Final Report

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Identifying Sources of Fecal Pollution in Shellfish and Nontidal Waters in Maryland Watersheds

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INTRODUCTION

Microbial Source Tracking. Microbial Source Tracking (MST) is a relatively recent scientific and technological innovation designed to distinguish the origins of enteric microorganisms found in environmental waters. Several different methods and a variety of different indicator organisms (both bacteria and viruses) have successfully been used for MST, as described in recent reviews (Scott *et al.*, 2002; Simpson *et al.*, 2002). When the indicator organism is bacteria, the term Bacterial Source Tracking (BST) is often used. Some common bacterial indicators for BST analysis include: *E. coli*, *Enterococcus* spp., *Bacteroides-Prevotella,* and *Bifidobacterium* spp.

Techniques for MST can be grouped into one of the following three categories: molecular (genotypic) methods, biochemical (phenotypic) methods, or chemical methods. Ribotyping, Pulsed-Field Gel Electrophoresis (PFGE), and Randomly-Amplified Polymorphic DNA (RAPD) are examples of molecular techniques. Biochemical methods include Antibiotic Resistance Analysis (ARA), F-specific coliphage typing, and Carbon Source Utilization (CSU) analysis. Chemical techniques detect chemical compounds associated with human activities, but do not provide any information regarding nonhuman sources. Examples of this type of technology include detection of optical brighteners from laundry detergents or caffeine (Simpson *et al*., 2002).

Many of the molecular and biochemical methods of MST are "library-based," requiring the collection of a database of fingerprints or patterns obtained from indicator organisms isolated from known sources. Statistical analysis determines fingerprints/patterns of known-source species or categories of species (*i.e.*, human, livestock, pet, wildlife). Indicator isolates collected from water samples are analyzed using the same MST method to obtain their fingerprints or patterns, which are then statistically compared to those in the library. Based upon this comparison, the final results are expressed in terms of the "statistical probability" that the water isolates came from a given source (Simpson *et al*. 2002).

In this BST project, we studied the following Maryland tidal watersheds/shellfish harvesting areas: Furnace Creek, Magothy River, Marley Creek, Rhode River, Severn River, South River, West Chesapeake Bay, and West River. The methodology used was the ARA, with *Enterococcus* spp. as the indicator organism. Previous BST publications have demonstrated the predictive value of using this particular technique and indicator organism (Price *et al*., 2006; Hagedorn, 1999; Wiggins, 1999). A subset of deer scat isolates collected from watersheds across Maryland were analyzed using PFGE (a genotypic method) to augment a pilot study begun during the 2007 BST project.

Antibiotic Resistance Analysis. A variety of different host species can potentially contribute to the fecal contamination found in natural waters. Many years ago, scientists speculated on the possibility of using resistance to antibiotics as a way of determining the sources of this fecal contamination (Bell *et al.*, 1983; Krumperman, 1983). In ARA, the premise is that bacteria

isolated from different hosts can be discriminated based upon differences in the selective pressure of microbial populations found in the gastrointestinal tract of those hosts (humans, livestock, pets, wildlife) (Wiggins, 1996). Microorganisms isolated from the fecal material of wildlife would be expected to have a much lower level of resistance to antibiotics than isolates collected from the fecal material of humans, livestock and pets. In addition, depending upon the specific antibiotics used in the analysis, isolates from humans, livestock and pets could be differentiated from each other.

In ARA, isolates from known sources are tested for resistance or sensitivity against a panel of antibiotics and antibiotic concentrations. This information is then used to construct a library of antibiotic resistance patterns from known-source bacterial isolates. Microbial isolates collected from water samples are then tested and their resistance results are recorded. Based upon a comparison of resistance patterns of water and library isolates, a statistical analysis can predict the likely host source of the water isolates (Hagedorn 1999; Price *et al*., 2006; Wiggins 1999).

LABORATORY METHODS

Isolation of *Enterococcus* from Known-Source Samples. Fecal samples, identified to source, were delivered to the Salisbury University (SU) BST lab by Maryland Department of the Environment (MDE) personnel. Fecal material suspended in phosphate-buffered saline was plated onto selective m-Enterococcus agar. After incubation at 37° C, up to eight (8) *Enterococcus* isolates were randomly selected from each fecal sample for ARA testing.

Isolation of Enterococcus from Water Samples. Water samples were collected by MDE staff and shipped overnight to MapTech Inc, Blacksburg, Va. Bacterial isolates were collected by membrane filtration. Up to 24 randomly selected *Enterococcus* isolates were collected from each water sample and all isolates were then shipped to the SU BST lab.

Antibiotic Resistance Analysis. Each bacterial isolate from both water and scat were grown in Enterococcosel® broth (Becton Dickinson, Sparks, MD) prior to ARA testing. *Enterococci* are capable of hydrolyzing esculin, turning this broth black. Only esculin-positive isolates were tested for antibiotic resistance.

Bacterial isolates were plated onto tryptic soy agar plates, each containing a different concentration of a given antibiotic. Plates were incubated overnight at 37° C and isolates then scored for growth (resistance) or no growth (sensitivity). Data consisting of a “1” for resistance or “0” for sensitivity for each isolate at each concentration of each antibiotic was then entered into a spread-sheet for statistical analysis.

The following table includes the antibiotics and concentrations used for isolates in analyses for all the study watersheds.

Table 1. Antibiotics and concentrations used for ARA.

Antibiotic	Concentration (µg/ml)
Amoxicillin	0.625
Bacitracin	25, 50
Cephalothin	10, 15, 30, 50
Chloramphenicol	10
Chlortetracycline	60, 80, 100
Erythromycin	10
Gentamycin	5, 10, 15
Kanamycin	25, 50
Neomycin	40, 60, 80
Oxytetracycline	20, 40, 60, 80, 100
Streptomycin	40, 60, 80, 100
Tetracycline	10, 30, 50, 100
Vancomycin	2.5

KNOWN-SOURCE LIBRARY

Construction and Use. Fecal samples (scat) from known sources in each watershed were collected during the study period by MDE personnel and delivered to the BST Laboratory at SU. *Enterococcus* isolates were obtained from known sources (i.e., human, livestock (chicken, cow, donkey, emu, goat, horse, llama, peacock, pig, sheep), pet (cat, dog), wildlife (beaver, deer, duck, fox, goose, muskrat, opossum, pigeon, rabbit, raccoon, seagull, squirrel)). For each watershed, a library of patterns of *Enterococcus* isolate responses to the panel of antibiotics was analyzed using the statistical software CART® (Salford Systems, San Diego, CA). *Enterococcus* isolate response patterns were also obtained from bacteria in water samples collected at the monitoring stations in each basin. Using statistical techniques, these patterns were then compared to those in the appropriate library to identify the probable source of each water isolate. No combined known-source libraries were used for any shellfish harvesting area; a known-source isolate library collected from each area was used for the particular watershed.

STATISTICAL ANALYSIS

Our objective is to estimate the probability distribution for bacterial sources likely to contaminate surface waters. We use a statistical method, tree classification, to analyze watershed

library data consisting of ARA isolate profiles.[1] The statistical analysis results are predictive relationships between isolate characteristics and bacterial sources. These predictive relationships, collectively referred to as a model, are used to estimate probabilities of bacterial source origin for each isolate. When applied to a water sample isolate of unknown origin, these relationships lead to an estimate of the probability of source origin for each source. For example, if there were four sources of bacteria potentially contaminating surface waters, the model applied to a specific water sample isolate would produce four probabilities, the probabilities of source origin for that water sample isolate. Averaging each of the four probabilities across all water sample isolates produces an estimate of the probability distribution for bacteria sources. The source probability distribution estimate may be used to determine the source or sources responsible the largest contributions of bacterial contamination to surface waters.

1 The tree classification method we employed is known as CART®, Classification and Regression Trees, Salford Systems, San Diego, CA. For details, refer to: Breiman L, et al. *Classification and Regression Trees*. Pacific Grove: Wadsworth, 1984; Steinberg D and Colla P. *CART—Classification and Regression Trees.* San Diego, CA: Salford Systems, 1997; and The Elements of Statistical Learning: Data Mining, Inference, and Prediction; Hastie T, Tibshirani R, and Friedman J. Springer 2001.

Furnace Creek Watershed ARA Results

Known-Source Library. A 565 known-source isolate library was constructed from sources in the Furnace Creek Watershed. The number of unique antibiotic resistance patterns was calculated, and the known sources in the library were grouped into four categories: human, livestock (none), pet (dog), and wildlife (deer, fox, rabbit, raccoon) (Table 2-FUR). The library was analyzed for its ability to take a subset of the library isolates and correctly predict the identity of their host sources when they were treated as unknowns.

Table 2-FUR: Furnace Creek. Source category, total number, and number of unique patterns in the Furnace Creek known-source library.

Source category	Potential Sources	Total Isolates	Unique Patterns
Human	human	185	133
Livestock	n/a	0	0
Pet	dog	173	105
Wildlife	deer, fox, rabbit, raccoon	207	72
Total		565	310

For Furnace Creek Watershed, the rates of correct classification (RCC) were calculated and the average rate of correct classification (ARCC) for the library was determined as described above in the "Statistical Analysis" section of this document. (Table 3-FUR).

Table 3-FUR. Furnace Creek. Actual versus predicted sources, with rates of correct classification (RCC) for each source. No cutoffs applied.

	Predicted				% Correct
Actual	Human	Pet	Wildlife	Total	(RCC*)
Human	150	18	17	185	81.1%
Pet	23	115	35	173	66.5%
Wildlife	22	22	163	207	78.7%
Total	195	155	215	565	
				ARCC:	75.8%

*RCC = Actual number of predicted species category / Total number predicted. Example: 150 human correctly predicted; 185 total number predicted for human = 150/185 = 81.1%.

Furnace Creek Water Samples. Monthly monitoring from one (1) monitoring station on Furnace Creek was the source of water samples. The maximum number of *Enterococcus* isolates per water sample was 24, although the number of isolates that actually grew was sometimes less than 24. A total of 258 *Enterococcus* isolates were analyzed by statistical analysis. The BST results by species category is shown in Table 4-FUR.

Table 4-FUR. Furnace Creek. Predicted host source distribution of water isolates.

Source	Distribution
Human	32.5%
Livestock	0.0%
Pet	29.4%
Wildlife	38.1%
Total	**100.0%**

The seasonal distribution of water isolates from samples collected at the sampling station is shown below in Table 5-FUR.

Table 5-FUR: Furnace Creek. *Enterococcus* isolates obtained from water collected during the spring, summer, fall, and winter seasons at the Furnace Creek monitoring station.

	Season				
Station	Spring	Summer	Fall	Winter	Total
FUR-1	53	69	69	67	258
Total	53	69	69	67	258

Tables 6-FUR and 7-FUR on the following pages show the numbers and percentages, respectively, of probable sources for the monitoring station by date.

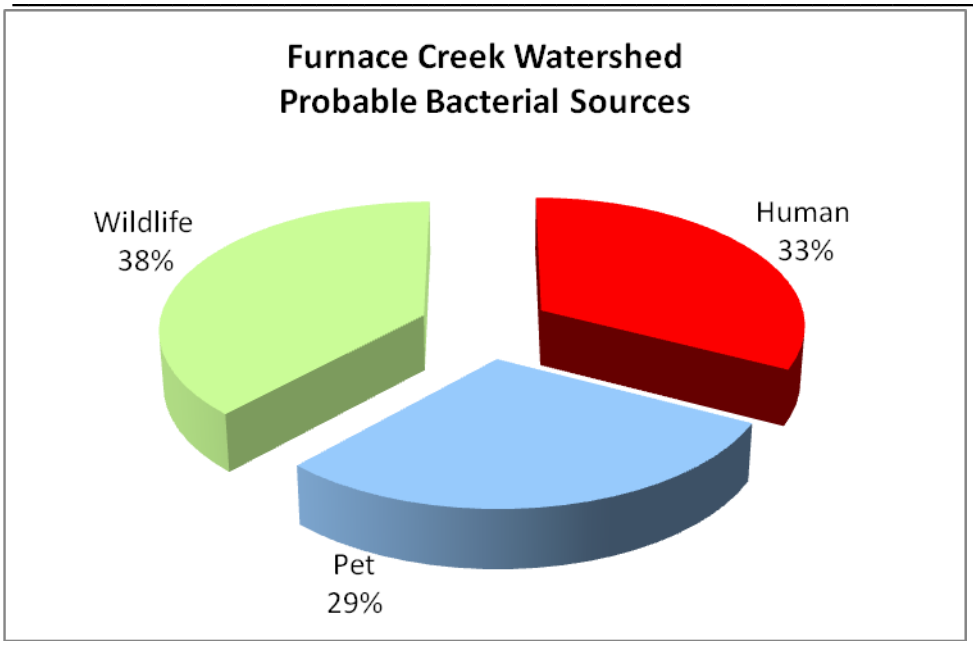
Table 6-FUR. Furnace Creek. BST Analysis: Number of isolates per station per date.

		Predicted Source			
Station	Date	Human	Pet	Wildlife	Total
FUR1	12/05/07	10.81	4.49	6.70	22.00
FUR1	01/10/08	5.13	3.77	15.10	24.00
FUR1	02/11/08	8.55	9.73	5.72	24.00
FUR1	03/05/08	9.75	4.64	4.61	19.00
FUR1	04/08/08	1.81	7.18	2.01	11.00
FUR1	05/07/08	4.58	5.73	7.69	18.00
FUR1	06/04/08	7.79	8.12	8.09	24.00
FUR1	07/09/08	8.05	5.95	9.99	24.00
FUR1	08/06/08	7.31	3.86	12.83	24.00
FUR1	09/04/08	5.86	9.44	5.70	21.00
FUR1	10/16/08	7.81	8.59	7.60	24.00
FUR1	11/20/08	6.49	4.33	12.17	23.00
Total		83.93	75.85	98.22	258.00

Table 7-FUR. Furnace Creek. Predicted source distribution by station and date.

		Predicted Source			
Station	Date	Human	Pet	Wildlife	Total
FUR1	12/05/07	49%	20%	30%	100%
FUR1	01/10/08	21%	16%	63%	100%
FUR1	02/11/08	36%	41%	24%	100%
FUR1	03/05/08	51%	24%	24%	100%
FUR1	04/08/08	16%	65%	18%	100%
FUR1	05/07/08	25%	32%	43%	100%
FUR1	06/04/08	32%	34%	34%	100%
FUR1	07/09/08	34%	25%	42%	100%
FUR1	08/06/08	30%	16%	53%	100%
FUR1	09/04/08	28%	45%	27%	100%
FUR1	10/16/08	33%	36%	32%	100%
FUR1	11/20/08	28%	19%	53%	100%
Total		33%	29%	38%	100%

Figure 1-FUR. Furnace Creek Watershed relative contributions by probable sources of *Enterococcus* contamination



Furnace Creek Summary

The use of ARA was successful for identification of probable bacterial sources in the Furnace Creek Watershed. The highest RCC for the library was for human (81%), followed by wildlife (79%), and pet (67%). The largest category of potential sources in the watershed as a whole was wildlife, followed by human, then pet (Fig. 1-FUR).

Magothy River Watershed ARA Results

Known-Source Library. A 1,117 known-source isolate library was constructed from sources in the Magothy River Watershed. The number of unique antibiotic resistance patterns was calculated, and the known sources in the library were grouped into four categories: human, livestock (cow, horse), pet (dog), and wildlife (deer, fox, goose, muskrat, rabbit, raccoon) (Table 2-MAG). The library was analyzed for its ability to take a subset of the library isolates and correctly predict the identity of their host sources when they were treated as unknowns.

Table 2-MAG. Magothy River. Source category, total number, and number of unique patterns.

Source category	Potential Sources	Total Isolates	Unique Patterns
Human	human	295	171
Livestock	cow, horse	267	82
Pet	dog	280	134
Wildlife	deer, fox, goose, muskrat, rabbit, raccoon	275	105
Total		1117	492

For Magothy River Watershed, the rates of correct classification (RCC) were calculated and the average rate of correct classification (ARCC) for the library was determined as described above in the "Statistical Analysis" section of this document. (Table 3-MAG).

Table 3-MAG. Magothy River. Actual versus predicted sources, with rates of correct classification (RCC) for each source. No cutoffs applied.

	Predicted					
Actual	Human	Livestock	Pet	Wildlife	Total	% Correct (RCC*)
Human	237	16	23	19	295	80.3%
Livestock	27	197	11	32	267	73.8%
Pet	18	50	168	44	280	60.0%
Wildlife	11	67	14	183	275	66.6%
Total	293	330	216	278	1117	
					ARCC:	70.3%

*RCC = Actual number of predicted species category / Total number predicted. Example: 237 human correctly predicted; 295 total number predicted for human = 237/295 = 80.3%.

Magothy River Water Samples. Monthly monitoring from ten (10) monitoring stations on Magothy River was the source of water samples. The maximum number of *Enterococcus* isolates per water sample was 24, although the number of isolates that actually grew was sometimes less than 24. A total of 271 *Enterococcus* isolates were analyzed by statistical analysis. The BST results by species category is shown in Table 4-MAG.

Table 4-MAG. Magothy River. Predicted host source distribution of water isolates.

Source	Distribution
Human	20.2%
Livestock	26.2%
Pet	26.0%
Wildlife	27.6%
Total	100.0%

The seasonal distribution of water isolates from samples collected at the sampling stations is shown below in Table 5-MAG.

Table 5-MAG: Magothy River. *Enterococcus* isolates obtained from water collected during the spring, summer, fall, and winter seasons at the Magothy River monitoring stations.

	Season				
Station	Spring	Summer	Fall	Winter	Total
03-01-001	7	6	8	9	30
03-01-001A	5	21	30	20	76
03-01-001C	0	0	5	5	10
03-01-005C	4	3	3	7	17
03-01-006B	2	16	4	2	24
03-01-009A	7	12	10	3	32
03-01-011	17	1	26	8	52
03-01-800	1	4	1	0	6
03-01-801	1	1	2	0	4
03-01-802	0	13	5	2	20
Total	44	77	94	56	271

Tables 6-MAG and 7-MAG below show the numbers and percentages, respectively, of the probable sources for the monitoring stations by date.

Table 6-MAG. Magothy River. Predicted source distribution by station and date.

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-01-001	11/08/07	0.79	0.14	0.07	0.00	1.00
03-01-001	01/08/08	0.00	0.47	0.15	1.38	2.00
03-01-001	02/05/08	1.24	0.70	1.72	1.34	5.00
03-01-001	03/04/08	0.83	0.49	0.37	0.31	2.00
03-01-001	05/05/08	1.05	2.09	1.19	1.67	6.00
03-01-001	06/03/08	0.13	0.28	0.00	0.60	1.00
03-01-001	07/08/08	2.22	0.82	0.89	1.07	5.00
03-01-001	09/03/08	0.18	0.20	0.48	0.15	1.00
03-01-001	10/15/08	1.69	1.77	1.13	2.40	7.00
03-01-001A	11/08/07	12.77	2.72	3.66	0.85	20.00
03-01-001A	12/10/07	0.08	0.46	1.15	0.31	2.00
03-01-001A	01/08/08	0.29	2.15	6.00	1.56	10.00
03-01-001A	02/05/08	1.07	0.94	2.92	2.07	7.00
03-01-001A	03/04/08	0.66	0.99	0.74	0.61	3.00
03-01-001A	06/03/08	2.37	1.16	0.72	0.75	5.00
03-01-001A	07/08/08	0.54	1.42	0.44	1.60	4.00
03-01-001A	08/05/08	1.00	0.24	1.08	0.69	3.00
03-01-001A	09/03/08	1.08	5.23	3.81	3.87	14.00
03-01-001A	10/15/08	1.50	2.35	1.30	2.84	8.00
03-01-001C	11/08/07	0.36	0.98	1.52	1.15	4.00
03-01-001C	02/05/08	0.22	0.73	2.93	0.12	4.00
03-01-001C	03/04/08	0.77	0.00	0.00	0.23	1.00
03-01-001C	10/15/08	0.65	0.18	0.00	0.17	1.00
03-01-005C	01/08/08	2.43	0.76	0.08	2.73	6.00
03-01-005C	02/05/08	0.57	0.29	0.14	0.00	1.00
03-01-005C	04/03/08	0.05	0.58	0.38	0.99	2.00
03-01-005C	06/03/08	0.01	0.90	0.22	0.87	2.00
03-01-005C	07/08/08	0.24	0.00	0.50	0.26	1.00
03-01-005C	09/03/08	0.08	1.25	0.17	0.49	2.00
03-01-005C	10/15/08	0.26	0.23	1.02	1.49	3.00
03-01-006B	11/08/07	0.00	0.24	0.08	0.69	1.00
03-01-006B	01/08/08	0.00	0.00	1.00	0.00	1.00
03-01-006B	02/05/08	0.05	0.35	0.30	0.31	1.00
03-01-006B	04/03/08	0.00	0.24	0.08	0.69	1.00
03-01-006B	06/03/08	0.13	0.28	0.00	0.60	1.00
03-01-006B	07/15/08	0.00	0.03	0.28	0.69	1.00
03-01-006B	08/05/08	0.34	3.80	0.79	2.07	7.00
03-01-006B	09/03/08	1.85	2.97	1.20	1.98	8.00
03-01-006B	10/15/08	0.66	0.99	0.74	0.6	3.00

Table 6-MAG. Magothy River. Predicted source distribution by station and date (continued).

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-01-009A	11/08/07	0.05	1.83	0.81	2.31	5.00
03-01-009A	12/10/07	1.05	1.01	0.44	0.49	3.00
03-01-009A	01/08/08	1.00	0.00	0.00	0.00	1.00
03-01-009A	02/05/08	0.05	0.35	0.30	0.31	1.00
03-01-009A	03/04/08	0.05	0.35	0.30	0.31	1.00
03-01-009A	05/05/08	0.22	0.38	2.79	1.61	5.00
03-01-009A	06/03/08	0.05	1.29	0.23	0.43	2.00
03-01-009A	07/08/08	2.92	0.51	0.07	1.49	5.00
03-01-009A	08/05/08	0.00	0.10	0.83	2.07	3.00
03-01-009A	09/03/08	0.29	1.18	0.09	2.44	4.00
03-01-009A	10/15/08	0.25	0.66	0.65	0.44	2.00
03-01-011	11/08/07	0.08	1.60	0.39	0.93	3.00
03-01-011	12/10/07	0.00	0.00	0.00	1.00	1.00
03-01-011	02/05/08	0.43	1.01	4.08	1.48	7.00
03-01-011	03/04/08	0.57	0.29	0.14	0.00	1.00
03-01-011	04/03/08	0.00	0.34	0.22	0.44	1.00
03-01-011	05/05/08	1.58	3.83	0.81	4.78	11.00
03-01-011	06/03/08	0.77	1.39	1.42	1.42	5.00
03-01-011	09/03/08	0.05	0.35	0.30	0.31	1.00
03-01-011	10/15/08	1.23	8.56	3.75	8.47	22.00
03-01-800	06/03/08	0.18	0.00	0.70	0.12	1.00
03-01-800	07/08/08	0.00	0.08	0.76	0.16	1.00
03-01-800	09/03/08	1.19	0.66	0.84	0.30	3.00
03-01-800	10/15/08	0.18	0.00	0.70	0.12	1.00
03-01-801	12/10/07	0.01	0.66	0.14	0.18	1.00
03-01-801	06/03/08	0.13	0.28	0.00	0.60	1.00
03-01-801	09/03/08	0.65	0.18	0.00	0.17	1.00
03-01-801	10/15/08	0.18	0.00	0.70	0.12	1.00
03-01-802	01/08/08	0.00	1.00	0.00	0.00	1.00
03-01-802	03/04/08	0.05	0.35	0.30	0.31	1.00
03-01-802	07/08/08	0.05	1.01	0.44	0.49	2.00
03-01-802	09/03/08	1.47	1.14	7.13	1.26	11.00
03-01-802	10/15/08	1.91	1.07	1.95	0.07	5.00
Total		54.75	70.93	70.50	74.82	271.00

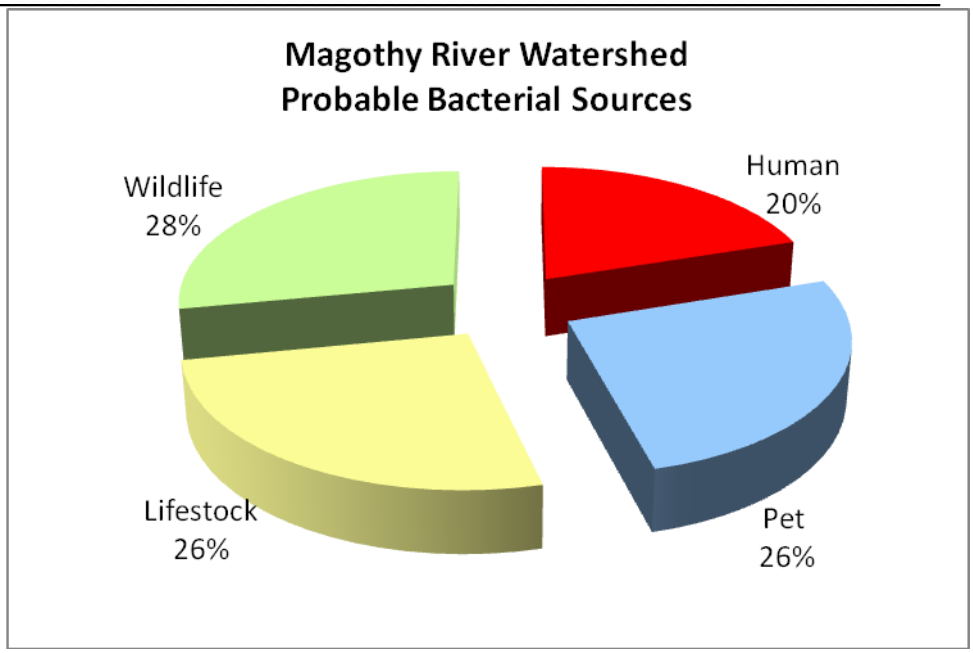
Table 7-MAG. Magothy River. Predicted source distribution by station and date.

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-01-001	11/08/07	79%	14%	7%	0%	100%
03-01-001	01/08/08	0%	24%	8%	69%	100%
03-01-001	02/05/08	25%	14%	34%	27%	100%
03-01-001	03/04/08	42%	25%	18%	15%	100%
03-01-001	05/05/08	17%	35%	20%	28%	100%
03-01-001	06/03/08	13%	28%	0%	60%	100%
03-01-001	07/08/08	44%	16%	18%	21%	100%
03-01-001	09/03/08	18%	20%	48%	15%	100%
03-01-001	10/15/08	24%	25%	16%	34%	100%
03-01-001A	11/08/07	64%	14%	18%	4%	100%
03-01-001A	12/10/07	4%	23%	57%	15%	100%
03-01-001A	01/08/08	3%	21%	60%	16%	100%
03-01-001A	02/05/08	15%	13%	42%	30%	100%
03-01-001A	03/04/08	22%	33%	25%	20%	100%
03-01-001A	06/03/08	47%	23%	14%	15%	100%
03-01-001A	07/08/08	13%	36%	11%	40%	100%
03-01-001A	08/05/08	33%	8%	36%	23%	100%
03-01-001A	09/03/08	8%	37%	27%	28%	100%
03-01-001A	10/15/08	19%	29%	16%	35%	100%
03-01-001C	11/08/07	9%	24%	38%	29%	100%
03-01-001C	02/05/08	6%	18%	73%	3%	100%
03-01-001C	03/04/08	77%	0%	0%	23%	100%
03-01-001C	10/15/08	65%	18%	0%	17%	100%
03-01-005C	01/08/08	40%	13%	1%	46%	100%
03-01-005C	02/05/08	57%	29%	14%	0%	100%
03-01-005C	04/03/08	2%	29%	19%	50%	100%
03-01-005C	06/03/08	0%	45%	11%	44%	100%
03-01-005C	07/08/08	24%	0%	50%	26%	100%
03-01-005C	09/03/08	4%	63%	9%	25%	100%
03-01-005C	10/15/08	9%	8%	34%	50%	100%
03-01-006B	11/08/07	0%	24%	8%	69%	100%
03-01-006B	01/08/08	0%	0%	100%	0%	100%
03-01-006B	02/05/08	5%	35%	30%	31%	100%
03-01-006B	04/03/08	0%	24%	8%	69%	100%
03-01-006B	06/03/08	13%	28%	0%	60%	100%
03-01-006B	07/15/08	0%	3%	28%	69%	100%
03-01-006B	08/05/08	5%	54%	11%	30%	100%
03-01-006B	09/03/08	23%	37%	15%	25%	100%

Table 7-MAG. Magothy River. Predicted source distribution by station and date (continued).

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-01-009A	11/08/07	1%	37%	16%	46%	100%
03-01-009A	12/10/07	35%	34%	15%	16%	100%
03-01-009A	01/08/08	100%	0%	0%	0%	100%
03-01-009A	02/05/08	5%	35%	30%	31%	100%
03-01-009A	03/04/08	5%	35%	30%	31%	100%
03-01-009A	05/05/08	4%	8%	56%	32%	100%
03-01-009A	06/03/08	2%	65%	11%	22%	100%
03-01-009A	07/08/08	58%	10%	1%	30%	100%
03-01-009A	08/05/08	0%	3%	28%	69%	100%
03-01-009A	09/03/08	7%	30%	2%	61%	100%
03-01-009A	10/15/08	12%	33%	32%	22%	100%
03-01-011	11/08/07	3%	53%	13%	31%	100%
03-01-011	12/10/07	0%	0%	0%	100%	100%
03-01-011	02/05/08	6%	14%	58%	21%	100%
03-01-011	03/04/08	57%	29%	14%	0%	100%
03-01-011	04/03/08	0%	34%	22%	44%	100%
03-01-011	05/05/08	14%	35%	7%	43%	100%
03-01-011	06/03/08	15%	28%	28%	28%	100%
03-01-011	09/03/08	5%	35%	30%	31%	100%
03-01-011	10/15/08	6%	39%	17%	39%	100%
03-01-800	06/03/08	18%	0%	70%	12%	100%
03-01-800	07/08/08	0%	8%	76%	16%	100%
03-01-800	09/03/08	40%	22%	28%	10%	100%
03-01-800	10/15/08	18%	0%	70%	12%	100%
03-01-801	12/10/07	1%	66%	14%	18%	100%
03-01-801	06/03/08	13%	28%	0%	60%	100%
03-01-801	09/03/08	65%	18%	0%	17%	100%
03-01-801	10/15/08	18%	0%	70%	12%	100%
03-01-802	01/08/08	0%	100%	0%	0%	100%
03-01-802	03/04/08	5%	35%	30%	31%	100%
03-01-802	07/08/08	3%	51%	22%	25%	100%
03-01-802	09/03/08	13%	10%	65%	11%	100%
03-01-802	10/15/08	38%	21%	39%	1%	100%
Total		20%	26%	26%	28%	100%

Figure 1-MAG. Magothy River Watershed relative contributions by probable sources of *Enterococcus* contamination



Magothy River Summary.

The use of ARA was successful for identification of probable bacterial sources in the Magothy River Watershed. The highest RCC for the library was for human (80%), followed by livestock (74%), wildlife (67%), then pet (60%). The largest category of potential sources in the watershed as a whole was wildlife, followed by livestock and pet, and, lastly, human, (Fig. 1-MAG).

Marley Creek Watershed ARA Results

Known-Source Library. A 473 known-source isolate library was constructed from sources in the Marley Creek Watershed. The number of unique antibiotic resistance patterns was calculated, and the known sources in the library were grouped into four categories: human, livestock (none), pet (dog), and wildlife (beaver, deer, fox) (Table 2-MCK). The library was analyzed for its ability to take a subset of the library isolates and correctly predict the identity of their host sources when they were treated as unknowns.

Table 2-MCK: Marley Creek. Source category, total number, and number of unique patterns in the Marley Creek known-source library.

Source category	Potential Sources	Total Isolates	Unique Patterns
Human	human	166	126
Livestock	n/a	0	0
Pet	dog	155	81
Wildlife	beaver, deer, fox	152	44
Total		473	251

For Marley Creek Watershed, the rates of correct classification (RCC) were calculated and the average rate of correct classification (ARCC) for the library was determined as described above in the "Statistical Analysis" section of this document. (Table 3-MCK).

Table 3-MCK. Marley Creek. Actual versus predicted sources, with rates of correct classification (RCC) for each source. No cutoffs applied.

Actual	Predicted Human	Pet	Wildlife	Total	% Correct (RCC*)
Human	111	16	39	166	66.9%
Pet	17	100	38	155	64.5%
Wildlife	10	12	130	152	85.5%
Total	138	128	207	473	
				ARCC:	72.1%

*RCC = Actual number of predicted species category / Total number predicted. Example: 111 human correctly predicted; 166 total number predicted for human = 111/166 = 66.9%.

Marley Creek Water Samples. Monthly monitoring from one (1) monitoring station on Marley Creek was the source of water samples. The maximum number of *Enterococcus* isolates per water sample was 24, although the number of isolates that actually grew was sometimes less than 24. A total of 135 *Enterococcus* isolates were analyzed by statistical analysis. The BST results by species category is shown in Table 4-MCK.

Table 4-MCK. Marley Creek. Predicted host source distribution of water isolates.

Source	Distribution
Human	34.2%
Livestock	0.0%
Pet	34.6%
Wildlife	31.2%
Total	100.0%

The seasonal distribution of water isolates from samples collected at the sampling station is shown below in Table 5-MCK.

Table 5-MCK: Marley Creek. *Enterococcus* isolates obtained from water collected during the spring, summer, fall, and winter seasons for the Marley Creek monitoring station.

	Season				
Station	Spring	Summer	Fall	Winter	Total
MCK-1	34	48	40	13	135
Total	34	48	40	13	135

Tables 6-MCK and 7-MCK on the following pages show the numbers and percentages, respectively, of the probable sources for the monitoring station by date.

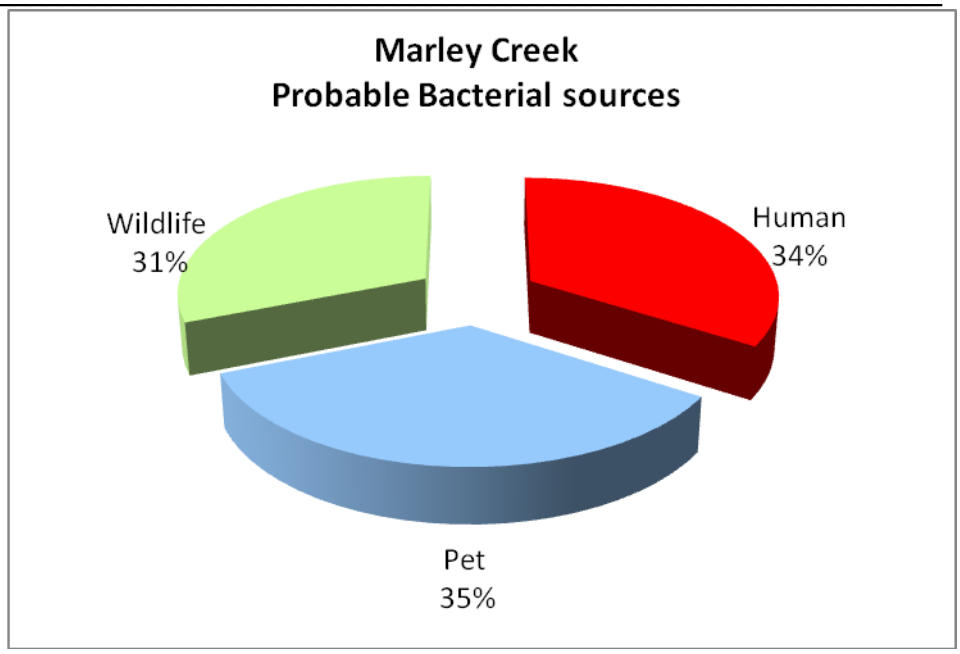
Table 6-MCK. Marley Creek. BST Analysis: Number of isolates per station per date.

		Predicted Source			
Station	Date	Human	Pet	Wildlife	Total
MSC1	12/05/07	6.02	9.17	7.80	23.00
MSC1	01/10/08	1.12	1.76	0.11	3.00
MSC1	02/11/08	1.13	0.81	0.06	2.00
MSC1	03/05/08	2.54	2.31	3.15	8.00
MSC1	04/08/08	0.99	0.96	0.06	2.00
MSC1	05/07/08	0.93	4.05	3.02	8.00
MSC1	06/04/08	5.61	8.41	9.99	24.00
MSC1	07/09/08	7.30	3.62	2.08	13.00
MSC1	08/06/08	7.70	1.68	3.62	13.00
MSC1	09/04/08	5.12	10.47	6.41	22.00
MSC1	10/16/08	5.08	1.54	3.38	10.00
MSC1	11/20/08	2.63	1.96	2.40	7.00
Total		46.18	46.74	42.08	135.00

Table 7-MCK. Marley Creek. Predicted source distribution by station and date.

		Predicted Source			
Station	Date	Human	Pet	Wildlife	Total
MSC1	12/05/07	26%	40%	34%	100%
MSC1	01/10/08	37%	59%	4%	100%
MSC1	02/11/08	57%	40%	3%	100%
MSC1	03/05/08	32%	29%	39%	100%
MSC1	04/08/08	49%	48%	3%	100%
MSC1	05/07/08	12%	51%	38%	100%
MSC1	06/04/08	23%	35%	42%	100%
MSC1	07/09/08	56%	28%	16%	100%
MSC1	08/06/08	59%	13%	28%	100%
MSC1	09/04/08	23%	48%	29%	100%
MSC1	10/16/08	51%	15%	34%	100%
MSC1	11/20/08	38%	28%	34%	100%
Total		34%	35%	31%	100%

Figure 1-MCK: Marley Creek Watershed relative contributions by probable sources of *Enterococcus* contamination.



Marley Creek Summary

The use of ARA was successful for identification of probable bacterial sources in the Marley Creek Watershed. The highest RCC for the library was for wildlife (86%), followed by human (67%), and wildlife (65%). The largest category of potential sources in the watershed as a whole was pet, followed by human, then wildlife (Fig. 1-MCK).

Rhode River Watershed ARA Results

Known-Source Library. A 605 known-source isolate library was constructed from sources in the Rhode River Watershed. The number of unique antibiotic resistance patterns was calculated, and the known sources in the library were grouped into four categories: human, livestock (chicken, cow, horse), pet (dog), and wildlife (deer, fox, goose, opossum, raccoon) (Table 2-RHO). The library was analyzed for its ability to take a subset of the library isolates and correctly predict the identity of their host sources when they were treated as unknowns.

Table 2-RHO: Rhode River. Source category, total number, and number of unique patterns in the Rhode River Creek known-source library.

Source category	Potential Sources	Total Isolates	Unique Patterns
Human	human	116	66
Livestock	chicken, cow, horse	141	67
Pet	cat, dog	135	64
Wildlife	deer, fox, goose, opossum, raccoon	213	73
Total		605	270

For Rhode River Watershed, the rates of correct classification (RCC) were calculated and the average rate of correct classification (ARCC) for the library was determined as described above in the "Statistical Analysis" section of this document. (Table 3-RHO).

Table 3-RHO: Rhode River. Actual versus predicted sources, with % correct (RCC) shown for each category.

	Predicted					
Actual	Human	Livestock	Pet	Wildlife	Total	% Correct (RCC*)
Human	78	19	10	9	116	67.2%
Livestock	13	92	17	19	141	65.3%
Pet	17	3	108	7	135	80.0%
Wildlife	13	41	30	129	213	60.6%
Total	121	155	165	164	605	
					ARCC:	67.3%

*RCC = Actual number of predicted species category / Total number predicted. Example: 78 human correctly predicted; 116 total number predicted for human = 78/116 = 67.2%.

Rhode River Water Samples. Monthly monitoring from two (2) monitoring stations on Rhode River was the source of water samples. The maximum number of *Enterococcus* isolates per water sample was 24, although the number of isolates that actually grew was sometimes less than 24. A total of 186 *Enterococcus* isolates were analyzed by statistical analysis. The BST results by species category is shown in Table 4-RHO.

Table 4-RHO. Rhode River. Predicted host source distribution of water isolates.

Source	Distribution
Human	22.5%
Livestock	23.6%
Pet	18.3%
Wildlife	35.6%
Total	100.0%

The seasonal distribution of water isolates from samples collected at the sampling stations is shown below in Table 5-RHO.

Table 5-RHO: Rhode River. *Enterococcus* isolates obtained from water collected during the spring, summer, fall, and winter seasons at the Rhode River monitoring station.

	Season				
Station	Spring	Summer	Fall	Winter	Total
03-07-019	44	38	19	9	110
03-07-120A	34	23	13	6	76
Total	78	61	32	15	186

Tables 6-RHO and 7-RHO on the following pages show the numbers and percentages, respectively, of the probable sources for each monitoring station by date.

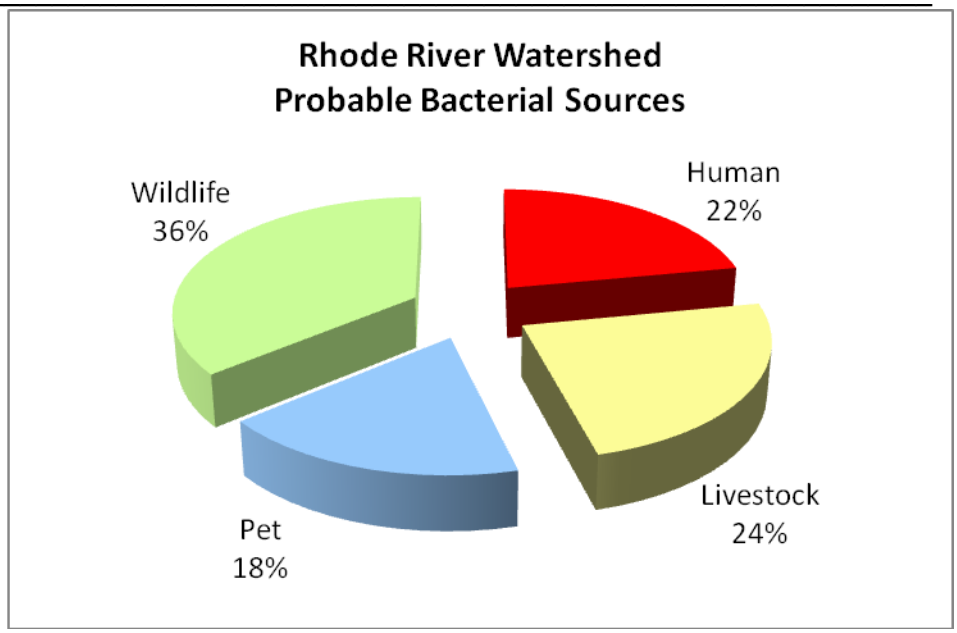
Table 7-RHO. Predicted source distribution by station and date

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-07-019	11/07/07	3.57	3.53	0.37	5.53	13.00
03-07-019	12/05/07	0.51	0.21	0.00	0.28	1.00
03-07-019	01/10/08	0.49	0.00	0.42	0.09	1.00
03-07-019	02/11/08	2.62	0.94	2.01	1.43	7.00
03-07-019	03/05/08	0.07	0.26	0.00	0.67	1.00
03-07-019	04/08/08	0.71	1.98	3.73	6.58	13.00
03-07-019	05/07/08	1.31	2.72	2.32	1.65	8.00
03-07-019	06/04/08	1.95	6.68	3.61	10.76	23.00
03-07-019	07/10/08	2.03	3.65	4.23	6.09	16.00
03-07-019	08/06/08	3.92	2.55	0.42	3.10	10.00
03-07-019	09/04/08	3.00	1.92	5.58	1.50	12.00
03-07-019	10/16/08	0.59	0.41	1.66	2.34	5.00
03-07-120A	11/07/07	1.35	1.05	1.00	1.60	5.00
03-07-120A	12/05/07	0.59	0.24	0.00	0.16	1.00
03-07-120A	01/10/08	0.15	0.00	0.77	0.08	1.00
03-07-120A	02/11/08	0.00	0.00	0.86	0.14	1.00
03-07-120A	03/05/08	0.64	0.84	1.44	1.08	4.00
03-07-120A	05/07/08	3.36	3.75	0.83	4.06	12.00
03-07-120A	06/04/08	6.97	4.36	3.03	7.64	22.00
03-07-120A	07/10/08	0.73	1.65	0.62	2.00	5.00
03-07-120A	08/06/08	1.22	2.45	0.38	3.94	8.00
03-07-120A	09/04/08	5.06	3.04	0.40	1.50	10.00
03-07-120A	10/16/08	0.99	1.64	0.34	4.03	7.00
Total		41.84	43.87	34.03	66.25	186.00

Table 7-RHO. Rhode River. Predicted source distribution by station and date.

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-07-019	11/07/07	27%	27%	3%	43%	100%
03-07-019	12/05/07	51%	21%	0%	28%	100%
03-07-019	01/10/08	49%	0%	42%	9%	100%
03-07-019	02/11/08	37%	13%	29%	20%	100%
03-07-019	03/05/08	7%	26%	0%	67%	100%
03-07-019	04/08/08	5%	15%	29%	51%	100%
03-07-019	05/07/08	16%	34%	29%	21%	100%
03-07-019	06/04/08	8%	29%	16%	47%	100%
03-07-019	07/10/08	13%	23%	26%	38%	100%
03-07-019	08/06/08	39%	26%	4%	31%	100%
03-07-019	09/04/08	25%	16%	46%	13%	100%
03-07-019	10/16/08	12%	8%	33%	47%	100%
03-07-120A	11/07/07	27%	21%	20%	32%	100%
03-07-120A	12/05/07	59%	24%	0%	16%	100%
03-07-120A	01/10/08	15%	0%	77%	8%	100%
03-07-120A	02/11/08	0%	0%	86%	14%	100%
03-07-120A	03/05/08	16%	21%	36%	27%	100%
03-07-120A	05/07/08	28%	31%	7%	34%	100%
03-07-120A	06/04/08	32%	20%	14%	35%	100%
03-07-120A	07/10/08	15%	33%	12%	40%	100%
03-07-120A	08/06/08	15%	31%	5%	49%	100%
03-07-120A	09/04/08	51%	30%	4%	15%	100%
03-07-120A	10/16/08	14%	23%	5%	58%	100%
Total		22%	24%	18%	36%	100%

Figure 1-RHO. Rhode River Watershed relative contributions by probable sources of *Enterococcus* contamination.



Rhode River Summary

The use of ARA was successful for identification of probable bacterial sources in the Rhode River Watershed. The highest RCC for the library was for pet (80%), followed by human (67%), livestock (65%), then wildlife (61%). The largest category of potential sources in the watershed as a whole was wildlife, followed by livestock, human, and then pet (Fig. 1-RHO).

Severn River Watershed ARA Results

Known-Source Library. A 2,188 known-source isolate library was constructed from sources in the Severn River Watershed. The number of unique antibiotic resistance patterns was calculated, and the known sources in the library were grouped into four categories: human, livestock (chicken, cow, goat, horse, pig, sheep), pet (dog), and wildlife (deer, fox goose, muskrat, pigeon, rabbit, raccoon) (Table 2-SEV). The library was analyzed for its ability to take a subset of the library isolates and correctly predict the identity of their host sources when they were treated as unknowns.

Table 2-SEV: Severn River. Source category, total number, and number of unique patterns in the Severn River Creek known-source library.

Source category	Potential Sources	Total Isolates	Unique Patterns
Human	human	554	285
Livestock	chicken, cow, goat, horse, pig, sheep	526	123
Pet	dog	540	199
Wildlife	deer, fox, goose, muskrat, pigeon, rabbit, raccoon	568	161
Total		2188	768

For Severn River Watershed, the rates of correct classification (RCC) were calculated and the average rate of correct classification (ARCC) for the library was determined as described above in the "Statistical Analysis" section of this document. (Table 3-SEV).

Table 3-SEV: Severn River. Actual versus predicted sources, with % correct (RCC) shown for each category.

	Predicted					
Actual	Human	Livestock	Pet	Wildlife	Total	% Correct (RCC*)
Human	408	30	66	50	554	73.7%
Livestock	48	298	40	140	526	56.7%
Pet	72	15	390	63	540	72.2%
Wildlife	55	103	54	356	568	62.7%
Total	583	446	550	609	2188	
					ARCC:	66.4%

*RCC = Actual number of predicted species category / Total number predicted. Example: 408 human correctly predicted; 554 total number predicted for human = 408/554 = 73.7%.

Severn River Water Samples. Monthly monitoring from nine (9) monitoring stations on Severn River was the source of water samples. The maximum number of *Enterococcus* isolates per water sample was 24, although the number of isolates that actually grew was sometimes less than 24. A total of 680 *Enterococcus* isolates were analyzed by statistical analysis. The BST results by species category is shown in Table 4-SEV.

Table 4-SEV. Severn River. Predicted host source distribution of water isolates.

Source	Distribution
Human	24.3%
Livestock	24.2%
Pet	23.1%
Wildlife	28.4%
Total	100.0%

The seasonal distribution of water isolates from samples collected at the sampling stations is shown below in Table 5-SEV.

Table 5-SEV: Severn River. *Enterococcus* isolates obtained from water collected during the spring, summer, fall, and winter seasons at the Severn River monitoring stations.

	Season				
Station	Spring	Summer	Fall	Winter	Total
03-03-005	25	39	23	21	108
03-03-005A	25	39	25	22	111
03-03-006	25	3	21	29	78
03-04-002A	24	0	1	23	48
03-04-005	25	1	5	10	41
03-04-008	24	12	6	30	72
03-04-011	24	1	2	21	48
03-04-150	26	4	9	26	65
03-04-152	24	8	49	28	109
Total	222	107	141	210	680

Tables 6-SEV and 7-SEV below show the number and percent, respectively, of probable sources for the monitoring stations by date.

Table 6-SEV. Predicted source distribution by station and date.

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-03-005	11/05/07	0.09	1.69	0.06	1.17	3.00
03-03-005	12/12/07	0.07	0.25	0.04	0.64	1.00
03-03-005	02/04/08	0.45	0.72	0.15	0.68	2.00
03-03-005	03/13/08	5.59	6.20	3.04	4.16	19.00
03-03-005	04/03/08	0.32	0.68	0.00	0.00	1.00
03-03-005	05/13/08	6.81	3.66	5.49	8.04	24.00
03-03-005	07/08/08	1.76	4.23	2.59	4.42	13.00
03-03-005	08/06/08	0.02	0.62	0.01	1.35	2.00
03-03-005	09/06/08	8.49	0.00	14.77	0.74	24.00
03-03-005	10/01/08	6.52	4.99	2.68	4.81	19.00
03-03-005A	11/05/07	0.36	0.38	0.56	0.70	2.00
03-03-005A	12/12/07	0.35	2.08	0.20	1.37	4.00
03-03-005A	02/04/08	0.00	0.11	0.78	0.11	1.00
03-03-005A	03/13/08	4.37	5.71	4.88	6.04	21.00
03-03-005A	04/03/08	0.15	0.33	0.16	0.36	1.00
03-03-005A	05/13/08	4.67	3.68	8.22	7.43	24.00
03-03-005A	06/09/08	1.77	2.25	0.35	1.64	6.00
03-03-005A	07/08/08	2.11	3.68	1.07	3.13	10.00
03-03-005A	08/06/08	1.14	2.10	3.94	2.82	10.00
03-03-005A	09/06/08	0.87	4.91	1.97	5.26	13.00
03-03-005A	10/01/08	1.17	3.02	7.31	7.49	19.00
03-03-006	11/05/07	0.07	0.58	0.01	0.34	1.00
03-03-006	12/12/07	0.86	2.61	0.72	4.81	9.00
03-03-006	02/04/08	2.58	4.19	1.93	2.30	11.00
03-03-006	03/13/08	7.41	4.42	1.99	4.18	18.00
03-03-006	04/03/08	0.00	0.00	0.34	0.66	1.00
03-03-006	05/13/08	7.22	3.48	5.57	7.72	24.00
03-03-006	07/08/08	0.25	0.26	0.34	0.16	1.00
03-03-006	08/06/08	0.07	0.58	0.01	0.34	1.00
03-03-006	09/06/08	0.08	0.84	0.08	0.00	1.00
03-03-006	10/01/08	3.62	1.24	3.21	2.93	11.00
03-04-002A	12/12/07	0.07	0.58	0.01	0.34	1.00
03-04-002A	01/14/08	1.47	0.96	0.52	1.05	4.00
03-04-002A	02/04/08	2.51	3.90	6.86	3.73	17.00
03-04-002A	03/13/08	0.15	0.33	1.16	0.36	2.00
03-04-002A	05/13/08	6.78	7.77	4.91	4.54	24.00
03-04-005	12/12/07	0.33	1.35	0.40	1.92	4.00
03-04-005	01/14/08	0.15	0.32	0.08	0.45	1.00

Table 6-SEV. Predicted source distribution by station and date (continued).

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-04-005	02/04/08	2.96	1.88	0.94	2.22	8.00
03-04-005	03/13/08	1.15	1.33	0.16	0.36	3.00
03-04-005	04/03/08	0.15	0.65	0.05	0.15	1.00
03-04-005	05/13/08	8.29	4.78	5.27	5.66	24.00
03-04-008	11/05/07	0.32	0.46	0.71	0.50	2.00
03-04-008	12/12/07	1.00	0.00	0.00	0.00	1.00
03-04-008	02/04/08	5.09	2.54	6.80	9.57	24.00
03-04-008	03/13/08	1.14	1.49	1.84	1.54	6.00
03-04-008	05/13/08	3.54	9.84	3.43	7.20	24.00
03-04-008	08/06/08	2.51	0.55	3.79	4.15	11.00
03-04-008	09/06/08	0.75	0.00	0.20	0.05	1.00
03-04-008	10/01/08	0.47	0.44	0.15	1.94	3.00
03-04-011	11/05/07	0.02	0.62	0.01	1.35	2.00
03-04-011	01/14/08	0.15	0.32	0.08	0.45	1.00
03-04-011	02/04/08	0.22	1.79	0.17	0.82	3.00
03-04-011	03/13/08	3.53	4.77	3.73	4.97	17.00
03-04-011	05/13/08	5.04	5.51	4.90	8.55	24.00
03-04-011	09/06/08	0.04	0.76	0.04	0.16	1.00
03-04-150	11/05/07	0.02	0.46	0.01	0.50	1.00
03-04-150	12/12/07	1.00	0.00	0.00	0.00	1.00
03-04-150	01/14/08	0.75	0.00	0.25	0.00	1.00
03-04-150	02/04/08	8.94	6.84	3.46	4.76	24.00
03-04-150	03/13/08	0.42	0.25	0.14	0.18	1.00
03-04-150	04/03/08	0.82	0.33	0.50	0.36	2.00
03-04-150	05/13/08	6.21	6.13	4.93	6.73	24.00
03-04-150	08/06/08	0.15	0.76	0.83	0.26	2.00
03-04-150	09/06/08	1.07	0.58	0.01	0.34	2.00
03-04-150	10/01/08	3.16	1.71	0.30	1.83	7.00
03-04-152	11/05/07	1.94	5.39	0.46	4.21	12.00
03-04-152	12/12/07	2.50	4.14	2.01	4.35	13.00
03-04-152	01/14/08	0.80	0.34	1.76	0.11	3.00
03-04-152	02/04/08	4.57	6.09	7.49	5.85	24.00
03-04-152	03/13/08	0.15	0.33	0.16	0.36	1.00
03-04-152	05/13/08	8.07	3.87	6.47	5.59	24.00
03-04-152	06/09/08	0.15	0.32	0.08	0.45	1.00
03-04-152	07/08/08	0.15	0.32	0.08	0.45	1.00
03-04-152	08/06/08	0.15	0.33	0.16	0.36	1.00
03-04-152	09/06/08	1.09	0.41	1.40	2.11	5.00
03-04-152	10/01/08	6.39	3.27	7.92	6.42	24.00
Total		165.56	164.28	157.16	193.00	680.00

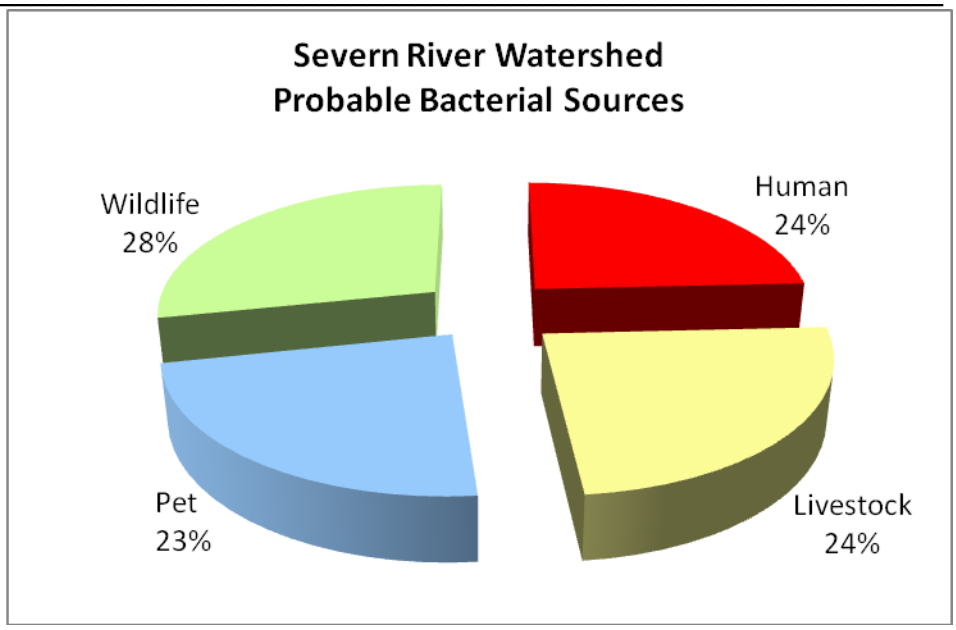
Table 7-SEV. Severn River. Predicted source distribution by station and date.

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-03-005	11/05/07	3%	56%	2%	39%	100%
03-03-005	12/12/07	7%	25%	4%	64%	100%
03-03-005	02/04/08	22%	36%	8%	34%	100%
03-03-005	03/13/08	29%	33%	16%	22%	100%
03-03-005	04/03/08	32%	68%	0%	0%	100%
03-03-005	05/13/08	28%	15%	23%	34%	100%
03-03-005	07/08/08	14%	33%	20%	34%	100%
03-03-005	08/06/08	1%	31%	0%	68%	100%
03-03-005	09/06/08	35%	0%	62%	3%	100%
03-03-005	10/01/08	34%	26%	14%	25%	100%
03-03-005A	11/05/07	18%	19%	28%	35%	100%
03-03-005A	12/12/07	9%	52%	5%	34%	100%
03-03-005A	02/04/08	0%	11%	78%	11%	100%
03-03-005A	03/13/08	21%	27%	23%	29%	100%
03-03-005A	04/03/08	15%	33%	16%	36%	100%
03-03-005A	05/13/08	19%	15%	34%	31%	100%
03-03-005A	06/09/08	29%	37%	6%	27%	100%
03-03-005A	07/08/08	21%	37%	11%	31%	100%
03-03-005A	08/06/08	11%	21%	39%	28%	100%
03-03-005A	09/06/08	7%	38%	15%	40%	100%
03-03-005A	10/01/08	6%	16%	38%	39%	100%
03-03-006	11/05/07	7%	58%	1%	34%	100%
03-03-006	12/12/07	10%	29%	8%	53%	100%
03-03-006	02/04/08	23%	38%	18%	21%	100%
03-03-006	03/13/08	41%	25%	11%	23%	100%
03-03-006	04/03/08	0%	0%	34%	66%	100%
03-03-006	05/13/08	30%	15%	23%	32%	100%
03-03-006	07/08/08	25%	26%	34%	16%	100%
03-03-006	08/06/08	7%	58%	1%	34%	100%
03-03-006	09/06/08	8%	84%	8%	0%	100%
03-03-006	10/01/08	33%	11%	29%	27%	100%
03-04-002A	12/12/07	7%	58%	1%	34%	100%
03-04-002A	01/14/08	37%	24%	13%	26%	100%
03-04-002A	02/04/08	15%	23%	40%	22%	100%
03-04-002A	03/13/08	8%	16%	58%	18%	100%
03-04-002A	05/13/08	28%	32%	20%	19%	100%
03-04-005	12/12/07	8%	34%	10%	48%	100%
03-04-005	01/14/08	15%	32%	8%	45%	100%

Table 7-SEV. Severn River. Predicted source distribution by station and date (continued).

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-04-005	02/04/08	37%	23%	12%	28%	100%
03-04-005	03/13/08	38%	44%	5%	12%	100%
03-04-005	04/03/08	15%	65%	5%	15%	100%
03-04-005	05/13/08	35%	20%	22%	24%	100%
03-04-008	11/05/07	16%	23%	36%	25%	100%
03-04-008	12/12/07	100%	0%	0%	0%	100%
03-04-008	02/04/08	21%	11%	28%	40%	100%
03-04-008	03/13/08	19%	25%	31%	26%	100%
03-04-008	05/13/08	15%	41%	14%	30%	100%
03-04-008	08/06/08	23%	5%	34%	38%	100%
03-04-008	09/06/08	75%	0%	20%	5%	100%
03-04-008	10/01/08	16%	15%	5%	65%	100%
03-04-011	11/05/07	1%	31%	0%	68%	100%
03-04-011	01/14/08	15%	32%	8%	45%	100%
03-04-011	02/04/08	7%	60%	6%	27%	100%
03-04-011	03/13/08	21%	28%	22%	29%	100%
03-04-011	05/13/08	21%	23%	20%	36%	100%
03-04-011	09/06/08	4%	76%	4%	16%	100%
03-04-150	11/05/07	2%	46%	1%	50%	100%
03-04-150	12/12/07	100%	0%	0%	0%	100%
03-04-150	01/14/08	75%	0%	25%	0%	100%
03-04-150	02/04/08	37%	28%	14%	20%	100%
03-04-150	03/13/08	42%	25%	14%	18%	100%
03-04-150	04/03/08	41%	16%	25%	18%	100%
03-04-150	05/13/08	26%	26%	21%	28%	100%
03-04-150	08/06/08	8%	38%	42%	13%	100%
03-04-150	09/06/08	53%	29%	1%	17%	100%
03-04-150	10/01/08	45%	24%	4%	26%	100%
03-04-152	11/05/07	16%	45%	4%	35%	100%
03-04-152	12/12/07	19%	32%	15%	33%	100%
03-04-152	01/14/08	27%	11%	59%	4%	100%
03-04-152	02/04/08	19%	25%	31%	24%	100%
03-04-152	03/13/08	15%	33%	16%	36%	100%
03-04-152	05/13/08	34%	16%	27%	23%	100%
03-04-152	06/09/08	15%	32%	8%	45%	100%
03-04-152	07/08/08	15%	32%	8%	45%	100%
03-04-152	08/06/08	15%	33%	16%	36%	100%
03-04-152	09/06/08	22%	8%	28%	42%	100%
03-04-152	10/01/08	27%	14%	33%	27%	100%
Total		24%	24%	23%	28%	100%

Figure 1-SEV. Severn River Watershed relative contributions by probable sources of *Enterococcus* contamination.



Severn River Summary

The use of ARA was successful for identification of probable bacterial sources in the Severn River Watershed. The highest RCC for the library was for human (74%), followed by pet (72%), wildlife (63%), then livestock (57%). The largest category of potential sources in the watershed as a whole was wildlife, followed by livestock and human, then pet (Fig. 1-SEV).

South River Watershed ARA Results

Known-Source Library. A 1,150 known-source isolate library was constructed from sources in the South River Watershed. The number of unique antibiotic resistance patterns was calculated, and the known sources in the library were grouped into four categories: human, livestock (cow, donkey, emu, goat, horse, llama, peacock, pig), pet (cat, dog), and wildlife (beaver, bird, deer, duck, fox, goose, raccoon) (Table 2-SOU). The library was analyzed for its ability to take a subset of the library isolates and correctly predict the identity of their host sources when they were treated as unknowns.

Table 2-SOU: South River. Source category, total number, and number of unique patterns in the South River Creek known-source library.

Source category	Potential Sources	Total Isolates	Unique Patterns
Human	human	256	135
Livestock	cow, donkey, emu, goat, horse, llama, peacock, pig	270	87
Pet	cat, dog	295	158
Wildlife	beaver, bird, deer, duck, fox, goose, raccoon	329	108
Total		1150	488

For South River Watershed, the rates of correct classification (RCC) were calculated and the average rate of correct classification (ARCC) for the library was determined as described above in the "Statistical Analysis" section of this document. (Table 3-SOU).

Table 3-SOU: South River. Actual versus predicted sources, with % correct (RCC) shown for each category.

	Predicted					
Actual	Human	Livestock	Pet	Wildlife	Total	% Correct (RCC*)
Human	183	22	21	30	256	71.5%
Livestock	14	168	21	67	270	62.2%
Pet	34	11	212	38	295	71.9%
Wildlife	17	61	25	226	329	68.7%
Total	248	262	279	361	1150	
					ARCC:	68.6%

*RCC = Actual number of predicted species category / Total number predicted. Example: 183 human correctly predicted; 256 total number predicted for human = 183/256 = 71.5%.

South River Water Samples. Monthly monitoring from 11 monitoring stations on South River was the source of water samples. The maximum number of *Enterococcus* isolates per water sample was 24, although the number of isolates that actually grew was sometimes less than 24. A total of 620 *Enterococcus* isolates were analyzed by statistical analysis. The BST results by species category is shown in Table 4-SOU.

Table 4-SOU. South River. Predicted host source distribution of water isolates.

Source	Distribution
Human	21.0%
Livestock	23.9%
Pet	33.2%
Wildlife	21.9%
Total	100.0%

The seasonal distribution of water isolates from samples collected at the sampling stations is shown below in Table 5-SOU.

Table 5-SOU: South River. *Enterococcus* isolates obtained from water collected during the spring, summer, fall, and winter seasons at the South River monitoring stations.

	Season				
Station	Spring	Summer	Fall	Winter	Total
03-06-002	25	9	2	29	65
03-06-013A	32	6	2	10	50
03-06-104	37	11	7	16	71
03-06-110	27	10	10	24	71
03-06-111	33	4	2	7	46
03-06-115	29	3	2	12	46
03-06-115A	37	17	4	13	71
03-06-205	26	4	4	19	53
03-06-208	25	6	4	10	45
03-06-211	24	7	6	24	61
03-06-801	27	2	4	8	41
Total	322	79	47	172	620

Tables 6-SOU and 7-SOU below show the percent and number, respectively, of probable sources for each monitoring station by date.

Table 6-SOU. Predicted source distribution by station and date.

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-06-002	11/05/07	0.82	0.05	0.10	0.03	1.00
03-06-002	01/07/08	0.26	1.37	0.54	2.82	5.00
03-06-002	02/04/08	3.99	7.92	8.67	3.43	24.00
03-06-002	04/01/08	0.00	0.79	0.00	0.21	1.00
03-06-002	06/05/08	6.54	1.79	8.93	6.74	24.00
03-06-002	08/04/08	0.65	5.38	0.28	2.69	9.00
03-06-002	10/14/08	0.18	0.07	0.70	0.05	1.00
03-06-013A	01/07/08	0.06	0.00	0.55	0.39	1.00
03-06-013A	02/04/08	1.43	2.86	3.90	0.80	9.00
03-06-013A	05/08/08	0.61	3.00	1.31	3.08	8.00
03-06-013A	06/05/08	5.79	3.18	10.35	4.68	24.00
03-06-013A	07/07/08	0.06	0.60	0.04	0.30	1.00
03-06-013A	08/04/08	0.30	1.03	1.56	1.11	4.00
03-06-013A	09/02/08	0.13	0.63	0.08	0.16	1.00
03-06-013A	10/14/08	0.24	0.30	0.84	0.62	2.00
03-06-104	11/05/07	0.46	3.00	0.21	2.33	6.00
03-06-104	01/07/08	0.26	1.18	0.00	0.56	2.00
03-06-104	02/04/08	4.98	2.47	4.93	1.62	14.00
03-06-104	04/01/08	0.19	0.22	0.73	0.86	2.00
03-06-104	05/08/08	0.83	4.19	3.38	2.60	11.00
03-06-104	06/05/08	5.31	5.65	9.76	3.27	24.00
03-06-104	07/07/08	0.12	0.27	0.10	0.51	1.00
03-06-104	08/04/08	3.46	3.94	1.38	1.22	10.00
03-06-104	10/14/08	0.14	0.64	0.12	0.11	1.00
03-06-110	11/05/07	0.00	0.56	0.02	1.42	2.00
03-06-110	12/03/07	0.70	1.84	1.33	2.13	6.00
03-06-110	02/04/08	5.15	9.97	3.24	4.64	23.00
03-06-110	03/03/08	0.00	0.37	0.02	0.62	1.00
03-06-110	04/01/08	0.82	0.05	0.10	0.03	1.00
03-06-110	05/08/08	0.82	0.27	0.41	0.51	2.00
03-06-110	06/05/08	4.01	3.59	10.97	5.43	24.00
03-06-110	08/04/08	0.75	3.97	1.06	2.23	8.00
03-06-110	09/02/08	0.77	0.19	0.30	0.74	2.00
03-06-110	10/14/08	0.12	0.03	0.73	1.12	2.00
03-06-111	11/05/07	0.00	0.37	0.02	0.62	1.00
03-06-111	02/04/08	0.78	1.34	2.91	1.98	7.00
03-06-111	04/01/08	0.00	1.00	0.00	0.00	1.00
03-06-111	05/08/08	2.02	1.54	3.10	1.34	8.00

Table 6-SOU. Predicted source distribution by station and date (continued).

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-06-111	06/05/08	7.13	1.40	11.76	3.71	24.00
03-06-111	08/04/08	0.25	1.64	0.10	1.00	3.00
03-06-111	09/02/08	0.00	0.00	0.87	0.13	1.00
03-06-111	10/14/08	0.23	0.22	0.50	0.06	1.00
03-06-115	11/05/07	0.12	0.27	0.10	0.51	1.00
03-06-115	12/03/07	0.00	0.79	0.00	0.21	1.00
03-06-115	02/04/08	1.37	5.19	2.13	3.31	12.00
03-06-115	04/01/08	0.07	0.19	0.00	0.74	1.00
03-06-115	05/08/08	0.13	1.94	0.11	1.83	4.00
03-06-115	06/05/08	5.49	4.41	11.86	2.25	24.00
03-06-115	07/07/08	0.61	0.00	0.39	0.00	1.00
03-06-115	08/04/08	0.83	0.00	0.54	0.63	2.00
03-06-115A	12/03/07	1.06	0.72	0.84	0.38	3.00
03-06-115A	01/07/08	0.18	0.98	0.65	1.19	3.00
03-06-115A	02/04/08	1.58	2.82	3.07	2.52	10.00
03-06-115A	04/01/08	4.06	0.68	1.03	0.23	6.00
03-06-115A	05/08/08	0.67	2.47	0.40	3.45	7.00
03-06-115A	06/05/08	3.51	1.98	12.92	5.59	24.00
03-06-115A	07/07/08	1.80	1.82	1.28	2.10	7.00
03-06-115A	08/04/08	0.66	2.32	3.31	1.70	8.00
03-06-115A	09/02/08	0.24	0.54	0.21	1.01	2.00
03-06-115A	10/14/08	0.06	0.00	0.55	0.39	1.00
03-06-205	12/03/07	0.30	0.65	0.37	1.68	3.00
03-06-205	01/07/08	0.82	0.05	0.10	0.03	1.00
03-06-205	02/04/08	2.09	4.75	7.63	3.52	18.00
03-06-205	04/01/08	0.12	0.56	0.67	0.66	2.00
03-06-205	06/05/08	6.52	2.39	12.08	3.00	24.00
03-06-205	08/04/08	0.85	1.05	0.36	1.75	4.00
03-06-205	10/14/08	0.06	0.60	0.04	0.30	1.00
03-06-208	11/05/07	0.82	0.05	0.10	0.03	1.00
03-06-208	02/04/08	1.56	3.76	2.87	1.81	10.00
03-06-208	05/08/08	0.33	0.00	0.15	0.52	1.00
03-06-208	06/05/08	7.47	4.10	9.28	3.15	24.00
03-06-208	07/07/08	0.12	0.27	0.10	0.51	1.00
03-06-208	08/04/08	0.96	1.41	0.73	0.90	4.00
03-06-208	09/02/08	0.70	0.00	0.30	0.00	1.00
03-06-208	10/14/08	1.60	0.08	0.83	0.49	3.00
03-06-211	11/05/07	0.57	0.10	0.28	0.06	1.00
03-06-211	12/03/07	0.95	0.64	0.10	0.31	2.00
03-06-211	02/04/08	4.21	6.53	9.19	4.07	24.00
03-06-211	06/05/08	5.42	2.60	8.78	7.20	24.00
03-06-211	08/04/08	0.43	2.08	0.74	0.76	4.00

Table 6-SOU. Predicted source distribution by station and date (continued).

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-06-211	09/02/08	1.07	0.92	0.14	0.87	3.00
03-06-211	10/14/08	0.70	1.02	1.28	0.00	3.00
03-06-801	12/03/07	0.06	0.00	0.55	1.39	2.00
03-06-801	01/07/08	0.06	0.00	0.55	0.39	1.00
03-06-801	02/04/08	1.40	2.05	2.51	1.04	7.00
03-06-801	04/01/08	0.42	1.27	0.23	1.08	3.00
03-06-801	06/05/08	6.60	4.11	9.66	3.63	24.00
03-06-801	08/04/08	0.85	0.30	0.73	0.12	2.00
03-06-801	10/14/08	0.18	0.87	0.14	0.80	2.00
Total		129.99	148.20	205.78	136.03	620.00

Table 7-SOU. Predicted source distribution by station and date.

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-06-002	11/05/07	82%	5%	10%	3%	100%
03-06-002	01/07/08	5%	27%	11%	56%	100%
03-06-002	02/04/08	17%	33%	36%	14%	100%
03-06-002	04/01/08	0%	79%	0%	21%	100%
03-06-002	06/05/08	27%	7%	37%	28%	100%
03-06-002	08/04/08	7%	60%	3%	30%	100%
03-06-002	10/14/08	18%	7%	70%	5%	100%
03-06-013A	01/07/08	6%	0%	55%	39%	100%
03-06-013A	02/04/08	16%	32%	43%	9%	100%
03-06-013A	05/08/08	8%	37%	16%	38%	100%
03-06-013A	06/05/08	24%	13%	43%	20%	100%
03-06-013A	07/07/08	6%	60%	4%	30%	100%
03-06-013A	08/04/08	7%	26%	39%	28%	100%
03-06-013A	09/02/08	13%	63%	8%	16%	100%
03-06-013A	10/14/08	12%	15%	42%	31%	100%
03-06-104	11/05/07	8%	50%	4%	39%	100%
03-06-104	01/07/08	13%	59%	0%	28%	100%
03-06-104	02/04/08	36%	18%	35%	12%	100%
03-06-104	04/01/08	9%	11%	37%	43%	100%
03-06-104	05/08/08	8%	38%	31%	24%	100%
03-06-104	06/05/08	22%	24%	41%	14%	100%
03-06-104	07/07/08	12%	27%	10%	51%	100%
03-06-104	08/04/08	35%	39%	14%	12%	100%
03-06-104	10/14/08	14%	64%	12%	11%	100%

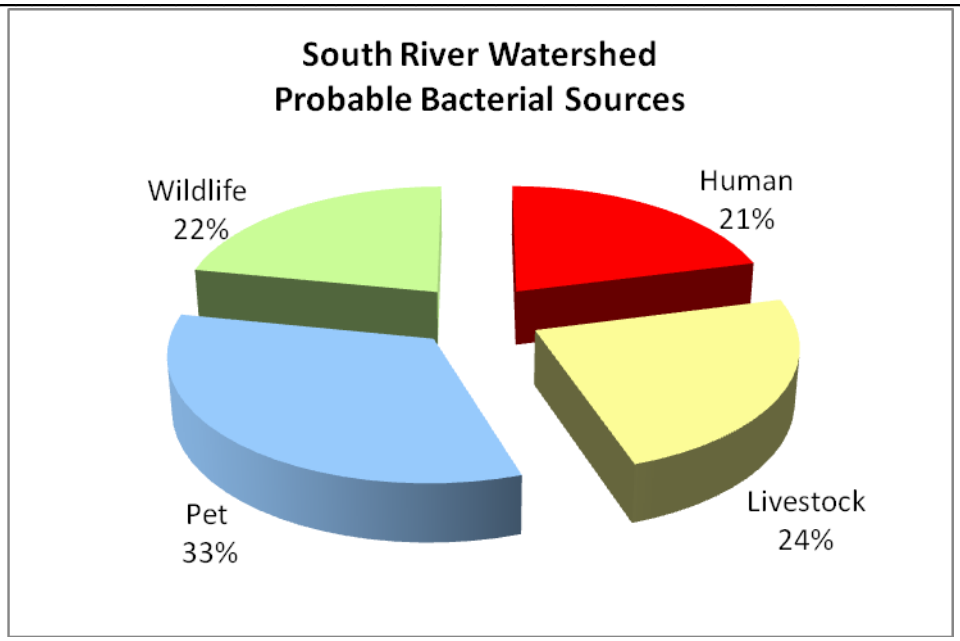
Table 7-SOU. Predicted source distribution by station and date (continued).

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-06-110	02/04/08	22%	43%	14%	20%	100%
03-06-110	06/05/08	17%	15%	46%	23%	100%
03-06-110	08/04/08	9%	50%	13%	28%	100%
03-06-110	09/02/08	38%	10%	15%	37%	100%
03-06-110	10/14/08	6%	1%	37%	56%	100%
03-06-111	11/05/07	0%	37%	2%	62%	100%
03-06-111	02/04/08	11%	19%	42%	28%	100%
03-06-111	04/01/08	0%	100%	0%	0%	100%
03-06-111	05/08/08	25%	19%	39%	17%	100%
03-06-111	06/05/08	30%	6%	49%	15%	100%
03-06-111	08/04/08	8%	55%	3%	33%	100%
03-06-111	09/02/08	0%	0%	87%	13%	100%
03-06-111	10/14/08	23%	22%	50%	6%	100%
03-06-115	11/05/07	12%	27%	10%	51%	100%
03-06-115	12/03/07	0%	79%	0%	21%	100%
03-06-115	02/04/08	11%	43%	18%	28%	100%
03-06-115	04/01/08	7%	19%	0%	74%	100%
03-06-115	05/08/08	3%	48%	3%	46%	100%
03-06-115	06/05/08	23%	18%	49%	9%	100%
03-06-115	07/07/08	61%	0%	39%	0%	100%
03-06-115	08/04/08	42%	0%	27%	32%	100%
03-06-115A	12/03/07	35%	24%	28%	13%	100%
03-06-115A	01/07/08	6%	33%	22%	40%	100%
03-06-115A	02/04/08	16%	28%	31%	25%	100%
03-06-115A	04/01/08	68%	11%	17%	4%	100%
03-06-115A	05/08/08	10%	35%	6%	49%	100%
03-06-115A	06/05/08	15%	8%	54%	23%	100%
03-06-115A	07/07/08	26%	26%	18%	30%	100%
03-06-115A	08/04/08	8%	29%	41%	21%	100%
03-06-115A	09/02/08	12%	27%	10%	51%	100%
03-06-115A	10/14/08	6%	0%	55%	39%	100%
03-06-205	12/03/07	10%	22%	12%	56%	100%
03-06-205	01/07/08	82%	5%	10%	3%	100%
03-06-205	02/04/08	12%	26%	42%	20%	100%
03-06-205	04/01/08	6%	28%	33%	33%	100%
03-06-205	06/05/08	27%	10%	50%	12%	100%
03-06-205	08/04/08	21%	26%	9%	44%	100%
03-06-205	10/14/08	6%	60%	4%	30%	100%
03-06-208	11/05/07	82%	5%	10%	3%	100%
03-06-208	02/04/08	16%	38%	29%	18%	100%
03-06-208	05/08/08	33%	0%	15%	52%	100%
03-06-208	06/05/08	31%	17%	39%	13%	100%
03-06-208	07/07/08	12%	27%	10%	51%	100%

Table 7-SOU. Predicted source distribution by station and date (continued).

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-06-208	08/04/08	24%	35%	18%	22%	100%
03-06-208	09/02/08	70%	0%	30%	0%	100%
03-06-208	10/14/08	53%	3%	28%	16%	100%
03-06-211	11/05/07	57%	10%	28%	6%	100%
03-06-211	12/03/07	47%	32%	5%	16%	100%
03-06-211	02/04/08	18%	27%	38%	17%	100%
03-06-211	06/05/08	23%	11%	37%	30%	100%
03-06-211	08/04/08	11%	52%	19%	19%	100%
03-06-211	09/02/08	36%	31%	5%	29%	100%
03-06-211	10/14/08	23%	34%	43%	0%	100%
03-06-801	12/03/07	3%	0%	27%	70%	100%
03-06-801	01/07/08	6%	0%	55%	39%	100%
03-06-801	02/04/08	20%	29%	36%	15%	100%
03-06-801	04/01/08	14%	42%	8%	36%	100%
03-06-801	06/05/08	28%	17%	40%	15%	100%
03-06-801	08/04/08	42%	15%	37%	6%	100%
03-06-801	10/14/08	9%	44%	7%	40%	100%
Total		21%	24%	33%	22%	100%

Figure 1-SOU. South River Watershed relative contributions by probable sources of *Enterococcus* contamination.



South River Summary.

The use of ARA was successful for identification of probable bacterial sources in the South River Watershed. The highest RCC for the library was for pet and human (72% each), followed by wildlife (69%), and livestock (62%). The largest category of potential sources in the watershed as a whole was pet, followed by livestock, wildlife, then human (Fig. 1-SOU).

West Chesapeake Bay Watershed ARA Results

Known-Source Library. A 995 known-source isolate library was constructed from sources in the West Chesapeake Bay Watershed. The number of unique antibiotic resistance patterns was calculated, and the known sources in the library were grouped into four categories: human, livestock (chicken, horse) pet (cat, dog), and wildlife (deer, duck, fox, raccoon) (Table 2-WCB). The library was analyzed for its ability to take a subset of the library isolates and correctly predict the identity of their host sources when they were treated as unknowns.

Table 2-WCB: West Chesapeake Bay. Source category, total number, and number of unique patterns in the West Chesapeake Bay Creek known-source library.

Source category	Potential Sources	Total Isolates	Unique Patterns
Human	human	226	130
Livestock	chicken, horse	235	92
Pet	cat, dog	267	106
Wildlife	deer, duck, fox, raccoon	267	119
Total		995	447

For West Chesapeake Bay Watershed, the rates of correct classification (RCC) were calculated and the average rate of correct classification (ARCC) for the library was determined as described above in the "Statistical Analysis" section of this document. (Table 3-WCB).

Table 3-WCB: West Chesapeake Bay. Actual versus predicted sources, with % correct (RCC) shown for each category.

	Predicted					
Actual	Human	Livestock	Pet	Wildlife	Total	% Correct (RCC*)
Human	204	5	5	12	226	90.3%
Livestock	18	154	7	56	235	65.5%
Pet	24	17	187	39	267	70.0%
Wildlife	20	34	22	191	267	71.5%
Total	266	210	221	298	995	
					ARCC:	74.0%

*RCC = Actual number of predicted species category / Total number predicted.
Example: 204 human correctly predicted; 226 total number predicted for human = 204/226 = 90.3%.

West Chesapeake Bay Water Samples. Monthly monitoring from two (2) monitoring stations on West Chesapeake Bay was the source of water samples. The maximum number of *Enterococcus* isolates per water sample was 24, although the number of isolates that actually grew was sometimes less than 24. A total of 263 *Enterococcus* isolates were analyzed by statistical analysis. The BST results by species category is shown in Table 4-WCB.

Table 4-WCB. West Chesapeake Bay. Predicted host source distribution of water isolates.

Source	Distribution
Human	22.6%
Livestock	20.6%
Pet	24.7%
Wildlife	32.1%
Total	100.0%

The seasonal distribution of water isolates from samples collected at the sampling stations is shown below in Table 5-WCB.

Table 5-WCB: West Chesapeake Bay. *Enterococcus* isolates obtained from water collected during the spring, summer, fall, and winter seasons at the West Chesapeake Bay monitoring stations.

	Season				
Station	Spring	Summer	Fall	Winter	Total
05-01-004	69	35	30	16	150
05-01-004A	43	26	41	3	113
Total	112	61	71	19	263

Tables 6-WCB and 7-WCB below show the numbers and percentages, respectively, of probable sources for each monitoring station by date.

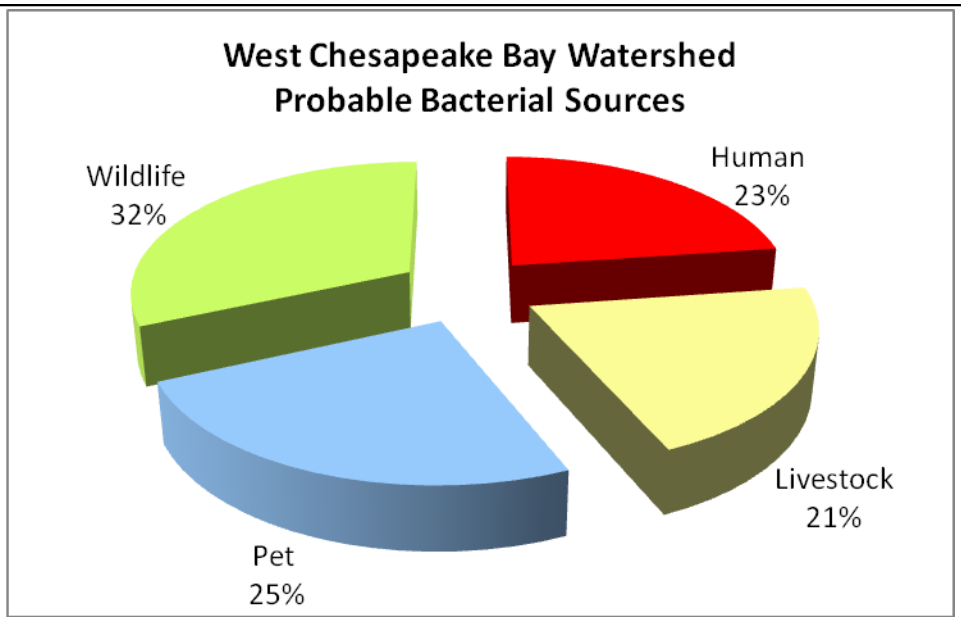
Table 6-WCB. Predicted source distribution by station and date.

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
05-01-004	11/07/07	0.20	0.46	1.86	1.48	4.00
05-01-004	12/05/07	0.10	1.43	0.00	0.47	2.00
05-01-004	01/10/08	0.07	0.83	0.06	3.04	4.00
05-01-004	02/11/08	2.78	3.15	0.53	2.53	9.00
05-01-004	03/05/08	1.06	0.12	1.11	0.71	3.00
05-01-004	04/08/08	2.86	11.29	3.65	6.20	24.00
05-01-004	05/07/08	1.67	8.58	4.36	6.40	21.00
05-01-004	06/04/08	4.96	2.07	8.43	8.54	24.00
05-01-004	07/10/08	4.63	0.50	6.27	12.60	24.00
05-01-004	08/06/08	0.00	0.00	1.25	1.75	3.00
05-01-004	09/04/08	3.10	0.98	1.61	2.31	8.00
05-01-004	10/16/08	4.56	2.77	10.82	5.85	24.00
05-01-004A	11/07/07	3.13	0.99	1.27	0.61	6.00
05-01-004A	12/05/07	3.27	1.30	2.91	3.51	11.00
05-01-004A	01/10/08	3.00	0.00	0.00	0.00	3.00
05-01-004A	04/08/08	0.99	0.53	1.76	0.72	4.00
05-01-004A	05/07/08	2.73	6.76	0.78	5.74	16.00
05-01-004A	06/04/08	3.44	6.32	5.99	7.25	23.00
05-01-004A	07/10/08	8.17	3.18	6.05	6.60	24.00
05-01-004A	08/06/08	0.00	0.00	1.00	0.00	1.00
05-01-004A	09/04/08	0.44	0.24	0.05	0.27	1.00
05-01-004A	10/16/08	8.31	2.62	5.15	7.92	24.00
Total		59.45	54.14	64.91	84.50	263.00

Table 7-WCB. West Chesapeake Bay. Predicted source distribution by station and date.

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
05-01-004	11/07/07	5%	12%	47%	37%	100%
05-01-004	12/05/07	5%	71%	0%	23%	100%
05-01-004	01/10/08	2%	21%	2%	76%	100%
05-01-004	02/11/08	31%	35%	6%	28%	100%
05-01-004	03/05/08	35%	4%	37%	24%	100%
05-01-004	04/08/08	12%	47%	15%	26%	100%
05-01-004	05/07/08	8%	41%	21%	30%	100%
05-01-004	06/04/08	21%	9%	35%	36%	100%
05-01-004	07/10/08	19%	2%	26%	53%	100%
05-01-004	08/06/08	0%	0%	42%	58%	100%
05-01-004	09/04/08	39%	12%	20%	29%	100%
05-01-004	10/16/08	19%	12%	45%	24%	100%
05-01-004A	11/07/07	52%	17%	21%	10%	100%
05-01-004A	12/05/07	30%	12%	26%	32%	100%
05-01-004A	01/10/08	100%	0%	0%	0%	100%
05-01-004A	04/08/08	25%	13%	44%	18%	100%
05-01-004A	05/07/08	17%	42%	5%	36%	100%
05-01-004A	06/04/08	15%	27%	26%	32%	100%
05-01-004A	07/10/08	34%	13%	25%	28%	100%
05-01-004A	08/06/08	0%	0%	100%	0%	100%
05-01-004A	09/04/08	44%	24%	5%	27%	100%
05-01-004A	10/16/08	35%	11%	21%	33%	100%
Total		23%	21%	25%	32%	100%

Figure 1-WCB. West Chesapeake Bay Watershed relative contributions by probable sources of *Enterococcus* contamination.



West Chesapeake Bay Summary.

The use of ARA was successful for identification of probable bacterial sources in the West Chesapeake Bay Watershed. The highest RCC for the library was for human (90%), followed by wildlife (72%), pet (70%), then livestock (66%). The largest category of potential sources in the watershed as a whole was wildlife, followed by pet, human, and livestock (Fig. 1-WCB).

West River Watershed ARA Results

Known-Source Library. A 529 known-source isolate library was constructed from sources in the West River Watershed. The number of unique antibiotic resistance patterns was calculated, and the known sources in the library were grouped into four categories: human, livestock (alpaca, horse, sheep), pet (dog), and wildlife (deer, fox, goose, raccoon, seagull) (Table 2-WRV). The library was analyzed for its ability to take a subset of the library isolates and correctly predict the identity of their host sources when they were treated as unknowns.

Table 2-WRV: West River. Source category, total number, and number of unique patterns in the West River known-source library.

Source category	Potential Sources	Total Isolates	Unique Patterns
Human	human	98	63
Livestock	alpaca, horse, sheep	126	36
Pet	dog	175	79
Wildlife	deer, fox, goose, raccoon, seagull	130	35
Total		529	213

For West River Watershed, the rates of correct classification (RCC) were calculated and the average rate of correct classification (ARCC) for the library was determined as described above in the "Statistical Analysis" section of this document. (Table 3-WRV).

Table 3-WRV: West River. Actual versus predicted sources, with % correct (RCC) shown for each category.

	Predicted					
Actual	Human	Livestock	Pet	Wildlife	Total	% Correct (RCC*)
Human	72	12	5	9	98	73.5%
Livestock	6	80	15	25	126	63.5%
Pet	11	24	115	25	175	65.7%
Wildlife	6	20	15	89	130	68.5%
Total	95	136	150	148	529	
					ARCC:	67.3%

*RCC = Actual number of predicted species category / Total number predicted. Example: 115 pet correctly predicted; 175 total number predicted for pet = 115/175 = 65.7%.

West River Water Samples. Monthly monitoring from two (2) monitoring stations on West River was the source of water samples. The maximum number of *Enterococcus* isolates per water sample was 24, although the number of isolates that actually grew was sometimes less than 24. A total of 233 *Enterococcus* isolates were analyzed by statistical analysis. The BST results by species category is shown in Table 4-WRV.

Table 4-WRV. West River. Predicted host source distribution of water isolates.

Source	Distribution
Human	28.4%
Livestock	15.3%
Pet	37.3%
Wildlife	19.0%
Total	100.0%

The seasonal distribution of water isolates from samples collected at the sampling stations is shown below in Table 5-WRV.

Table 5-WRV: West River. *Enterococcus* isolates obtained from water collected during the spring, summer, fall, and winter seasons at the West River monitoring stations.

	Season				
Station	Spring	Summer	Fall	Winter	Total
03-07-011	53	46	29	5	133
03-07-205	37	36	11	16	100
Total	90	82	40	21	233

Tables 6-WRV and 7-WRV below show the numbers and percentages, respectively, of probable sources for each monitoring station by date.

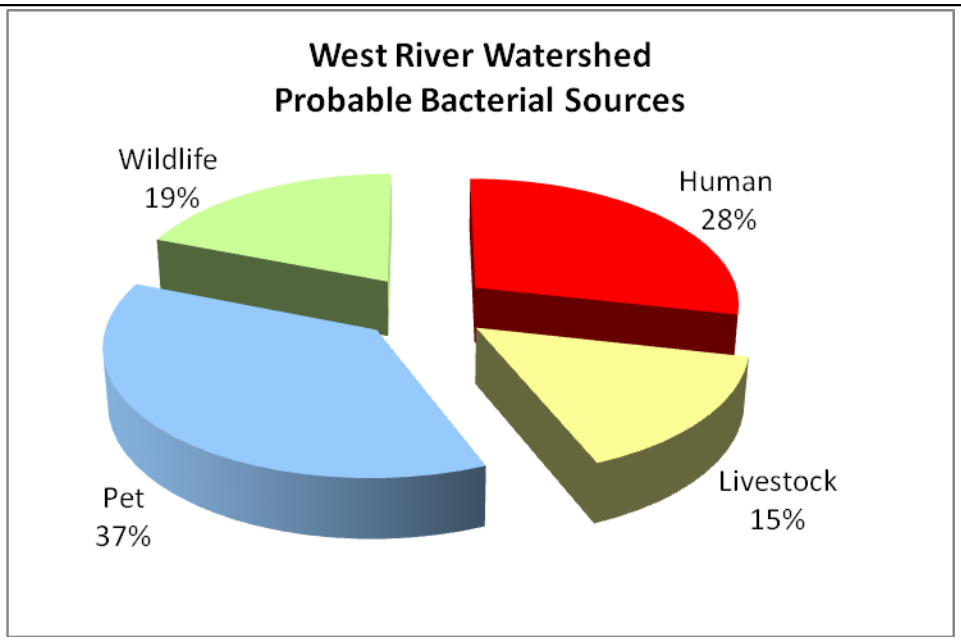
Table 6-WRV. Predicted source distribution by station and date.

		Predicted Source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
03-07-011	11/07/07	0.05	0.57	0.08	1.30	2.00
03-07-011	12/05/07	0.03	0.99	1.73	0.25	3.00
03-07-011	01/10/08	0.00	0.00	0.27	0.73	1.00
03-07-011	02/11/08	1.02	0.14	0.39	0.45	2.00
03-07-011	03/05/08	0.27	0.47	0.25	1.01	2.00
03-07-011	04/08/08	7.02	6.23	4.39	2.36	20.00
03-07-011	05/07/08	1.60	0.26	2.55	4.60	9.00
03-07-011	06/04/08	5.11	4.83	8.57	5.50	24.00
03-07-011	07/10/08	5.26	0.60	6.40	2.74	15.00
03-07-011	08/06/08	4.49	0.13	1.51	0.87	7.00
03-07-011	09/04/08	17.96	0.00	6.04	0.00	24.00
03-07-011	10/16/08	6.24	0.69	14.37	2.70	24.00
03-07-205	11/07/07	0.35	0.83	0.36	0.45	2.00
03-07-205	12/05/07	0.74	1.10	0.67	0.49	3.00
03-07-205	02/11/08	1.00	0.00	0.00	0.00	1.00
03-07-205	03/05/08	5.66	1.72	5.71	1.91	15.00
03-07-205	04/08/08	0.85	1.27	2.24	1.65	6.00
03-07-205	05/07/08	0.62	2.44	1.31	2.63	7.00
03-07-205	06/04/08	3.26	6.76	5.32	8.65	24.00
03-07-205	07/10/08	2.57	1.06	0.84	2.53	7.00
03-07-205	08/06/08	0.54	0.28	22.27	0.91	24.00
03-07-205	09/04/08	0.23	2.54	0.47	1.76	5.00
03-07-205	10/16/08	1.33	2.80	1.06	0.81	6.00
Total		66.20	35.71	86.79	44.29	233.00

Table 7-WRV. West River. Predicted source distribution by station and date.

		Predicted source				
Station	Date	Human	Livestock	Pet	Wildlife	Total
05-01-004	11/07/07	5%	12%	47%	37%	100%
05-01-004	12/05/07	5%	71%	0%	23%	100%
05-01-004	01/10/08	2%	21%	2%	76%	100%
05-01-004	02/11/08	31%	35%	6%	28%	100%
05-01-004	03/05/08	35%	4%	37%	24%	100%
05-01-004	04/08/08	12%	47%	15%	26%	100%
05-01-004	05/07/08	8%	41%	21%	30%	100%
05-01-004	06/04/08	21%	9%	35%	36%	100%
05-01-004	07/10/08	19%	2%	26%	53%	100%
05-01-004	08/06/08	0%	0%	42%	58%	100%
05-01-004	09/04/08	39%	12%	20%	29%	100%
05-01-004	10/16/08	19%	12%	45%	24%	100%
05-01-004A	11/07/07	52%	17%	21%	10%	100%
05-01-004A	12/05/07	30%	12%	26%	32%	100%
05-01-004A	01/10/08	100%	0%	0%	0%	100%
05-01-004A	04/08/08	25%	13%	44%	18%	100%
05-01-004A	05/07/08	17%	42%	5%	36%	100%
05-01-004A	06/04/08	15%	27%	26%	32%	100%
05-01-004A	07/10/08	34%	13%	25%	28%	100%
05-01-004A	08/06/08	0%	0%	100%	0%	100%
05-01-004A	09/04/08	44%	24%	5%	27%	100%
05-01-004A	10/16/08	35%	11%	21%	33%	100%
Total		23%	21%	25%	32%	100%

Figure 1-WRV. West River Watershed relative contributions by probable sources of *Enterococcus* contamination.



West River Summary

The use of ARA was successful for identification of probable bacterial sources in the West River Watershed. The highest RCC for the library was for human (74%), followed by wildlife (69%), pet (66%), and livestock (64%). The largest category of potential sources in the watershed as a whole was pet, followed by human, wildlife, and livestock (Fig. 1-WRV).

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