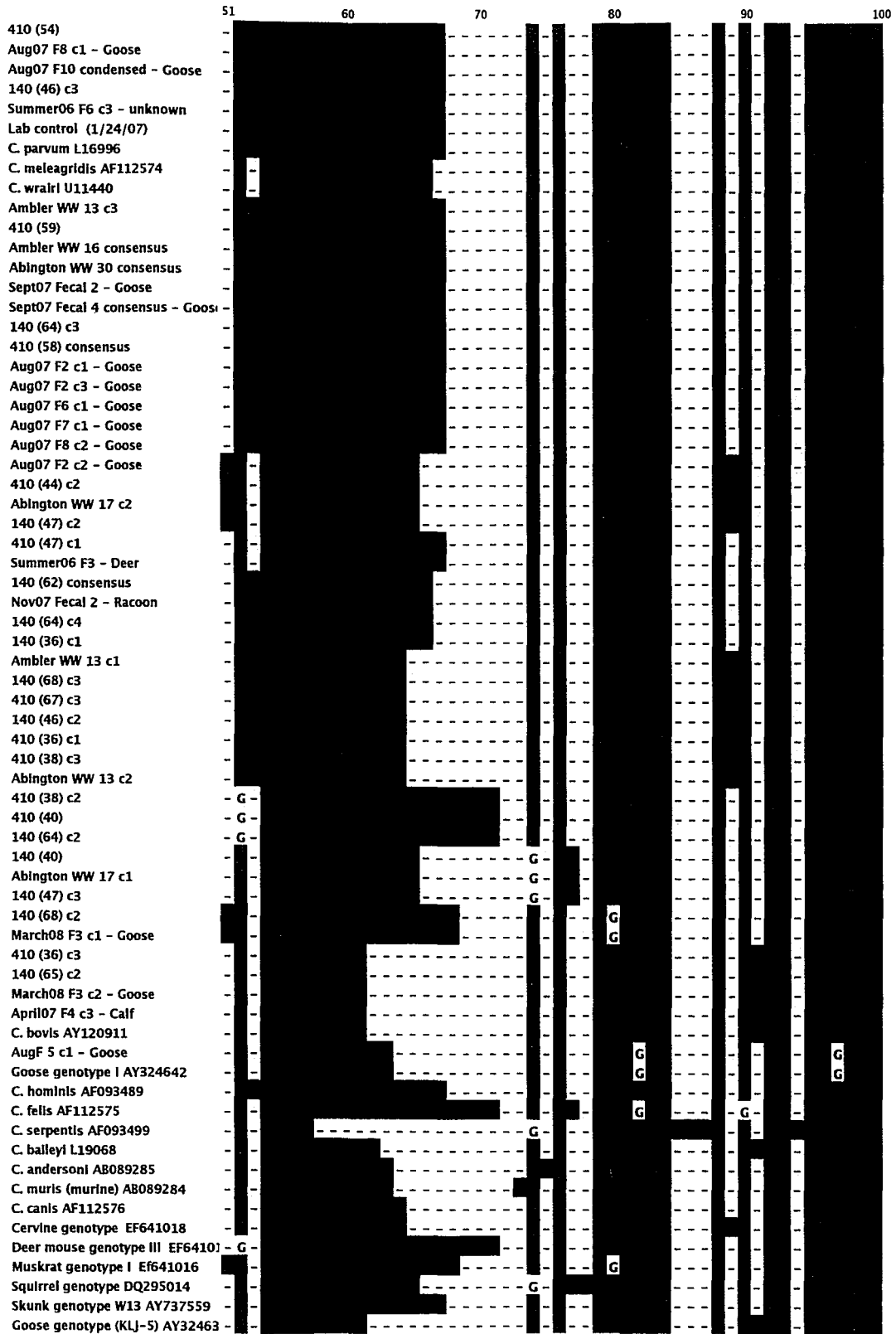
Spaces added in are shown in white.

The base pair number is located at the top of the page.

Helix 1 is located from base pair 20 through 99.

Helix 2 is located from base pair 103 through 149.

	1	10	20	30	40	50
410 (54)	G	G	GG	GG		
Aug07 F8 c1 - Goose	G	G	GG	GG		
Aug07 F10 condensed - Goose	G	G	GG	GG		
140 (46) c3	G	G	GG	GG		
Summer06 F6 c3 - unknown	G	G	GG	GG		
Lab control (1/24/07)	G	G	GG	GG		
C. parvum L16996	G	G	GG	GG		
C. meleagridis AF112574	G	G	GG	GG		
C. wrairi U11440	G	G	GG	GG		
Ambler WW 13 c3	G	G	GG	GG		
410 (59)	G	G	GG	GG		
Ambler WW 16 consensus	G	G	GG	GG		
Abington WW 30 consensus	G	G	GG	GG		
Sept07 Fecal 2 - Goose	G	G	GG	GG		
Sept07 Fecal 4 consensus - Goose	G	G	GG	GG		
140 (64) c3	G	G	GG	GG		
410 (58) consensus	G	G	GG	GG		
Aug07 F2 c1 - Goose	G	G	GG	GG		
Aug07 F2 c3 - Goose	G	G	GG	GG		
Aug07 F6 c1 - Goose	G	G	GG	GG		
Aug07 F7 c1 - Goose	G	G	GG	GG		
Aug07 F8 c2 - Goose	G	G	GG	GG		
Aug07 F2 c2 - Goose	G	G	GG	GG		
410 (44) c2	G	G	GG	GG		
Abington WW 17 c2	G	G	GG	GG		
140 (47) c2	G	G	GG	GG		
410 (47) c1	G	G	GG	GG		
Summer06 F3 - Deer	G	G	GG	GG		
140 (62) consensus	G	G	GG	GG		
Nov07 Fecal 2 - Raccoon	G	G	GG	GG		
140 (64) c4	G	G	GG	GG		
140 (36) c1	G	G	GG	GG		
Ambler WW 13 c1	G	G	GG	GG		
140 (68) c3	G	G	GG	GG		
410 (67) c3	G	G	GG	GG		
140 (46) c2	G	G	GG	GG		
410 (36) c1	G	G	GG	GG		
410 (38) c3	G	G	GG	GG		
Abington WW 13 c2	G	G	GG	GG		
410 (38) c2	G	G	GG	GG		
410 (40)	G	G	GG	GG		
140 (64) c2	G	G	GG	GG		
140 (40)	G	G	GG	GG		
Abington WW 17 c1	G	G	GG	GG		
140 (47) c3	G	G	GG	GG		
140 (68) c2	G	G	GG	GG		
March08 F3 c1 - Goose	G	G	GG	GG		
410 (36) c3	G	G	GG	GG		
140 (65) c2	G	G	GG	GG		
March08 F3 c2 - Goose	G	G	GG	GG		
April07 F4 c3 - Calf	G	G	GG	GG		
C. bovis AY120911	G	G	GG	GG		
AugF 5 c1 - Goose	G	G	GG	GG		
Goose genotype I AY324642	G	G	GG	GG		
C. hominis AF093489	G	G	GG	GG		
C. felis AF112575	G	G	GG	GG		
C. serpentis AF093499	G	G	GG	GG		
C. baileyi L19068	G	G	GG	GG		
C. andersoni AB089285	G	G	GG	GG		
C. muris (murine) AB089284	G	G	GG	GG		
C. canis AF112576	G	G	GG	GG		
Cervine genotype EF641018	G	G	GG	GG		
Deer mouse genotype III EF64101	G	G	GG	GG		
Muskrat genotype I Ef641016	G	G	GG	GG		
Squirrel genotype DQ295014	G	G	GG	GG		
Skunk genotype W13 AY737559	G	G	GG	GG		
Goose genotype (KLJ-5) AY32463	G	G	GG	GG		



INTENTIONAL SECOND EXPOSURE

419 (34)	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Aug07 F1 c1 - Goose	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Aug07 F10 - condensed - Goose	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
140 (36) c1	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Summer06 F3 c3 - unknown	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Lab control 11 23 (67)	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
C. parvum 116996	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
C. macrogadis AF112574	A	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
C. wratislaviensis 11440	A	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Ambler WW 11 c3	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
130 (59)	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Ambler WW 16 consensus	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Abington WW 10 consensus	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Sept07 fecal 2 - Goose	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Sept07 fecal 3 consensus - Goose	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
140 (64) c1	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
410 (40) consensus	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Aug07 F2 c1 - Goose	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Aug07 F3 c3 - Goose	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Aug07 F4 c1 - Goose	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Aug07 F5 c1 - Goose	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Aug07 F6 c2 - Goose	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Aug07 F7 c2 - Goose	AT	TTT	GAT	GAATA	A	T	TTATAT	AGT	AT	TAACAT	
410 (43) c2	AT	TTT	GAT	GAATA	A	T	TTATAT	AGT	AT	TAACAT	
Abington WW 17 c2	AT	TTT	GAT	GAATA	A	T	TTATAT	AGT	AT	TAACAT	
140 (47) c1	AT	TTT	GAT	GAATA	A	T	TTATAT	AGT	AT	TAACAT	
410 (47) c1	A	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Summer06 F3 - Deer	A	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
140 (52) consensus	AT	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Nov07 fecal 2 - Raccoon	AT	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
140 (64) c4	AT	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
140 (39) c1	AT	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Ambler WW 13 c1	AT	TTT	GAT	GAATA	A	T	TTATAT	AGT	AT	TAACAT	
140 (68) c3	AT	TTT	GAT	GAATA	A	T	TTATAT	AGT	AT	TAACAT	
410 (67) c3	AT	TTT	GAT	GAATA	A	T	TTATAT	AGT	AT	TAACAT	
140 (46) c2	AT	TTT	GAT	GAATA	A	T	TTATAT	AGT	AT	TAACAT	
410 (36) c1	AT	TTT	GAT	GAATA	A	T	TTATAT	AGT	AT	TAACAT	
410 (38) c3	AT	TTT	GAT	GAATA	A	T	TTATAT	AGT	AT	TAACAT	
Abington WW 11 c2	AT	TTT	GAT	GAATA	A	T	TTATAT	AGT	AT	TAACAT	
410 (38) c2	G	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
410 (40)	G	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
140 (64) c2	G	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
140 (40)	C	TTT	GAT	GAATA	G	AT	TTATAA	A	T	AT	TAACAT
Abington WW 17 c1	C	TTT	GAT	GAATA	G	AT	TTATAA	A	T	AT	TAACAT
140 (37) c3	C	TTT	GAT	GAATA	G	AT	TTATAA	A	T	AT	TAACAT
140 (62) c2	TA	TTT	GAT	GAATA	T	T	AGTATA	A	T	AT	TAACAC
March08 F3 c1 - Goose	TA	TTT	GAT	GAATA	T	T	AGTATA	A	T	AT	TAACAC
410 (36) c2	T	TTT	GAT	GAATA	A	T	TTATAT	A	AGAT	TAACAT	
140 (65) c2	T	TTT	GAT	GAATA	A	T	TTATAT	A	AGAT	TAACAT	
March08 F3 c2 - Goose	T	TTT	GAT	GAATA	A	T	TTATAT	A	AGAT	TAACAT	
April07 F3 c1 - Sali	T	TTT	GAT	GAATA	A	T	TTATAT	A	AGAT	TAACAT	
C. bovis AY120911	T	TTT	GAT	GAATA	A	T	TTATAT	A	AGAT	TAACAT	
Aug1 5 c1 - Goose	A	TTT	GAT	GAATA	T	T	TATGTA	A	A	AT	TAGCAT
Goose genotype 1 AY324642	A	TTT	GAT	GAATA	T	T	TATGTA	A	A	AT	TAGCAT
C. hominis AF093489	AA	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
C. felis AF112575	A	TTT	GAT	GAATA	T	A	TATGTA	A	G	AT	TAACAT
C. serpenti AF093499	T	TTT	GAT	GAATA	G	T	AATATT	A	A	TATCAACAT	
C. baileyi F9048	A	TTT	GAT	GAATA	A	T	TTATAT	A	AGAT	TAACAT	
C. andersoni AB089285	T	TTT	GAT	GAATA	ATT	ATTATA	T	T	AT	CAACAT	
C. muris (murina) AB089284	T	TTT	GAT	GAATA	TA	T	ATTATA	T	T	AT	CAACAT
C. canis AF112576	A	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Cervine genotype 1 F641018	T	TTT	GAT	GAATA	A	T	TTATAT	AGT	AT	TAACAT	
Deer mouse genotype III F641019	G	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Muskat genotype 1 F641016	TA	TTT	GAT	GAATA	T	T	AGTATA	A	T	AT	TAACAC
Squirrel genotype DQ295014	C	TTT	GAT	GAATA	G	AT	TTATAA	A	T	AT	TAACAT
Skunk genotype W13 AY237539	A	TTT	GAT	GAATA	T	T	TATATA	A	T	AT	TAACAT
Goose genotype (13) 3) AY52465	T	TTT	GAT	GAATA	A	T	TTATAT	A	AGAT	TAACAT	

INTENTIONAL SECOND EXPOSURE

410 (84)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Aug12 12 (1) - Goose	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Aug07 110 condensed - Goose	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
410 (85) (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Summer06 10 (1) - unknown	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
140 (60) (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
C. parvum 115996	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
C. melbaeaudis 0112573	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
C. weari 011440	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Swabber WW 11 (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
410 (79)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Ambler WW 16 consensus	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Minion WW 30 consensus	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Sep02 16 (1) - Goose	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Sep02 16 (1) - Goose	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
140 (60) (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
410 (82) (1) - Goose	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Aug07 117 (1) - Goose	AATTCATATT A	CTATATATTTT	AG T	ATATGAA
Sep07 12 (1) - Goose	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Aug07 116 (1) - Goose	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Aug07 117 (1) - Goose	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Aug07 116 (2) - Goose	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Aug07 117 (2) - Goose	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
410 (44) (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Abington WW 12 (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
140 (67) (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
410 (47) (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Summer06 11 - Deer	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
140 (62) consensus	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Nov 07 14 (1) - Ratoon	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
140 (63) (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
140 (64) (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Ambler WW 12 (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
140 (68) (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
410 (67) (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
140 (46) (2)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
410 (38) (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
410 (35) (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Abington WW 12 (2)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
410 (18) (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
410 (40)	AATTCATATT A	CTATATATTTT	AG T	ATATGAA
140 (64) (2)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
140 (60)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Abington WW 12 (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
140 (47) (1)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
140 (65) (2)	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
Mar 08 11 (1) - Goose	AATTCATATT A	CTATATATTTT	AG T	ATATGAAA
410 (36) (1)	AATTCGCAAT A	CTTTAT	G T	ATGCCGAAA
140 (65) (1)	AATTCGCAAT A	CTTTAT	G T	ATGCCGAAA
March08 11 (2) - Goose	AATTCGCAAT A	CTTTAT	G T	ATGCCGAAA
Aug07 14 (1) - Goose	AATTCATATT A	CTTTT	AG T	ATATGAAA
C. bovis AY120911	AATTCATATT A	CTTTT	G T	ATATGAAA
Aug1 5 (1) - Goose	AATCCGCAAT A	CCTGGC	G T	ATGCCGAAA
Goose genotype 1 AY157401	AATCCGCAAT A	CCTGGC	G T	ATGCCGAA
C. necatrix M091489	AATTCATATT A	CTATATATTTT	G T	ATATGAAA
C. felis AI112575	AATTCATAT TTTT	TAAAGACTGAATTTTACTTTTG	ATA	ATATGAAA
C. serpentina F091499	CCTTCCTATT A	TATTTTAAAT	A T	ATAGGAAA
C. helix 115068	AATTCACATT A	CTTTTAAA	AG T	ATGTGAAA
C. andersoni A089283	CCTTCCTATT A	TATTTCTAAAT	A T	ATAGGAAA
C. muris (marina) A089284	CCTTCCTATT A	TATTTCTAAAT	A T	ATAGGAAA
C. canis AI112576	AATTCATATT A	CTATATATTTT	G T	ATATGAAA
Cervine genotype 116-1018	AATTCATATT A	CTATATATTTT	G T	ATATGAAA
Deer mouse genotype III 116-410	AATTCATATT A	CTATATATTTT	G T	ATATGAAA
Squirrel genotype 1 116-410-16	AATTCATATT A	CTATATATTTT	G T	ATATGAAA
Squirrel genotype DQ295014	AATTCATATT A	CTATATATTTT	G T	ATATGAAA
Skunk genotype W18 AY237559	AATTCATATT A	CTATATATTTT	G T	ATATGAAA
Goose genotype (UK) 01-01-02-953	AATTCGCAAT A	CTTTAT	G T	ATGCCGAAA

	151	160	170	180	190	200
410 (54)	G	G	G	G	G	G
Aug07 F8 c1 - Goose	G	G	G	G	G	G
Aug07 F10 condensed - Goose	G	G	G	G	G	G
140 (46) c3	G	G	G	G	G	G
Summer06 F6 c3 - unknown	G	G	G	G	G	G
Lab control (1/24/07)	G	G	G	G	G	G
C. parvum L16996	G	G	G	G	G	G
C. meleagridis AF112574	G	G	G	G	G	G
C. wrairi U11440	G	G	G	G	G	G
Ambler WW 13 c3	G	G	G	G	G	G
410 (59)	G	G	G	G	G	G
Ambler WW 16 consensus	G	G	G	G	G	G
Abington WW 30 consensus	G	G	G	G	G	G
Sept07 Fecal 2 - Goose	G	G	G	G	G	G
Sept07 Fecal 4 consensus - Goose	G	G	G	G	G	G
140 (64) c3	G	G	G	G	G	G
410 (58) consensus	G	G	G	G	G	G
Aug07 F2 c1 - Goose	G	G	G	G	G	G
Aug07 F2 c3 - Goose	G	G	G	G	G	G
Aug07 F6 c1 - Goose	G	G	G	G	G	G
Aug07 F7 c1 - Goose	G	G	G	G	G	G
Aug07 F8 c2 - Goose	G	G	G	G	G	G
Aug07 F2 c2 - Goose	G	G	G	G	G	G
410 (44) c2	G	G	G	G	G	G
Abington WW 17 c2	G	G	G	G	G	G
140 (47) c2	G	G	G	G	G	G
410 (47) c1	G	G	G	G	G	G
Summer06 F3 - Deer	G	G	G	G	G	G
140 (62) consensus	G	G	G	G	G	G
Nov07 Fecal 2 - Raccoon	G	G	G	G	G	G
140 (64) c4	G	G	G	G	G	G
140 (36) c1	G	G	G	G	G	G
Ambler WW 13 c1	G	G	G	G	G	G
140 (68) c3	G	G	G	G	G	G
410 (67) c3	G	G	G	G	G	G
140 (46) c2	G	G	G	G	G	G
410 (36) c1	G	G	G	G	G	G
410 (38) c3	G	G	G	G	G	G
Abington WW 13 c2	G	G	G	G	G	G
410 (38) c2	G	G	G	G	G	G
410 (40)	G	G	G	G	G	G
140 (64) c2	G	G	G	G	G	G
140 (40)	G	G	G	G	G	G
Abington WW 17 c1	G	G	G	G	G	G
140 (47) c3	G	G	G	G	G	G
140 (68) c2	G	G	G	G	G	G
March08 F3 c1 - Goose	G	G	G	G	G	G
410 (36) c3	G	G	G	G	G	G
140 (65) c2	G	G	G	G	G	G
March08 F3 c2 - Goose	G	G	G	G	G	G
April07 F4 c3 - Calf	G	G	G	G	G	G
C. bovis AY120911	G	G	G	G	G	G
AugF 5 c1 - Goose	G	G	G	G	G	G
Goose genotype I AY324642	G	G	G	G	G	G
C. hominis AF093489	G	G	G	G	G	G
C. felis AF112575	G	G	G	G	G	G
C. serpentis AF093499	G	G	G	G	G	G
C. baileyi L19068	G	G	G	G	G	G
C. andersoni AB089285	G	G	G	G	G	G
C. muris (murine) AB089284	G	G	G	G	G	G
C. canis AF112576	G	G	G	G	G	G
Cervine genotype EF641018	G	G	G	G	G	G
Deer mouse genotype III EF641019	G	G	G	G	G	G
Muskrat genotype I EF641016	G	G	G	G	G	G
Squirrel genotype DQ295014	G	G	G	G	G	G
Skunk genotype W13 AY37559	G	G	G	G	G	G
Goose genotype (KLJ-5) AY32463	G	G	G	G	G	G

	201	210	220	230	240	250
410 (54)	G	G G		G		G G
Aug07 F8 c1 - Goose	G	G G		G		G G
Aug07 F10 condensed - Goose	G	G G		G		G G
140 (46) c3	G	G G		G		G G
Summer06 F6 c3 - unknown	G	G G		G		G G
Lab control (1/24/07)	G	G G		G		G G
C. parvum L16996	G	G G		G		G G
C. meleagridis AF112574	G	G G		G		G G
C. wralri U11440	G	G G		G		G G
Ambler WW 13 c3	G	G G		G		G G
410 (59)	G	G G		G		G G
Ambler WW 16 consensus	G	G G	G	G		G G
Abington WW 30 consensus	G	G G		G		G G
Sept07 Fecal 2 - Goose	G	G G		G		G G
Sept07 Fecal 4 consensus - Goose	G	G G		G		G G
140 (64) c3	G	G G		G		G G
410 (58) consensus	G	G G		G		G G
Aug07 F2 c1 - Goose	G	G G		G		G G
Aug07 F2 c3 - Goose	G	G G		G		G G
Aug07 F6 c1 - Goose	G	G G		G		G G
Aug07 F7 c1 - Goose	G	G G		G		G G
Aug07 F8 c2 - Goose	G	G G		G		G G
Aug07 F2 c2 - Goose	G	G G		G		G G
410 (44) c2	G	G G		G		G G
Abington WW 17 c2	G	G G		G		G G
140 (47) c2	G	G G		G		G G
410 (47) c1	G	G G		G		G G
Summer06 F3 - Deer	G	G G		G		G G
140 (62) consensus	G	G G		G		G G
Nov07 Fecal 2 - Raccoon	G	G G		G		G G
140 (64) c4	G	G G		G		G G
140 (36) c1	G	G G		G		G G
Ambler WW 13 c1	G	G G		G		G G
140 (68) c3	G	G G		G		G G
410 (67) c3	G	G G		G		G G
140 (46) c2	G	G G		G		G G
410 (36) c1	G	G G		G		G G
410 (38) c3	G	G G		G		G G
Abington WW 13 c2	G	G G		G		G G
410 (38) c2	G	G G		G		G G
410 (40)	G	G G		G		G G
140 (64) c2	G	G G		G		G G
140 (40)	G	G G		G		G G
Abington WW 17 c1	G	G G		G		G G
140 (47) c3	G	G G		G		G G
140 (68) c2	G	G G		G		G G
March08 F3 c1 - Goose	G	G G		G		G G
410 (36) c3	G	G G		G		G G
140 (65) c2	G	G G		G		G G
March08 F3 c2 - Goose	G	G G		G		G G
April07 F4 c3 - Calf	G	G G		G		G G
C. bovis AY120911	G	G G		G		G G
AugF 5 c1 - Goose	G	G G		G		G G
Goose genotype I AY324642	G	G G		G		G G
C. hominis AF093489	G	G G		G		G G
C. felis AF112575	G	G G		G		G G
C. serpentis AF093499	G	G G		G		G G
C. balleyi L19068	G	G G		G		G G
C. andersoni AB089285	G	G G		G		G G
C. muris (murine) AB089284	G	G G		G		G G
C. canis AF112576	G	G G		G		G G
Cervine genotype EF641018	G	G G		G		G G
Deer mouse genotype III EF64101	G	G G		G		G G
Muskrat genotype I EF641016	G	G G		G		G G
Squirrel genotype DQ295014	G	G G		G		G G
Skunk genotype W13 AY737559	G	G G		G		G G
Goose genotype (KLJ-5) AY32463	G	G G		G		G G

	251	260	270	280	290	300
410 (54)	G	G	G	GGG	G	GGGGG
Aug07 F8 c1 - Goose	G	G	G	GGG	G	GGGGG
Aug07 F10 condensed - Goose	G	G	G	GGG	G	GGGGG
140 (46) c3	G	G	G	GGG	G	GGGGG
Summer06 F6 c3 - unknown	G	G	G	GGG	G	GGGGG
Lab control (1/24/07)	G	G	G	GGG	G	GGGGG
C. parvum L16996	G	G	G	GGG	G	GGGGG
C. meleagridis AF112574	G	G	G	GGG	G	GGGGG
C. wrairi U11440	G	G	G	GGG	G	GGGGG
Ambler WW 13 c3	G	G	G	GGG	G	GGGGG
410 (59)	G	G	G	GGG	G	GGGGG
Ambler WW 16 consensus	G	G	G	GGG	G	GGGGG
Abington WW 30 consensus	G	G	G	GGG	G	GGGGG
Sept07 Fecal 2 - Goose	G	G	G	GGG	G	GGGGG
Sept07 Fecal 4 consensus - Goose	G	G	G	GGG	G	GGGGG
140 (64) c3	G	G	G	GGG	G	GGGGG
410 (58) consensus	G	G	G	GGG	G	GGGGG
Aug07 F2 c1 - Goose	G	G	G	GGG	G	GGGGG
Aug07 F2 c3 - Goose	G	G	G	GGG	G	GGGGG
Aug07 F6 c1 - Goose	G	G	G	GGG	G	GGGGG
Aug07 F7 c1 - Goose	G	G	G	GGG	G	GGGGG
Aug07 F8 c2 - Goose	G	G	G	GGG	G	GGGGG
Aug07 F2 c2 - Goose	G	G	G	GGG	G	GGGGG
410 (44) c2	G	G	G	GGG	G	GGGGG
Abington WW 17 c2	G	G	G	GGG	G	GGGGG
140 (47) c2	G	G	G	GGG	G	GGGGG
410 (47) c1	G	G	G	GGG	G	GGGGG
Summer06 F3 - Deer	G	G	G	GGG	G	GGGGG
140 (62) consensus	G	G	G	GGG	G	GGGGG
Nov07 Fecal 2 - Raccoon	G	G	G	GGG	G	GGGGG
140 (64) c4	G	G	G	GGG	G	GGGGG
140 (36) c1	G	G	G	GGG	G	GGGGG
Ambler WW 13 c1	G	G	G	GGG	G	GGGGG
140 (68) c3	G	G	G	GGG	G	GGGGG
410 (67) c3	G	G	G	GGG	G	GGGGG
140 (46) c2	G	G	G	GGG	G	GGGGG
410 (36) c1	G	G	G	GGG	G	GGGGG
410 (38) c3	G	G	G	GGG	G	GGGGG
Abington WW 13 c2	G	G	G	GGG	G	GGGGG
410 (38) c2	G	G	G	GGG	G	GGGGG
410 (40)	G	G	G	GGG	G	GGGGG
140 (64) c2	G	G	G	GGG	G	GGGGG
140 (40)	G	G	G	GGG	G	GGGGG
Abington WW 17 c1	G	G	G	GGG	G	GGGGG
140 (47) c3	G	G	G	GGG	G	GGGGG
140 (68) c2	G	G	G	GGG	G	GGGGG
March08 F3 c1 - Goose	G	G	G	GGG	G	GGGGG
410 (36) c3	GG	G	G	GGG	G	GGGGG
140 (65) c2	GG	G	G	GGG	G	GGGGG
March08 F3 c2 - Goose	GG	G	G	GGG	G	GGGGG
April07 F4 c3 - Calf	G	G	G	GGG	G	GGGGG
C. bovis AY120911	G	G	G	GGG	G	GGGGG
AugF 5 c1 - Goose	GG	G	G	GGG	G	GGGGG
Goose genotype I AY324642	GG	G	G	GGG	G	GGGGG
C. hominis AF093489	G	G	G	GGG	G	GGGGG
C. felis AF112575	G	G	G	GGG	G	GGGGG
C. serpentis AF093499	GG	G	GG	GGG	G	GGGGG
C. baileyi L19068	GG	G	G	GGG	G	GGGGG
C. andersoni AB089285	GG	G	GG	GGG	G	GGGGG
C. muris (murine) AB089284	GG	G	GG	GGG	G	GGGGG
C. canis AF112576	G	G	G	GGG	G	GGGGG
Cervine genotype EF641018	G	G	G	GGG	G	GGGGG
Deer mouse genotype III EF641010	G	G	G	GGG	G	GGGGG
Muskrat genotype I EF641016	G	G	G	GGG	G	GGGGG
Squirrel genotype DQ295014	G	G	G	GGG	G	GGGGG
Skunk genotype W13 AY737559	G	G	G	GGG	G	GGGGG
Goose genotype (KL)-5 AY32463	GG	G	G	GGG	G	GGGGG

	301	310	320	330	340	350
410 (54)	G	G	G	G	G	G
Aug07 F8 c1 - Goose	G	G	G	G	G	G
Aug07 F10 condensed - Goose	G	G	G	G	G	G
140 (46) c3	G	G	G	G	G	G
Summer06 F6 c3 - unknown	G	G	G	G	G	G
Lab control (1/24/07)	G	G	G	G	G	G
C. parvum L16996	G	G	G	G	G	G
C. meleagridis AF112574	G	G	G	G	G	G
C. wralri U11440	G	G	G	G	G	G
Ambler WW 13 c3	G	G	G	G	G	G
410 (59)	G	G	G	G	G	G
Ambler WW 16 consensus	G	G	G	G	G	G
Abington WW 30 consensus	G	G	G	G	G	G
Sept07 Fecal 2 - Goose	G	G	G	G	G	G
Sept07 Fecal 4 consensus - Goose	G	G	G	G	G	G
140 (64) c3	G	G	G	G	G	G
410 (58) consensus	G	G	G	G	G	G
Aug07 F2 c1 - Goose	G	G	G	G	G	G
Aug07 F2 c3 - Goose	G	G	G	G	G	G
Aug07 F6 c1 - Goose	G	G	G	G	G	G
Aug07 F7 c1 - Goose	G	G	G	G	G	G
Aug07 F8 c2 - Goose	G	G	G	G	G	G
Aug07 F2 c2 - Goose	G	G	G	G	G	G
410 (44) c2	G	G	G	G	G	G
Abington WW 17 c2	G	G	G	G	G	G
140 (47) c2	G	G	G	G	G	G
410 (47) c1	G	G	G	G	G	G
Summer06 F3 - Deer	G	G	G	G	G	G
140 (62) consensus	G	G	G	G	G	G
Nov07 Fecal 2 - Raccoon	G	G	G	G	G	G
140 (64) c4	G	G	G	G	G	G
140 (36) c1	G	G	G	G	G	G
Ambler WW 13 c1	G	G	G	G	G	G
140 (68) c3	G	G	G	G	G	G
410 (67) c3	G	G	G	G	G	G
140 (46) c2	G	G	G	G	G	G
410 (36) c1	G	G	G	G	G	G
410 (38) c3	G	G	G	G	G	G
Abington WW 13 c2	G	G	G	G	G	G
410 (38) c2	G	G	G	G	G	G
410 (40)	G	G	G	G	G	G
140 (64) c2	G	G	G	G	G	G
140 (40)	G	G	G	G	G	G
Abington WW 17 c1	G	G	G	G	G	G
140 (47) c3	G	G	G	G	G	G
140 (68) c2	G	G	G	G	G	G
March08 F3 c1 - Goose	G	G	G	G	G	G
410 (36) c3	G	G	G	G	G	G
140 (65) c2	G	G	G	G	G	G
March08 F3 c2 - Goose	G	G	G	G	G	G
April07 F4 c3 - Calf	G	G	G	G	G	G
C. bovis AY120911	G	G	G	G	G	G
AugF 5 c1 - Goose	G	G	G	G	G	G
Goose genotype I AY324642	G	G	G	G	G	G
C. hominis AF093489	G	G	G	G	G	G
C. fells AF112575	G	G	G	G	G	G
C. serpentis AF093499	G	G	G	G	G	G
C. baileyi L19068	G	G	G	G	G	G
C. andersoni AB089285	G	G	G	G	G	G
C. muris (murine) AB089284	G	G	G	G	G	G
C. canis AF112576	G	G	G	G	G	G
Cervine genotype EF641018	G	G	G	G	G	G
Deer mouse genotype III EF641019	G	G	G	G	G	G
Muskrat genotype I EF641016	G	G	G	G	G	G
Squirrel genotype DQ295014	G	G	G	G	G	G
Skunk genotype W13 AY737559	G	G	G	G	G	G
Goose genotype (KLJ-5) AY32463	G	G	G	G	G	G

	351	360	370	380	390	400
410 (54)	G	G	G	G	G	GGGG
Aug07 F8 c1 - Goose	G	G	G	G	G	GGGG
Aug07 F10 condensed - Goose	G	G	G	G	G	GGGG
140 (46) c3	G	G	G	G	G	GGGG
Summer06 F6 c3 - unknown	G	G	G	G	G	GGGG
Lab control (1/24/07)	G	G	G	G	G	GGGG
C. parvum L16996	G	G	G	G	G	GGGG
C. meleagridis AF112574	G	G	G	G	G	GGGG
C. wralri U11440	G	G	G	G	G	GGGG
Ambler WW 13 c3	G	G	G	G	G	GGGG
410 (59)	G	G	G	G	G	GGGG
Ambler WW 16 consensus	G	G	G	G	G	GGGG
Abington WW 30 consensus	G	G	G	G	G	GGGG
Sept07 Fecal 2 - Goose	G	G	G	G	G	GGGG
Sept07 Fecal 4 consensus - Goose	G	G	G	G	G	GGGG
140 (64) c3	G	G	G	G	G	GGGG
410 (58) consensus	G	G	G	G	G	GGGG
Aug07 F2 c1 - Goose	G	G	G	G	G	GGGG
Aug07 F2 c3 - Goose	G	G	G	G	G	GGGG
Aug07 F6 c1 - Goose	G	G	G	G	G	GGGG
Aug07 F7 c1 - Goose	G	G	G	G	G	GGGG
Aug07 F8 c2 - Goose	G	G	G	G	G	GGGG
Aug07 F2 c2 - Goose	G	G	G	G	G	GGGG
410 (44) c2	G	G	G	G	G	GGGG
Abington WW 17 c2	G	G	G	G	G	GGGG
140 (47) c2	G	G	G	G	G	GGGG
410 (47) c1	G	G	G	G	G	GGGG
Summer06 F3 - Deer	G	G	G	G	G	GGGG
140 (62) consensus	G	G	G	G	G	GGGG
Nov07 Fecal 2 - Raccoon	G	G	G	G	G	GGGG
140 (64) c4	G	G	G	G	G	GGGG
140 (36) c1	G	G	G	G	G	GGGG
Ambler WW 13 c1	G	G	G	G	G	GGGG
140 (68) c3	G	G	G	G	G	GGGG
410 (67) c3	G	G	G	G	G	GGGG
140 (46) c2	G	G	G	G	G	GGGG
410 (36) c1	G	G	G	G	G	GGGG
410 (38) c3	G	G	G	G	G	GGGG
Abington WW 13 c2	G	G	G	G	G	GGGG
410 (38) c2	G	G	G	G	G	GGGG
410 (40)	G	G	G	G	G	GGGG
140 (64) c2	G	G	G	G	G	GGGG
140 (40)	G	G	G	G	G	GGGG
Abington WW 17 c1	G	G	G	G	G	GGGG
140 (47) c3	G	G	G	G	G	GGGG
140 (68) c2	G	G	G	G	G	GGGG
March08 F3 c1 - Goose	G	G	G	G	G	GGGG
410 (36) c3	G	G	G	G	G	GGGG
140 (65) c2	G	G	G	G	G	GGGG
March08 F3 c2 - Goose	G	G	G	G	G	GGGG
April07 F4 c3 - Calf	G	G	G	G	G	GGGG
C. bovis AY120911	G	G	G	G	G	GGGG
AugF 5 c1 - Goose	G	G	G	G	G	GGGG
Goose genotype I AY324642	G	G	G	G	G	GGGG
C. hominis AF093489	G	G	G	G	G	GGGG
C. felis AF112575	G	G	G	G	G	GGGG
C. serpentis AF093499	G	G	G	G	G	GGGG
C. baileyi L19068	G	G	G	G	G	GGGG
C. andersoni AB089285	G	G	G	G	G	GGGG
C. muris (murine) AB089284	G	G	G	G	G	GGGG
C. canis AF112576	G	G	G	G	G	GGGG
Cervine genotype EF641018	G	G	G	G	G	GGGG
Deer mouse genotype III EF641019	G	G	G	G	G	GGGG
Muskrat genotype I EF641016	G	G	G	G	G	GGGG
Squirrel genotype DQ295014	G	G	G	G	G	GGGG
Skunk genotype W13 AY737559	G	G	G	G	G	GGGG
Goose genotype (KLJ-5) AY32463	G	G	G	G	G	GGGG

	401	410	420	430	440	450
410 (54)	G	G	G			
Aug07 F8 c1 - Goose	G	G	G			
Aug07 F10 condensed - Goose	G	G	G			
140 (46) c3	G	G	G			
Summer06 F6 c3 - unknown	G	G	G			
Lab control (1/24/07)	G	G	G			
C. parvum L16996	G	G	G			
C. meleagridis AF112574	G	G	G			
C. wrairi U11440	G	G	G			
Ambler WW 13 c3	G	G	G			
410 (59)	G	G	G			
Ambler WW 16 consensus	G	G	G			
Abington WW 30 consensus	G	G	G			
Sept07 Fecal 2 - Goose	G	G	G			
Sept07 Fecal 4 consensus - Goose	G	G	G			
140 (64) c3	G	G	G			
410 (58) consensus	G	G	G			
Aug07 F2 c1 - Goose	G	G	G			
Aug07 F2 c3 - Goose	G	G	G			
Aug07 F6 c1 - Goose	G	G	G			
Aug07 F7 c1 - Goose	G	G	G			
Aug07 F8 c2 - Goose	G	G	G			
Aug07 F2 c2 - Goose	G	G	G			
410 (44) c2	G	G	G			
Abington WW 17 c2	G	G	G			
140 (47) c2	G	G	G			
410 (47) c1	G	G	G			
Summer06 F3 - Deer	G	G	G			
140 (62) consensus	G	G	G			
Nov07 Fecal 2 - Raccoon	G	G	G			
140 (64) c4	G	G	G			
140 (36) c1	G	G	G			
Ambler WW 13 c1	G	G	G			
140 (68) c3	G	G	G			
410 (67) c3	G	G	G			
140 (46) c2	G	G	G			
410 (36) c1	G	G	G		G	
410 (38) c3	G	G	G			
Abington WW 13 c2	G	G	G			
410 (38) c2	G	G	G			
410 (40)	G	G	G			
140 (64) c2	G	G	G			
140 (40)	G	G	G			
Abington WW 17 c1	G	G	G			
140 (47) c3	G	G	G			
140 (68) c2	G	G	G			
March08 F3 c1 - Goose	G	G	G			
410 (36) c3	G	G	G			G
140 (65) c2	G	G	G			G
March08 F3 c2 - Goose	G	G	G			G
April07 F4 c3 - Calf	G	G	G			G
C. bovis AY120911	G	G	G			G
AugF 5 c1 - Goose	G	G	G			G
Goose genotype I AY324642	G	G	G			G
C. hominis AF093489	G	G	G			G
C. felis AF112575	G	G	G			G
C. serpentis AF093499	G	G	G			G
C. baileyi L19068	G	G	G			G
C. andersoni AB089285	G	G	G			G
C. muris (murine) AB089284	G	G	G			G
C. canis AF112576	G	G	G			G
Cervine genotype EF641018	G	G	G			G
Deer mouse genotype III EF641010	G	G	G			G
Muskrat genotype I EF641016	G	G	G			G
Squirrel genotype DQ295014	G	G	G			G
Skunk genotype W13 AY737559	G	G	G			G
Goose genotype (KLJ-5) AY32463	G	G	G			G

	451	460	470	480	487
410 (54)	G	G	G	G	G
Aug07 F8 c1 - Goose	G	G	G	G	G
Aug07 F10 condensed - Goose	G	G	G	G	G
140 (46) c3	G	G	G	G	G
Summer06 F6 c3 - unknown	G	G	G	G	G
Lab control (1/24/07)	G	G	G	G	G
C. parvum L16996	G	G	G	G	G
C. meleagridis AF112574	G	G	G	G	G
C. wrarli U11440	G	G	G	G	G
Ambler WW 13 c3	G	G	G	G	G
410 (59)	G	G	G	G	G
Ambler WW 16 consensus	G	G	G	G	G
Abington WW 30 consensus	G	G	G	G	G
Sept07 Fecal 2 - Goose	G	G	G	G	G
Sept07 Fecal 4 consensus - Goose	G	G	G	G	G
140 (64) c3	G	G	G	G	G
410 (58) consensus	G	G	G	G	G
Aug07 F2 c1 - Goose	G	G	G	G	G
Aug07 F2 c3 - Goose	G	G	G	G	G
Aug07 F6 c1 - Goose	G	G	G	G	G
Aug07 F7 c1 - Goose	G	G	G	G	G
Aug07 F8 c2 - Goose	G	G	G	G	G
Aug07 F2 c2 - Goose	G	G	G	G	G
410 (44) c2	G	G	G	G	G
Abington WW 17 c2	G	G	G	G	G
140 (47) c2	G	G	G	G	G
410 (47) c1	G	G	G	G	G
Summer06 F3 - Deer	G	G	G	G	G
140 (62) consensus	G	G	G	G	G
Nov07 Fecal 2 - Raccoon	G	G	G	G	G
140 (64) c4	G	G	G	G	G
140 (36) c1	G	G	G	G	G
Ambler WW 13 c1	G	G	G	G	G
140 (68) c3	G	G	G	G	G
410 (67) c3	G	G	G	G	G
140 (46) c2	G	G	G	G	G
410 (36) c1	G	G	G	G	G
410 (38) c3	G	G	G	G	G
Abington WW 13 c2	G	G	G	G	G
410 (38) c2	G	G	G	G	G
410 (40)	G	G	G	G	G
140 (64) c2	G	G	G	G	G
140 (40)	G	G	G	G	G
Abington WW 17 c1	G	G	G	G	G
140 (47) c3	G	G	G	G	G
140 (68) c2	G	G	G	G	G
March08 F3 c1 - Goose	G	G	G	G	G
410 (36) c3	G	G	G	G	G
140 (65) c2	G	G	G	G	G
March08 F3 c2 - Goose	G	G	G	G	G
April07 F4 c3 - Calf	G	G	G	G	G
C. bovis AY120911	G	G			
AugF 5 c1 - Goose	G	G	G	G	G
Goose genotype I AY324642	G	G	G	G	G
C. hominis AF093489	G	G	G	G	G
C. felis AF112575	G	G	G	G	G
C. serpentina AF093499	G	G	G	G	G
C. baileyi L19068	G	G	G	G	G
C. andersoni AB089285	G	G	G	G	G
C. muris (murine) AB089284	G	G	G	G	G
C. canis AF112576	G	G	G	G	G
Cervine genotype EF641018	G	G	G	G	G
Deer mouse genotype III EF641010	G	G	G	G	G
Muskrat genotype I EF641016	G	G	G	G	G
Squirrel genotype DQ295014	G	G	G	G	G
Skunk genotype W13 AY737559	G	G	G	G	G
Goose genotype (KLJ-5) AY32463	G	G	G	G	G

Vita

About Amy Elizabeth Lynch:

Born Boston, MA on April 28, 1984 to David E. Lynch and Lorraine A. Lynch, she graduated high school from Tewksbury Memorial High School in May 2002. She graduated from Tufts University in Medford, MA with a Bachelor of Science in Environmental Engineering in May 2006. She received the Gibson Fellowship from Lehigh University Dept. of Civil and Environmental Engineering in September 2006. She completed her EIT certification in April 2006. She will go on to join Weston and Sampson Engineers, Inc. in Peabody MA as an Engineer with the completion of this thesis and graduation from Lehigh University.

**END OF
TITLE**